Université de Montréal

Étude de l'immunopathogenèse de la candidose oropharyngée chez la souris transgénique exprimant le génome du virus de l'immunodéficience humaine de type 1 (VIH-1)

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Université de Montréal

Faculté des études supérieures

Cette thèse intitulée:

Étude de l'immunopathogenèse de la candidose oropharyngée chez la souris transgénique exprimant le génome du virus de l'immunodéficience humaine de type 1 (VIH-1)

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Sommaire

La candidose oropharyngée (OPC) est l'infection fongique opportuniste la plus fréquente chez les patients infectés au virus de l'immunodéficience humaine (VIH). Bien qu'une réponse immunitaire à médiation cellulaire adaptée à l'égard de *Candida albicans* protège l'hôte contre l'OPC, les altérations de l'immunité innée et adaptée qui sont responsables de l'induction et de la persistance de la candidose buccale dans l'infection au VIH n'ont pas encore été précisément définies. Bien que les lymphocytes T CD4+ et les cellules de Langerhans, altérés dans l'infection au VIH, pourraient jouer un rôle central dans l'immunopathogenèse de l'OPC, des mécanismes compensatoires de défense de l'hôte, potentiellement préservés, pourraient limiter la prolifération de *C. albicans* à la surface des muqueuses et prévenir la dissémination systémique chez les patients infectés au VIH. La disponibilité de souris transgéniques (Tg) CD4C/HIV exprimant les produits des gènes du VIH-1 dans les cellules immunitaires et développant une maladie apparentée au SIDA est apparu comme une nouvelle opportunité d'élaborer un nouveau modèle de candidose buccale qui reproduit les principales manifestations cliniques et pathologiques de l'infection à *Candida* chez les patients infectés au VIH. Au cours de mes études de doctorat, nous avons pris avantage de ce nouveau modèle pour déterminer le rôle 1) des cellules dendritiques (DCs) et des lymphocytes T CD4+, qui expriment le transgène CD4C/ HIV^{Mut}, dans la perturbation de l'immunité protectrice des muqueuses buccales à l'égard de *C. albicans* et la persistance d'un état de porteur chronique de *C. albicans*, 2) des leucocytes polymorphonucléaires (PMNs) et des lymphocytes T CD8+ dans le contrôle de la charge buccale chronique et de la dissémination systémique de *C. albicans* aux organes profonds.

Dans la majeure partie de mes travaux, nous avons montré que la charge chronique de *C. albicans* requiert l'expression du transgène CD4C/HIV^{Mut} dans les DCs et les lymphocytes T CD4+, que la déplétion et l'altération fonctionnelle des ces populations cellulaires chez les souris Tg empêchent l'immunité adaptée spécifique à *C. albicans* in

vivo et in vitro, et que l'immunité protectrice à l'égard de *C. albicans* peut être restaurée par le transfert adoptif de lymphocytes T CD4+ sains chez les souris Tg. Les lymphocytes T CD4+ et les cellules de Langerhans, altérés, jouent donc un rôle central dans l'immunopathogenèse de la candidose buccale chez ces souris Tg exprimant le génome du VIH, et suggèrent que des perturbations similaires pourraient induire la susceptibilité à l'OPC chez les patients infectés au VIH.

En collaborant aux travaux de Miriam Marquis, nous avons montré que, bien que les PMNs provenant de souris Tg sont quantitativement augmentés et presque fonctionnellement intacts, ils sont néanmoins dispensables pour limiter la charge buccale chronique de *C. albicans*, et pour prévenir la dissémination systémique de *C. albicans* chez les souris Tg CD4C/HIV^{MutA} qui expriment les gènes *ENV*, *REV* et *NEF* du VIH. Nous avons également démontré que l'augmentation de la charge buccale de *C. albicans* chez les souris CD8 *knockout* (KO) apparaît chez les souris Tg CD4C/HIV^{MutG} qui expriment le gène *NEF* du VIH, mais pas chez les souris contrôles qui n'expriment pas le transgène. Ces résultats démontrent pour la première fois que les lymphocytes T CD8+ participent aux défenses de l'hôte contre *C. albicans* in vivo.

les mots clés : immunité des muqueuses, candidose, VIH, SIDA, souris transgéniques, cellules dendritiques

Summary

Oropharyngeal candidiasis (OPC) is the most frequent opportunistic fungal infection among human immunodeficiency virus (HIV)-infected patients. Although an intact adaptive cell-mediated immune response to *Candida albicans* is protective against OPC, the critical impairments of innate and adaptive immunity which are responsible for the onset and maintenance of mucosal candidiasis in HIV-infection have not as yet been precisely defined. Although defective CD4+ T-cells and Langerhans' cells are considered central to the immunopathogenesis of OPC in HIV-infection, completely or partly preserved compensatory host defense mechanisms most likely limit *C. albicans* proliferation to the mucosa and prevent systemic dissemination in HIV-infected patients. The availability of CD4C/HIV transgenic (Tg) mice expressing gene products of HIV-1 in immune cells and developing an AIDS-like disease has provided an opportunity to devise a novel model of mucosal candidiasis that closely mimics the clinical and pathological features of candidal infection in human HIV-infection. During my studies of doctorate, we have taken advantage of this novel model, to investigate the role of : 1) CD4C/HIV^{Mut} transgene expressing dendritic cells (DCs) and CD4+ T-cells in impaired induction of protective mucosal immunity and maintenance of a chronic carrier state of *C. albicans*, 2) polymorphonuclear leukocytes (PMNs) and CD8+ T-cells in limiting chronic oral carriage and systemic dissemination of *C. albicans* to deep organs.

In the major part of my work, we showed that chronic carriage of *C. albicans* requires CD4C/HIV^{Mut} transgene expression in at least DCs and CD4+ T-cells, that depletion and functional impairment of these cell populations present in Tg mice abrogates *Candida*-specific adaptive immunity in vivo and in vitro, and that protective immunity to *C. albicans* can be reconstituted by adoptively transferring intact CD4+ T-cells into Tg mice. Therefore, altered DCs and CD4+ T-cells are central to the immunopathogenesis of mucosal candidiasis in these Tg mice expressing HIV-1 and suggest that similar defects may underlie the susceptibility to OPC in human HIV-

infection.

While collaborating in work of Miriam Marquis, we showed that although PMNs from these Tg mice are quantitatively augmented and nearly intact functionally, they are nevertheless dispensable for limiting chronic oral carriage of *C. albicans* and for preventing systemic dissemination of *C. albicans* in CD4C/HIV^{MutA} Tg mice which express the env, rev and nef genes of HIV-1. We also demonstrated that augmentation of oral burdens of *C. albicans* in CD8 knockout (KO) mice occurs in CD4C/HIV^{MutG} Tg mice which express the nef gene of HIV-1, but not in control mice which do not express the transgene. These results represent the first-ever clear evidence indicating that CD8+ T-cells participate in host defense against *C. albicans* in vivo.

key words : mucosal immunity, candidiasis, HIV, AIDS, transgenic mice, dendritic cells

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a.a	acide aminé
ADN	acide désoxyribonucléique
Ag	antigène
APC	cellule présentatrice d'Ag
ARNm	acide ribonucléique messager
C. albicans	*Candida albicans*
CCR (X)	récepteur de chémokine
CD	déterminant de groupe
Cfu	unités de colonies viables
Cellule NK	cellule tueuse naturelle
CMH	complexe majeur d'histocompatibilité
CTL	lymphocyte T cytotoxique
CRD	domaine de reconnaissance des polyosides
DC	cellule dendritique
EGF	facteur de croissance épidermique
FcR	récepteur Fc
GM-CSF	facteur stimulant la formation des granulocytes et des macrophages
HAART	thérapies anti-rétrovirales hautement actives
Ig	immunoglobuline
IL-(X)	interleukine
KO	*knockout*
LC	cellule de Langerhans
LPS	lipopolysacharride
M-CSF	facteur stimulant la formation des macrophage
MAIDS	SIDA murin

MIP	protéine inflammatoire de macrophage
MR	recepteur au mannose
NO	monoxyde d'azote
OPC	candidose oropharyngée
PDGF	facteur de croissance dérivé des plaquettes
PMNs	polymorphonucléaires neutrophiles
Sap	protéase aspartyle sécrétée
SIDA	syndrome de l'immunodéficience acquise
TCR	récepteur des lymphocytes T
Tg	transgénique
TLR	récepteur apparenté au Toll
TNF-α	facteur α de nécrose des tumeurs
VIH	virus de l'immunodéficience humaine

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(dédicace française)

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À ma Betty,

à mes parents et à ma soeur,

à l'ensemble de ma famille,

Chapitre 1

Revue de littérature

1. Candida albicans et candidose oropharyngée

1.1 Candida albicans

Pendant des centaines d'années, il était incertain d'attribuer les symptômes de la candidose à un organisme infectieux ou à une condition humaine innée (464). Ce n'est qu'autour des années 1840, que les lésions buccales associées au «muguet» ont été attribuées à un champignon. Le nom de «*Candida albicans*» est apparu avec la codification du système de classification des espèces en 1924. Ce nom est dérivé de l'expression latine *toga candida* (la robe blanche des sénateurs romain) et d'*albicans* (la forme grammaticale de la couleur blanche).

1.1.1 Biologie de Candida

Candida albicans et *Saccharomyces cerevisiae* ont divergé d'un ancêtre commun il y a de cela 100 millions d'années (214, 258). *C. albicans* est un champignon diploïde imparfait et dimorphe dont le génome comprend environ 6500 gènes distribués sur 8 chromosomes (377). Les deux allèles de chaque gène de *C. albicans* ne sont pas nécessairement identiques (377, 563), résultant parfois en des variations de séquences en acides aminés des protéines qu'ils codent. Cependant, ces différences sont plus souvent observées dans les régions en amont des deux allèles d'un gène (698). *C. albicans* était considéré comme un champignon asexué jusqu'à très récemment, où des études menées sur les loci «*mating-type-like*» (MTL) ont montré qu'il était capable de «s'accoupler» in vitro (376) et in vivo chez la souris (277). La faible fréquence de cet accouplement (292) et l'absence d'information sur le rétablissement de la forme diploïde de *C. albicans* à partir de la forme tétraploïde (générée par l'accouplement) pourraient indiquer que ce phénomène serait dû aux manipulations des souches en laboratoire. Néanmoins, des

études récentes ont montré que le locus MTL contrôle l'apparition de la transition de couleur blanche-opaque (fréquence d'apparition de 1/1000 divisions cellulaires) chez des souches cliniques de *C. albicans* (292, 362), indiquant que cette transition pourrait être nécessaire à l'accouplement de *C. albicans* et expliquerait sa faible fréquence d'apparition.

C. albicans peut exister sous trois formes, réversibles in vitro: la forme levure unicellulaire (blastospore), la forme pseudo-hyphe, et la forme hyphe ***(Figure 1.1)***. Ce champignon réside chez l'homme de façon commensal dans le tractus gastro-intestinal, uro-génital et plus modérément sur la peau (51). *C. albicans* est un pathogène opportuniste responsable de la candidose chez les individus immuno-supprimés. Les facteurs qui prédisposent à la candidose peuvent être d'ordre physiologique (âge), hématologique (neutropénie, SIDA), endocrinologique (diabète) ou iatrogénique (antibiotique, stéroïde, chimiothérapie, cathéters endovasculaires ou urinaires) (89). La candidose peut être localisée à la surface de la peau (intertigo, perionyxis) ou au niveau des muqueuses vaginales (vaginite), gastro-intestinales (entérite, rectite) ou buccales (candidose oropharyngée ou muguet) (89). Les patients atteint de neutropénie profonde, ayant subi une greffe de moelle osseuse ou en phase avancée du SIDA peuvent présenter une dissémination systémique de *C. albicans* aux organes profonds (89).

La capacité de *C. albicans* de coloniser, de pénétrer et d'endommager les tissus de l'hôte dépend du déséquilibre entre les facteurs de virulence du champignon et le dysfonctionnement spécifique des défenses immunitaires de l'hôte. *C. albicans* possède des propriétés d'adhésion, de dimorphisme, de «*phenotypic switching*», de mimétisme moléculaire des intégrines des mammifères et de sécrétion d'enzymes hydrolytiques (464), pouvant jouer un rôle dans l'initiation de l'infection fongique chez les individus présentant un altération de leurs défenses immunitaires.

1.1.2 Les facteurs de virulence

C. albicans s'adapte aux différentes niches anatomiques de l'hôte en exprimant des

gènes spécifiques reliés à sa virulence en réponse aux signaux de son environnement. Les gènes *PHR1* et *PHR2* de *C. albicans* sont essentiels à sa survie et sont exprimés différemment en réponse au pH environnemental (427, 557). *PHR1* s'exprime in vitro et in vivo à un pH neutre (ex: le sang périphérique) et *PHR2* à un pH acide (ex: cavité vaginale). Le mutant homozygote nul pour le gène *PHR1* de *C. albicans* a une survie réduite dans un modèle de candidose systémique chez la souris et conservée dans un modèle d'infection vaginale, à l'inverse du mutant *PHR2* qui ne survit que dans la circulation sanguine (139).

L'adhérence de *C. albicans* aux cellules de l'hôte est considérée comme une étape précoce, essentielle dans l'établissement de l'infection. *C. albicans* exprime en surface des protéines d'adhésion capables de se lier à plusieurs protéines composant la matrice extracellulaire des cellules des mammifères telles que la fibronectine, la laminine, le fibrinogène, et le collagène de type I et IV (90). Ces protéines d'adhésion incluent ***(Figure 1.1)***:

- Les protéines possédant une «agglutinin-like-sequence» (ALSs). Les protéines Als1p et Als5p ont des fonctions d'adhésion aux cellules buccales humaines de l'épithélium et à la fibronectine respectivement (274).
- Les protéines «integrin-like» (Int1p) se liant aux protéines de la matrice extracellulaire et capables d'induire des changements morphologiques en réponse aux signaux extracellulaires (88, 324). La mutation du gène *INT1* chez *C. albicans* réduit son adhérence aux cellules épithéliales humaines de 40% et sa virulence chez la souris (324).
- La protéine Mnt1p possédant une activité α1,2 mannosyltransférase. La délétion du gène *MNT1* chez *C. albicans* affecte ses propriétés d'adhésion et de virulence (87). Les mannosyltransférases O-glycosylées (Pmt1p et Pmt6p) sont requises pour l'adhérence de *C. albicans* aux cellules épithéliales in vitro (637, 638). Les gènes *MNT1*, *PMT1* et *PMT6* sont impliqués dans la synthèse du mannan, constituant majeur de la paroi cellulaire fongique et les protéines Mnt1p, Pmt1p et Pmt6p pourraient être impliquées dans la reconnaissance de l'hôte (89).

- La protéine Hwp1p, servant de cible pour les transglutaminases de l'hôte et permettant ainsi la formation de liens covalents entre la levure et la surface de la cellules hôte (89).

La capacité de *C. albicans* de produire des enzymes hydrolytiques pourrait lui permettre d'endommager et d'envahir les tissus de l'hôte ***(Figure 1.1).*** L'activité des protéases aspartyles sécrétées (SAPs) et des phospholipases de *C. albicans* est synchronisée avec la progression de l'infection dans les différentes niches de l'hôte. Les Saps sont codées par 10 gènes (*SAP1* à *SAP10*) et sont responsables de la dégradation des protéines de l'hôte au site d'infection telles que l'hémoglobine, la kératine, les IgAs et les protéines du complément (276), et jouent un rôle dans l'adhérence de *C. albicans* et la dégradation des barrières des muqueuses buccales de l'hôte. In vitro les SAP1, 2 et 3 sont exprimées seulement par les blastoconidies, tandis que l'expression des SAP4 à 6 est attribuée à la forme hyphe (694). Lors de l'infection par *C. albicans* d'un épithélium humain reconstitué, les SAP1 et SAP3 sont exprimées précocement (42h après l'infection), suivi de la SAP6 (48h après l'infection) s'accompagnant de la formation d'hyphes, et enfin de l'expression de SAP2 et SAP8 (60h après l'infection) (562). Ces observations ont été validées par des résultats similaires obtenus avec des souches cliniques de *C. albicans* isolées de patients présentant une candidose buccale (562). Les isolats de *C. albicans* des patients infectés au VIH présentant une OPC produisent de plus hauts taux de Saps que ceux provenant d'individus sains (138, 467, 686). Les SAP2, SAP4, SAP5 et SAP6 sont uniformément exprimées chez ces patients (435). L'expression des gènes spécifiques de SAP est comparable chez les patients infectés ou non au VIH présentant une OPC (435) et chez les souris C3H, DBA/2, transgéniques (Tg) VIH-1 et non-Tg (518), indiquant que le VIH n'altère pas l'expression des SAPs. Néanmoins, la diminution de la prévalence de l'OPC chez les patients infectés au VIH traités aux inhibiteurs de protéases pourrait être causée en partie par la capacité d'inhibition de l'activité des SAPs de ces protéases, indiquant que les SAPs pourraient être impliquées dans la pathogénèse de l'OPC.

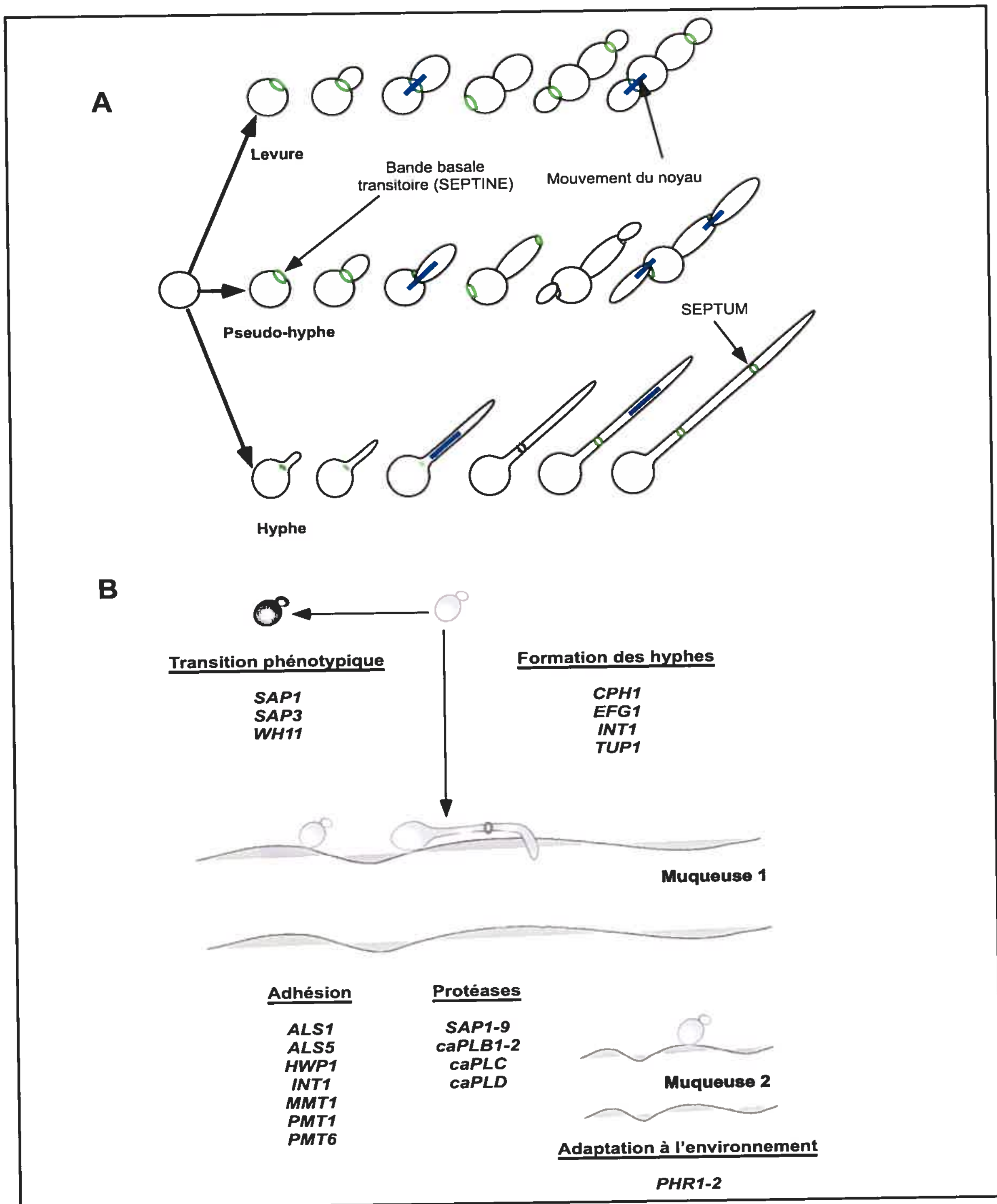

Figure 1.1 **:** A) Représentation schématique des trois formes que peut prendre le *C. albicans*: levure, hyphe, et pseudo-hyphe. Traduite de *Berman J et al., Nat Rev Genet. (2002)*, B) Les gènes impliqués dans la virulence de *C. albicans*. Traduite de *Yang YL, J Microbiol Immunol Infect (2003)*.

Parmi les phospholipases sécrétées par *C. albicans* ***(Figure 1.1)***, la phospholipase B, exprimée par deux gènes (*PLB1* et *PLB2*), contribue à la pathogénèse de la candidose en dégradant les tissus de l'hôte (351, 615). Les ARNm de *PLB1* ont été détectés durant toute la phase de porteur chronique de *C. albicans* chez la souris Tg VIH-1 (518). Les protéines caPlb1p sont sécrétées dans l'estomac (223) et dans les reins (351) des souris infectées à *C. albicans*. De plus, les blastoconidies, les pseudo-hyphes et les hyphes de *C. albicans* expriment de plus hauts taux d'ARNm de *PBL1* que les cellules formant des tubes germinatifs, suggérant que l'expression de caPLB1 est régulée en fonction de la transition morphogénique (271). L'atténuation de la virulence des mutants nul Plb1 dans différents modèles de souris (223, 351), combinée avec l'implication de *PLB1* dans la pénétration et l'endommagement des tissus de l'hôte (223, 351) indiquent que les phospholipases de *C. albicans* contribuent à sa virulence. Néanmoins, la détermination du rôle précis des produits des gènes *PLB* dans la pathogénèse de l'OPC chez les patients infectés au VIH requiert de futures expériences.

La capacité de *C. albicans* de changer de forme (hyphe-levure) en réponse aux signaux provenant de l'environnement pourrait être un facteur supplémentaire de virulence. Bien que la formation des hyphes de *C. albicans* ne soit pas requise pour sa pathogénicité, la transition de la forme levure à la forme hyphe pourrait favoriser sa pénétration des tissus de l'hôte (618) ***(Figure 1.1)***. La mutation des gènes *CPH1*, *EFG1*, *INT1* et/ou *TUP1* peut induire une réduction partielle, et dans certains cas complète, de la capacité de filamentation de *C. albicans*, inhibant ainsi sa capacité d'invasion des muqueuses (694).

Le « phenotypic switching » (blanche-opaque) est identifié in vitro comme un facteur de virulence pouvant gouverner la transition hyphe-levure (9), l'adhésion aux cellules épithéliales (653), et la sécrétion de Sap1 et Sap3 (340). Il serait responsable de l'expression différentielle des gènes essentiels à la virulence de *C. albicans* tels que le gène de résistance aux antifongiques *CDR3* (31), le gène spécifique à la formation des colonies blanches *WH11* (341) et opaques *OP4* (422), et le gène essentiel au développement de la

forme hyphe *EFG1* (589, 595).

1.2 Candidoses oropharyngée et oesophagienne dans l'infection au VIH

1.2.1 Les manifestations cliniques et la pathologie

La candidose buccale peut se présenter sous plusieurs variants de formes cliniques. Les formes pseudomembraneuse et érythémateuse de la candidose sont les plus communément retrouvées chez les patients infectés au VIH (555) et présentent avec la forme hyperplasique les trois variants principaux de candidose buccale (25). Lorsque deux ou plusieurs de ces variants apparaissent ensemble, le terme de candidose multifocalisée est utilisé (554) ***(Figure 1.2)***. D'autres variants cliniques associés à *C. albicans* comprennent la stomatite associée aux prothèses dentaires, la chéilite commissurale et la glossite médiane rhomboïde ***(Figure 1.2)***. Les symptômes se présentent chez les patients sous forme de sensation de brûlure, d'altération du goût, et de difficultés à ingérer les aliments liquides et solides (193). La candidose pseudomembraneuse peut être diagnostiquée en cultivant et en identifiant les espèces de *Candida* prélevées de la bouche des patients infectés au VIH. Néanmoins, d'autres formes telle que l'érythémateuse, de par la présence éparse des espèces de *Candida* dans la cavité buccale, requiert une biopsie et un marquage à l'acide de Schiff pour établir un diagnostique formel.

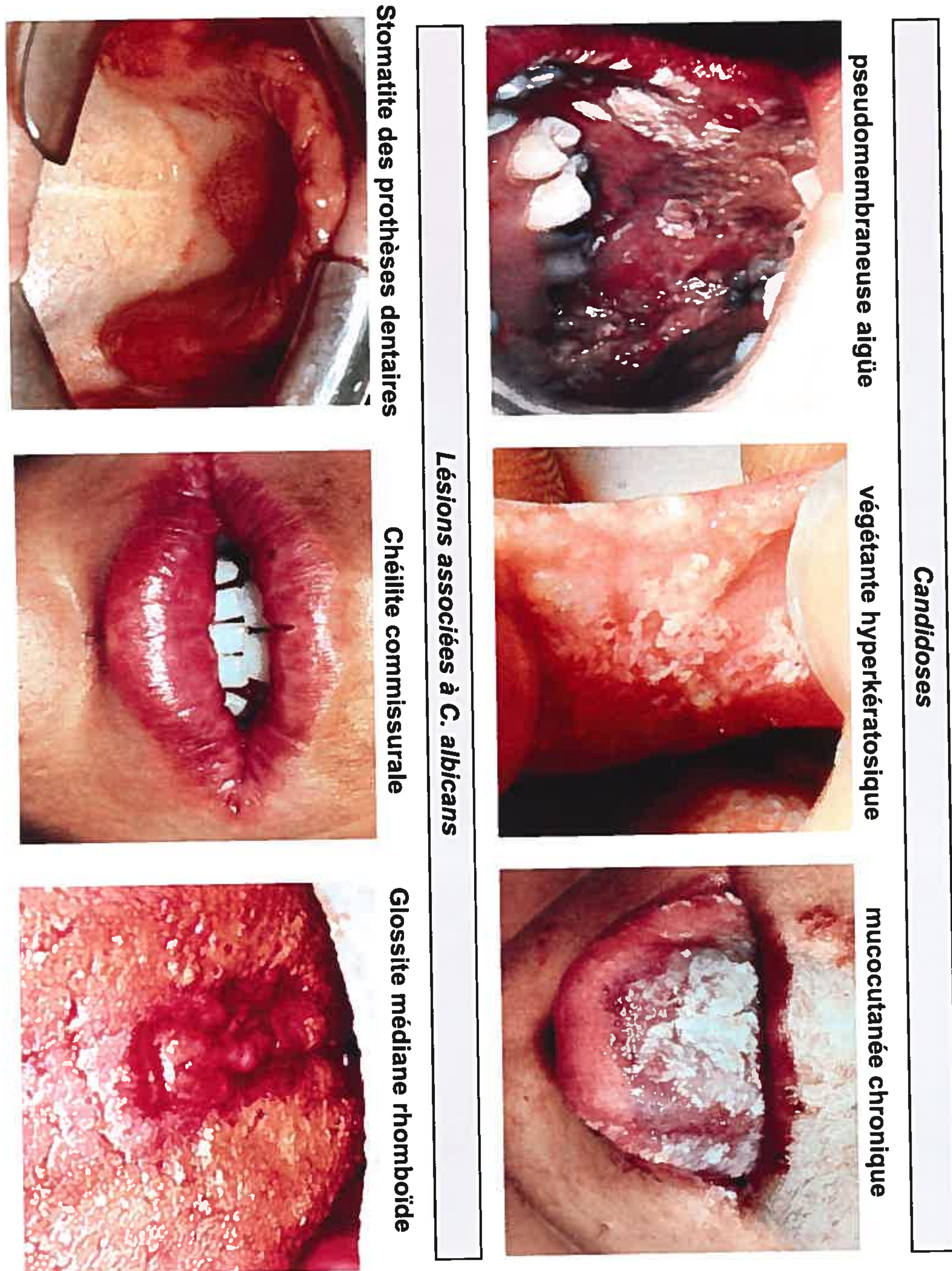

Figure 1.2 : Présentation clinique des différentes formes de candidose et de lésion associée à *C. albicans*. *Farah CS et al., Clinics in Dermatology (2000).*

Les patients infectés au VIH atteints d'OPC peuvent développer simultanément une candidose oesophagienne (75% des cas) (364), et présenter des symptômes liés à la déglutition (dysphagie, odynophagie) (116, 632). Bien que l'obtention de prélèvements buccaux chez ces patient soit restreinte pour des raisons éthiques, quelques études histopathologiques ont révélées des différences phénotypiques de *C. albicans* associées à certaines formes de candidose. Dans la candidose érythémateuse, les blastoconidies de *C. albicans* sont majoritairement observées à la surface de l'épithélium buccal, tandis que dans la candidose pseudomembraneuse on retrouve un plus grand nombre de formes hyphes en surface capables de pénétrer les espaces inter-épithélials (511, 512). La pénétration inter-cellulaire des hyphes est facilitée par le détachement des jonctions desmosomales des cellules épithéliales, qui pourrait être induit par les Saps et/ou la phospholipase sécrétées par *C. albicans* (511). Bien que ces deux formes de candidose entraînent une réponse inflammatoire d'intensité variable (plus prononcée pour la forme érythémateuse), l'infiltration de lymphocytes T CD8+ et de LCs CD1a+ prédomine chez les deux formes (523).

1.2.2 Épidémiologie

Des études longitudinales effectuées chez des patients infectés au VIH ont permis d'examiner les récurrences d'OPC en discriminant les souches de *Candida* par des techniques de biologie moléculaire (38, 153, 348, 492, 509, 556, 654). Soixante dix sept à cent pour-cent des patients présentent une OPC à *C. albicans*. Certains patients sont infectés avec une ou plusieurs espèces de *Candida* non-*albicans* (*C. tropicalis, C. parapsilosis, C. guillermondii, C. glabrata, C. dubliniensis*) ou en combinaison avec *C. albicans* (38, 348, 492, 509, 556, 654). Durant les épisodes de récurrences de l'OPC chez ces patients, les analyses sur les séries d'isolats ont révélé majoritairement une unique souche de *C. albicans*, présente de façon commensale dans la cavité buccale, responsable de l'infection (38, 348, 371, 654). Bien que les femmes peuvent développer une OPC et une

candidose vaginale, le risque d'OPC seul est augmenté par l'infection au VIH (569). L'empreinte moléculaire de *C. albicans*, prélevé chez des femmes infectées au VIH, a révélé dans tous les cas des différences entre les isolats vaginaux et buccaux, suggérant que les souches dominantes de *C. albicans* colonisant ces différents sites sont distinctes (135). Ces différences pourraient indiquer une capacité des génotypes spécifiques de *C. albicans* à coloniser différentes niches écologiques.

L'OPC et la candidose oesophagienne peuvent apparaitre au cours de la primo-infection au VIH, pendant la phase asymptomatique chronique ou plus tardivement durant la phase SIDA (1, 116, 191, 235, 329, 400, 478, 519, 569). Durant la phase asymptomatique chronique, les candidoses érythémateuse et pseudomembraneuse sont prédictives de la progression de l'immunodéficience et du début du SIDA, indépendamment du décompte des lymphocytes T CD4+ (161, 315, 326, 453). Les charges buccales de *C. albicans* sont augmentées chez les patients infectés au VIH avant même le premier épisode d'OPC (654, 647, 697) et continuent au cours de la progression du stade asymptomatique vers l'OPC (654). Ces observations suggèrent que les défenses normales de l'hôte à l'égard de *C. albicans* sont perturbées de façon précoce au cours de l'infection au VIH avant même l'apparition de la déplétion des lymphocytes T CD4+. Néanmoins, la réduction des décomptes cellulaires de lymphocytes T CD4+ (< à 200/mm^3 de sang) durant la phase avancée de l'infection au VIH entraîne un accroissement des risques des formes pseudomembraneuses de candidose (191, 329, 400, 478, 654) et de candidose oesophagienne (1).

1.2.3 Les thérapies anti-rétrovirales (HAART)

L'utilisation des thérapies anti-rétrovirales (HAART) a entraîné une diminution des incidences d'OPC (18, 102, 395) et de candidose oesophagienne (148, 284, 311), réduisant le risque de développer une candidose d'environ 30% sur 12 mois de traitement à l'aide d'inhibiteurs de protéases (18, 102, 395). Une attention particulière a été portée sur

l'influence des HAART sur l'incidence de l'OPC et la restauration des mécanismes de défense des muqueuses à l'égard de *C. albicans* perturbés lors de l'infection au VIH. En effet, la diminution de l'incidence de l'OPC chez les patients traités à l'aide des HAART est corrélée avec la remontée du nombre de lymphocytes T CD4+ (18, 311, 395). L'augmentation des lymphocytes T CD4+ mémoires et la restitution de la prolifération des lymphocytes T CD4+ en réponse aux Ag (23, 24, 414) pourraient conférer au HAART la capacité de restaurer l'hypersensibilité de type retardée à l'égard de *C. albicans*. De plus, la restitution de la production des cytokines (IL-12, IL-2, IL-10) en réponse aux mitogènes (12, 414) suggère que les HAART pourraient rectifier le profil Th2 non-protecteur à l'égard de *C. albicans*, résultant de l'infection au VIH, en un profil Th1 protecteur. Cependant, certains patients traités à l'aide des HAART se débarrassent de l'infection buccale à *C. albicans* avant même la remontée du nombre de lymphocytes T CD4+ circulants et la restitution de la prolifération à l'égard de ses Ag (100, 102). La diminution de l'incidence chez ces patients ne peut donc pas être entièrement attribuée à la capacité de reconstitution de l'immunité à médiation cellulaire à l'égard de *C. albicans* par les HAART. L'inhibition des Saps par les inhibiteurs de protéases (101) et/ou le rétablissement des fonctions des PMNs (178, 208, 673) pourraient contribuer à améliorer les défenses des patients infectés au VIH à l'égard de l'OPC et de la candidose oesophagienne.

1.3 Histologie de la cavité buccale

La muqueuse buccale est constituée à 60% d'un épithélium pavimenteux stratifié non-kératinisé (les joues, le dessous de la langue, la base de la bouche, le palais mou), à 25% kératinisé (les gencives et le palais dur) et à 15% mixte (face dorsale de la langue). Ces épithéliums reposent sur une lamina propria constituée de tissu conjonctif lache (497) séparés par une membrane basale et les couches cellulaires les composant varient suivant leur position et leur fonction dans la cavité buccale (181, 536, 594). L'épithélium pavimenteux stratifié contient une strate de cellules germinatives (stratum basale)

reposant sur la membrane basale, une strate de cellules pavimenteuses (stratum spinosum) et une strate de cellules granulaires (stratum granulosum). Il est généralement non-kératinisé à l'image de celui qui recouvre l'oesophage. A cause de l'abrasion entraînée par la mastication, les gencives et le palais dur présentent une strate additionnelle de cellules kératinisées ou para-kératinisées en surface de l'épithélium pavimenteux stratifié, similaire à celui de la peau sans la stratum lucidum (181, 536). La face dorsale de la langue contient d'abondantes papilles linguales de quatre types différents: filiformes, fungiformes, circumvallées (calciformes) et foliaires. La surface apicale des papilles est couverte d'un épithélium kératinisé, tandis que les régions inter-papillaires présentent un épithélium non-kératinisé (567). Sollicité continuellement lors des frictions créées par la mastication et les mouvements des aliments, l'épithélium buccal présente une épaisseur qui peut varier de 190±40 µm (base de la bouche) à 580±80 µm (les joues) dépendamment du degré de kératinisation, ortho- ou para-kératinisation (567). Chez la souris, l'épaisseur de l'épithélium buccal de chaque région est inférieure à celle de l'épithélium humain (265).

Le renouvellement complet des cellules de l'épithélium survient tous les 14 à 20 jours (594). Les kératinocytes attachés à la membrane basale prolifèrent sous l'influence de l'EGF, du TGF-α, du PDGF et de l'IL-1 (594), perdent l'expression de leurs intégrines, et se différentient au contact du Ca^{2+} extracellulaire, de l'acide rétinoïque, des esters de phorbol, et de la vitamine D3 (594). La migration des cellules à la surface de l'épithélium s'accompagne d'une augmentation de leur capacité de jonction (augmentation des desmosomes et des jonctions gap (497, 567)) permettant l'établissement d'une barrière physique. Les kératinocytes différenciés migrant dans les couches superficielles d'un épithélium kératinisé participent à son recouvrement de lipides lamellaires servant de barrière imperméable, tandis que les lipides intercellulaires d'un épithélium non-kératinisé sont non-lamellaires et consituent une barrière plus perméable (594, 675). La desquamation continue des kératinocytes à la surface de l'épithélium buccal joue un rôle important en limitant la colonisation et les infections à *C. albicans* (554). Dans plusieurs régions de la cavité buccale, des nodules de tissu lymphoïde forment des cryptes dans la

lamina propria à partir de l'invagination de l'épithélium. Ces régions sont infiltrées de lymphocytes pouvant jouer un rôle dans les défenses de l'hôte contre les infections buccales (554).

2. Cellules immunitaires des muqueuses buccales

2.1 Les cellules dendritiques

2.1.1 Ontogénie

Les cellules dendritiques (DCs), cellules allongées et étoilées, ont été observées pour la première fois dans la rate de souris de différentes lignées (DBA/2J, C3H, BALB/cJ, C57BL) et décrites dans les années 70 par Ralph Steinman (603-605, 607). Dérivées de progéniteurs hématopoïétiques CD34+ de moelle osseuse, les cellules de Langerhans (LCs) et les DCs interstitielles (ou dermales) partageraient un précurseur myéloïde commun avec les macrophages (36, 282), tandis que certaines DCs se différencieraient à partir d'un précurseur lymphoïde (16). La question d'un unique précurseur hématopoïétique commun reste encore d'actualité (579). Présentes dans les organes lymphoïdes et dans la plupart des tissus non-lymphoïdes, les DCs acquièrent une forme mature et se différencient sous l'influence de leur environnement local. Si l'expression ou non des marqueurs CD4 et/ou CD8αα permet de distinguer les différentes sous-types de DCs chez la souris (661), seul le marqueur CD4 a été retrouvé pour certaines DCs chez l'homme. La disponibilité de la totalité des tissus murins a rendu possible l'étude des sous-populations des DCs, mais il n'en est pas de même pour l'homme où la plupart des études sur les DCs proviennent de cellules différenciées in vitro. Ces cellules sont générées à partir de monocytes du sang ou de progéniteurs hématopoïétiques en présence de GM-CSF, GM-CSF+IL-4, GM-CSF+TNF-α ou GM-CSF+INF-γ permettant seulement une comparaison indirecte avec les DCs de souris. Néanmoins, ce sont des études in vitro à partir de monocytes humains qui ont permis la découverte d'une quatrième sous-population, les DCs plasmocytoïdes. Ces dernières se retrouvent également dans les ganglions lymphatiques des souris et peuvent être générées à partir de progéniteurs hématopoïétiques murins ou de monocytes du sang en présence

de GM-CSF+Flt3-ligand (241, 267, 462, 468). Bien que le CD8αα a longtemps été considéré comme un marqueur des DCs lymphoïdes, cette hypothèse est actuellement remise en question. Les précurseurs lymphoïdes et myéloïdes sont capables de générer in vitro des DCs CD8+ (643) et les DCs plasmocytoïdes capables d'exprimer le CD8 après stimulation (461).

2.1.2 Les cellules de Langerhans

Observées pour la première fois dans l'épiderme en 1868 par Paul Langerhans, les LCs ont été identifiées comme cellules nerveuses puis comme histiocytes en 1961 après la découverte d'organelles en forme de raquette de tennis par Birbeck (appelées communément "granules de Birbeck"). Les LCs ont été considérées comme appartenant à la famille des DCs seulement en 1985, suite aux travaux de Gerold Schuler et Ralph M. Steinman (568). Mediées par le processus inflammatoire, elles servent de sentinelle à l'entrée de pathogènes dans l'épithélium de la peau et des muqueuses. Les LCs, cellules présentatrices d' Ag (APCs) professionnelles, établissent le lien entre l'immunité innée et l'immunité acquise en capturant les pathogènes, en processant ces Ag et en les présentant aux lymphocytes B et T naïfs ou aux *Natural Killer* (NK) dans les organes lymphoïdes secondaires (119). Les LCs s'identifient par l'expression membranaire du CD1a, de Lag et de la langerin, bien que chez la souris aucune expression de CD1a n'ait été détectée en surface et que l'Ac anti-langerin murin ne soit pas encore commercialisé. Elles représentent 2 à 4% des cellules de l'épithélium, dans lequel elles résident dans les couches basales et supra-basales (142). Elles expriment les molécules du complexe majeur d'histocompatibilité de type I et II (CMH I et II) de façon constitutive, sont CD11b+ et CD11c+, et expriment différents co-récepteurs nécessaires à la phagocytose et à la présentation des Ag exogènes.

2.1.3 De la reconnaissance à la présentation des Ag

2.1.3.1 Phagocytose et co-récepteurs

Amenées par la circulation sanguine en provenance de la moelle osseuse, les DCs immatures résident dans les tissus, jusqu'au déclenchement de leur programme de maturation initié par la reconnaissance d'un pathogène ou des Ag du soi dans une situation de danger, et activent la réponse immune. Les DCs immatures sont capables de capturer les Ag par phagocytose, macropinocytose et endocytose médiée par récepteurs (242). Si la macropinocytose est constitutive chez les DCs immatures, la phagocytose et l'endocytose sont médiées par des récepteurs, l'endocytose étant plus complexe. Les LCs initient cette dernière en utilisant des récepteurs d'Ig, FcγRI et FcεRI (CD23), ou des *C-type Lectin Receptors* (CLRs). Les CLRs, constitués de deux feuillets β antiparallèles et de deux hélices α, s'organisent en domaines de reconnaissance (Ca2+ dépendant) des sucres portés par les protéines ou les lipides (*carbohydrate recognition domains* (CRDs)) (197). Le *macrophage mannose receptor* (MMR), et le DEC 205, constitués respectivement de 8 et 10 CRDs, appartiennent aux CLRs de type I, tandis que le *dendritic-cell-specific ICAM-3 grabbing non-integrin* (DC-SIGN) et la langerin (CD207), constitués de 1 CRD, appartiennent au type II. Le MMR utilise 2 CRDs pour la reconnaissance spécifique du mannose et du fucose (633), permettant une endocytose constitutive et l'acheminement des Ag aux endosomes. Le DEC 205 et le DC-SIGN reconnaissent des structures manosylées, les internalisent après l'interaction ligant-récepteur et acheminent ces Ag vers les endosomes tardifs ou vers les lysosomes (197). La langerin achemine les Ag vers les granules de Birbeck. Bien qu'elle soit capable de se lier au mannose contenu dans les carbohydrates de la protéine gp 120 du VIH, seuls le MMR et le DC-SIGN ont la capacité de capturer et transmettre le VIH aux lymphocytes T (221). L'expression de nef dans les DCs immatures est impliquée dans la redistribution du DC-SIGN, réduisant son internalisation et favorisant le contact et la transmission du virus aux lymphocytes T (586). Les LCs n'expriment pas le MMR et le DC-SIGN, mais ces derniers ont été observés respectivement

à la surface des macrophages et des DCs et pourraient jouer un rôle clé dans la dissémination du VIH (220, 452). Ces deux récepteurs semblent également impliqués dans la phagocytose de *C. albicans* par les DCs (91, 134), bien que des études récentes in vitro ont montré que le DC-SIGN murin n'interagissait pas avec le *C. albicans* tué (627). Au cours de leur maturation, les DCs diminuent leur capacité d'endocytose, l'expression membranaire de leurs récepteurs MMR et FcR, la macropinocytose et la phagocytose, limitant la diversité d'Ag qu'elles présenteront aux lymphocytes (546).

2.1.3.2 Activation, maturation et migration

L'engagement des FcR, à l'image du FcεRI induisant la phosphorylation de la tyrosine kinase Syk et la maturation des LCs, compte parmi trois autres processus d'activation des DCs comprenant: les récepteurs des cytokines de l'inflammation (IL-1β, TNF-α), le PGE2, les *Toll-like receptors* (TLRs) et les molécules de la famille des récepteurs du TNF (ex: CD40 Ligand (CD40L)) (221). Le LPS, les composants des membranes des bactéries GRAM+ et GRAM-, et les CpG non-methylés activent respectivement les TLR-4, TLR-2 et TLR-5, et TLR-9 via la reconnaissance de *pathogen-associated molecular patterns* (PAMPs) (221). Les PAMPs de *C. albicans* pourraient être reconnus par les TLR-2 et TLR-4 jouant un rôle dans l'induction et la production des cytokines et des chemokines de l'inflammation (298, 446). Si le LPS induit l'activation du NF-κB au cours de la maturation des DCs, l'interaction des Ag membranaires CD40 des DCs avec le CD40L des lymphocytes favorise l'activation des p38MAPK requise pour la production d'IL-12, cytokine impliquée dans la réponse immunitaire à médiation cellulaire (367). Au cours de la maturation, les LCs sécrètent de l'IL-12 mais également de l'IL-8 et les *macrophage inflamatory proteins* 1α et 1β (MIP-1α et MIP-1β), et expriment de hauts taux de CMH I et II et de molécules de co-stimulation CD54, CD58, CD80 et CD86 (36). La sur-expression du CD80 et/ou du CD86 pourrait être favorisée par l'augmentation des interactions CD40/CD40L (36).

Les DCs immatures, capables de migrer suivant un gradient de chemokines en

réponse à l'inflammation des tissus, seront exposées aux cytokines pro-inflammatoires (TNF-α, IL-1) et aux produits de pathogènes. La réduction d'expression des récepteurs membranaires des chemokines CCR1, CCR5 et CXCR1 et l'augmentation des récepteurs CXCR4, CCR4 et CCR7 traduisent un état de cellules matures capables de migrer vers les organes lymphoïdes secondaires (359). Des études ont montrées que les DCs matures réduiraient leurs récepteurs de chemokines par régulation autocrine en sécrétant du MIP-1α, du MIP-1β et RANTES (549). Le CCR7, exprimé également à la surface des lymphocytes B et T naïfs, permettrait aux DCs de migrer des tissus aux organes lymphoïdes secondaires (204). Le MIP-3α, exprimé par les kératinocytes stimulés au TNF-α dans la couche de cellules épineuses de l'épithélium (110), et les défensines reconnaissent le CCR6 exprimé uniquement à la surface des LCs (692), pouvant ainsi expliquer la position et l'accessibilité aux produits de pathogènes envahissant l'épiderme (133). l'IL-18, produit par les LCs et les kératinocytes, contribue également à la régulation de la migration des LCs orchestrée par le TNF-α et l'IL-1β (132).

2.1.3.3 Présentation

La présentation des Ag via le CMH II des LCs, sous l'action de l'IL-12 et de la collaboration de l'IL-18, induit la différentiation des lymphocytes CD4+ en lymphocytes Th1 producteurs d'IFN-γ, cytokine activant les propriétés anti-microbiennes des macrophages, et établit le premier pas vers l'induction de la réponse immunitaire à médiation cellulaire (36, 436). L'augmentation de l'expression des co-récepteurs des DCs B7-1 (CD80) et B7-2 (CD86) interagissant avec le CD28 des lymphocytes T CD4+ participe à l'initiation de leur prolifération alors que la liaison de ces co-récepteurs avec le CTL-4 en induit l'anergie (242). L'interaction des Ag OX40 et OX40L, à la surface des lymphocytes T CD4+ et des DCs respectivement, induit également la différenciation des lymphocytes (242).

L'interaction CD40L/CD40, qui s'établit entre les lymphocytes T auxiliaires et les LCs

respectivement, permet non seulement aux LCs de présenter les Ag via le CMH I aux lymphocytes T CD8+ cytotoxiques mais engendre un signal suffisant pour initier leur prolifération (36, 242). L'engagement du 4-1BBL des DCs avec le récepteur 4-1BB augmente l'activation des lymphocytes T CD8+ et leur production d'IFN-γ (581). Si les lymphocytes T CD4+ naïfs nécessitent le prolongement du signal via l'interaction de leurs molécules de co-stimulation jusqu'à 20h, les lymphocytes T CD8+ prolifèrent après un temps très court (1h) d'exposition aux Ag (280). Chez la souris, la muqueuse buccale est un site d'induction de la prolifération des lymphocytes T CD8+ via le CMH I (147).

2.1.4 Localisation

La densité des LCs est pratiquement identique dans la peau et dans les muqueuses buccales non kératinisées chez l'homme, est moins importante dans les muqueuses buccales kératinisées, mais ne semble pas différer de celle des muqueuses buccales des souris (84, 136, 652). Cependant, les LCs humaines présentent des morphologies variables dépendant de leur localisation dans l'épithélium. Formant un réseau de dendrites dans l'épithélium supérieur, les LCs CD1a+ diffèrent des formes arrondies et faiblement étoilées des LCs observées dans la couche basale et pourraient constituer une surveillance immune optimale (573). Les LCs peuvent être recrutées dans l'épithélium buccal en réponse à une infection bactérienne et leur densité est augmentée chez les patients atteints de péridontite chronique (70, 297). Chez l'homme, les LCs de l'épiderme activent les lymphocytes T spécifiques à *C. albicans* en engageant non seulement une interaction entre leur CMH avec le récepteur T, mais également entre leurs molécules d'adhésion CD54 et CD58 avec le CD11a et le CD2 respectivement (636).

L'infection des LCs des muqueuses buccales par le VIH pourrait contribuer à leur déplétion (118), perturber leur capacité à générer une réponse immune primaire (59) et contribuer au dysfonctionnement de l'immunité buccale protégeant des infections opportunistes par les pathogènes microbiens.

2.2 Les cellules lymphoïdes

2.2.1 Les lymphocytes T α/β

2.2.1.1 Le processus trans-migratoire

D'abord précurseurs hématopoïétiques lymphoïdes et se différenciant ensuite en cellules lymphocytaires dans le thymus, les lymphocytes T naïfs circulants expriment la L-sélectine, le récepteur de chemokine CCR7 et l'intégrine LFA-1. Ces protéines membranaires sont impliquées dans les étapes séquentielles des interactions aux cellules endothéliales. Si la L-sélectine et l'intégrine α4 médient le roulement des lymphocytes à la surface de l'endothélium (229, 596), LFA-1 limiterait leur mouvement en faveur d'un attachement aux ICAM-1 des cellules endothéliales et aux JAM-1 exprimés sur les *tight junctions* (471, 545). Ces récepteurs, les intégrines β1 et β2 transitoirement sur-exprimées sous l'influence des chemokines (125), les récepteurs non-intégrines CD31 et CD99 (495), et l'intégrine α4β1 (158) seraient impliqués dans le processus trans-migratoire et la diapédèse des lymphocytes T.

Les lymphocytes T naïfs ($CD62L^{hi}$, $CD45RB^{hi}$, $CD44^{lo}$), entrant dans la région paracorticale des ganglions lymphatiques où s'étend un réseau de fibres réticulées constituées en majeur partie de collagène de type I, II, III, et IV (305), présentent une forte mobilité permettant de patrouiller les populations de DCs (72, 610). Les gradients de chemokines et de cytokines du micro-environnement favorisent ce phénomène et participent au maintien de l'interaction lymphocyte T / DCs en présence d'Ag exogènes (76).

Le stimulus délivré via *le T cell receptor* (TCR), l'engagement de molécules de co-stimulation spécifiques et l'activation de leur voie de signalisation intracellulaire sont nécessaires aux lymphocytes T CD4+ naïfs pour progresser vers un profil Th1 ou Th2.

Suite à l'engagement du TCR, le type d'APC (124), la concentration d'Ag présenté, le temps d'interaction ou encore l'intensité du signal (630) sont d'autres facteurs influençant la polarisation Th1/Th2 des lymphocytes T CD4+.

2.2.1.2 Lymphocytes T α/β CD4: Th1 versus Th2

La sécrétion du facteur Eta1/osteopontin par les lymphocytes T CD4+ les différencierait directement en lymphocytes Th1 en induisant simultanément la production d'IL-12 et l'inhibition de la sécrétion d'IL-10 par les APC (19). L'IL-6 activerait la formation de lymphocytes CD4+ Th2 sécréteurs d'IL-4 (517). De même, les regroupements des récepteurs (TCR, CMH) à la surface de leurs cellules respectives, via les radeaux lipidiques, pourraient coordonner l'agrégation des molécules impliquées dans l'initiation et la régulation du signal de polarisation (30). La kinase Src, la kinase Tec, la PLCγ, le flux calcique (Ca^{2+}), l'IP3 et la famille des MAPK (ERK, JNK, p38 MAPKs) participent à la transduction des signaux chez les lymphocytes T CD4+ naïfs activés au contact du CMH II des APC exposant l'Ag cible (624).

Si les molécules de co-stimulation CD28, CTLA-4, OX40 ou les interactions LFA-1/ ICAM-1 semblent également impliquées dans le processus de différenciation Th (624), les cytokines de l'environnement des lymphocytes T CD4+ sont les plus déterminants. L'IL-12 et l'IL-4 ont été initialement caractérisées comme les cytokines influençant respectivement la différenciation en Th1 et Th2. Les lymphocytes CD4+ Th1 produisent l'IFN-γ, l'IL-2, le TNF-α et la lymphotoxine β (LT-β) favorisant l'immunité à médiation cellulaire, tandis que les lymphocytes CD4+ Th2 sécrètent l'IL-4, l'IL-5, l'IL-6, l'IL-9, l'IL-10 et l'IL-13 ayant un rôle important dans la réponse immunitaire menant à la production d'Ac (426, 460, 624).

2.2.1.3 Récepteurs et cytokines

2.2.1.3.1 IL-12R/IL-12

L'IL-12 est une molécule soluble hétérodimérique de deux sous-unités, p35 et p40, sécrétée par les APC activés incluant les DCs, les macrophages et les monocytes (624). Bien que le récepteur IL-12R, composé de deux chaînes IL-12Rβ1 et IL-12Rβ2, soit activé au contact de la forme p70 de l'IL-12 (la forme p40 sécrétée étant un antagoniste) (216, 494), il est absent de la surface des lymphocytes T CD4+ naïfs. En effet, l'activation via le TCR est nécessaire pour induire l'expression de l'IL-12Rβ1 et de l'IL-12Rβ2 formant un IL-12R fonctionnel (494). L'expression de l'IL-12Rβ2 est maintenue à la surface des lymphocytes CD4+ Th1 et inhibée durant le processus de différenciation des lymphocytes CD4+ Th2, perdant ainsi la capacité de répondre à l'IL-12 (521, 623). L'IL-12 peut initier la prolifération des cellules NK et des lymphocytes T exprimant un IL-12R fonctionnel permettant l'activation de la voie Jak/STAT et des facteurs de transcription STAT 1, 3, et 4(217, 650). Néanmoins, l'activation unique de STAT 4 pourrait être spécifique de l'action de l'IL-12 chez la souris (286). Cependant, l'observation in vitro et in vivo de lymphocytes CD4+ Th1 producteurs d'IFN-γ en absence d'IL-12 permettrait d'attribuer à l'IL-12 un rôle dans la sécrétion optimale d'IFN-γ dans les stades plus tardifs de l'infection par des micro-organismes exogènes plutôt que dans l'initiation de la réponse Th1 (624).

2.2.1.3.2 IL-18R/IL-18

L'IL-18, produit par les macrophages et les DCs, sert de cofacteur à l'IL-12 pour le développement de la voie Th1 et l'activation de la production d'IFN-γ par les lymphocytes CD4+ Th1 effecteurs. Le récepteur IL-18R, constitué de l'IL-18Rα et d'une sous-unité de transduction du signal (IL-18β), est absent sur les lymphocytes T CD4+ naïfs, mais son expression sur les lymphocytes CD4+ Th1 pourrait être induite par l'Il-12 (699). Bien que l'IL-18 ne soit pas essentiel à la différenciation des lymphocytes T CD4+ en Th1, il pourrait faciliter le développement de la voie Th1 induit par l'IL-12, en optimisant la production d'IFN-γ par activation du NFκB via son récepteur (624).

2.2.1.3.3 IFN-γR/IFN-γ

L'IFN-γ, cytokine pléïotropique impliquée dans la réponse immune innée et adaptive, est synthétisé par les cellules NK, les cellules γ/δ, les lymphocytes T CD8+ et CD4+ Th1, bien que les lymphocytes T CD4+ naïfs, les macrophages, les DCs et les lymphocytes B peuvent constituer une source additionnelle de sécrétion supposant une capacité de régulation autocrine (207, 431, 466, 699). Sous l'action de l'IFN-γ, son récepteur l'IFN-γR, composé de deux chaînes IFN-γR1/IFN-γR2 est exprimé sur la majeure partie des cellules lymphoïdes et non-lymphoïdes, utilise la voie JAK/STAT et particulièrement STAT1 pour conduire la transduction du signal (27, 63). Les souris déficientes en IFN-γ, IFN-γR1, IFN-γR2 ou STAT1 ont une réponse immunitaire perturbée in vivo, se traduisant par l'augmentation de la susceptibilité aux pathogènes microbiens (624). En effet, un des rôles essentiel de l'IFN-γ est d'activer les macrophages, résultant en une augmentation de la phagocytose, de l'expression du CMH I et II, et en l'induction d'IL-12, de monoxyde d'azote, et de la production de superoxyde, tous importants pour l'élimination de pathogènes intracellulaires (63). Bien que son rôle dans la différenciation Th1 soit controversé (624), son absence dans les cultures de lymphocytes T CD4+ serait important pour l'engagement dans la voie Th2 (624, 674). De plus, l'IFN-γ induit rapidement l'expression de T-bet, un facteur de transcription spécifique de la voie Th1 contrôlant la différenciation des lymphocytes T CD4+ (356).

2.2.1.3.4 IL-2R/IL-2

L'IL-2, cytokine composée de quatre hélices α, est produite majoritairement par les lymphocytes T CD4+ activés, et l'augmentation de la synthèse de ses ARNm serait en partie régulée par un signal via le TCR et le CD28 (493). Les lymphocytes T CD4+ et CD8+ naïfs, les DCs, et les cellules thymiques seraient également capable d'exprimer l'IL-2 (40,

114, 230, 459). Bien que les trois sous-unités IL-2Rα (CD25), IL-2Rβ (CD122) et la chaîne commune γ_c (CD132) constituent ensemble un récepteur de haute affinité de liaison de l'IL-2, l'IL-2Rβ et γ_c forment un récepteur compétent d'affinité intermédiaire pour l'IL-2 et partagé par l'IL-15 (373, 440). Le récepteur de l'IL-2 activé est associé à JAK/STAT3/ STAT5, permettant la phosphorylation des PI3K et des MAPK et l'induction de la prolifération, la survie, et la différenciation des lymphocytes T en cellules effectrices (379). L'interaction IL-2/IL-2R est responsable de l'expansion clonale des lymphocytes T effecteurs spécifiques à un Ag. Bien que l'IL-2 soit caractérisée comme une cytokine Th1, elle est également requise pour la prolifération des lymphocytes T régulateurs CD4+CD25+ (T_{Reg}) (380) incapables de produire de l'IL-2 et dépendant d'une régulation paracrine (379). De plus, l'IL-2, de par sa fonction de facteur de prolifération, est importante pour l'initiation de l'activation des lymphocytes CD4+ Th2 sécrétant l'IL-4 (129, 350).

2.2.1.3.5 IL-4R/IL-4

L'IL-4, cytokine produite par les mastocytes, les cellules basophiles, les cellules NK et les lymphocytes CD4+ Th2, est requise pour le développement de la voie Th2 (426) et cette synthèse induit la sur-expression du CMH II des lymphocytes B présentant un Ag, active la commutation de classe des Ac en IgE et IgG1, et sert de mitogène aux lymphocytes B (439). Son récepteur de type I, constitué de l'IL-4Rα et de γ_c, est exprimé à la surface des cellules hématopoïétiques, tandis que le type II, constitué de l'IL-4Rα et de l'IL-13Rα1, partage le signal avec l'IL-13 et est exprimé à la surface des cellules épithéliales et de certaines cellules du système immunitaire (426, 439). L'IL-4R est associé aux molécules de signalisation intracellulaire JAK/STAT6 qui, lorsqu'activées, entraînent la sur-expression de GATA-3, facteur de transcription spécifique de la voie Th2 (472).

2.2.1.3.6 IL-10R/IL-10

L'IL-10, cytokine produite par les lymphocytes CD4+ Th2 et les lymphocytes B, a été initialement caractérisée comme un inhibiteur de la production de cytokines Th1 (IL-2, IFN-γ), du TNF-α et du GM-CSF (198). Elle joue également un rôle important dans le blocage de la production des chemokines, des molécules de co-stimulation CD80, CD86 et du CMH II (482). Bien que la principale fonction de l'IL-10 soit de limiter l'amplitude de la réponse immunitaire évitant ainsi l'auto-immunité tel que dans la maladie de Crohn (335), le knock out du gène chez la souris augmente la réponse Th1 nécessaire à l'élimination de *C. albicans* (657). Son récepteur, composé de l'IL-10R1 et de l'IL-10R2, présent à la surface de la plupart des cellules lymphoïdes et non-lymphoïdes, est associé avec JAK/STAT3, mais son activation peut induire la phosphorylation des facteurs de transcription STAT1 et STAT5 et initier la transcription des gènes codant pour les *Suppressors Of Cytokine Signaling 1&3* (SOCS1 et SOCS3) (4, 157, 667). SOCS1 serait l'inhibiteur physiologique majeur des signaux de transduction via l'activation des récepteurs de l'IFN-γ, l'IL-10 et l'IL-4, émettant l'hypothèse de l'existance d'une auto-régulation entre IFN-γ et IL-10 (4, 482).

2.2.1.3.7 TNFR/TNF-α

Le TNF-α est produit sous forme d'une protéine transmembranaire homotrimérique stable de type II qui nécessite l'activité protéolytique de la metalloprotéase *TNF alpha converting enzyme* (TACE) pour être généré sous forme de cytokine soluble (sTNF) (57, 332). Deux récepteurs, TNFR1 et TNFR2, peuvent lier le TNF-α membranaire, le sTNF, et la LTα (665), bien que le TNFR2 semble être activé uniquement au contact du TNF-α membranaire (238). TNFR1 est exprimé constitutivement dans la majorité des tissus, alors que le TNFR2 est hautement régulé et exprimé à la surface des cellules du système immunitaire (238, 665). Le TNF-α est produit par les macrophages, mais également par les cellules lymphoïdes, les cellules endothéliales, les fibroblastes et les tissus neuronaux (383). Une fois activé, le TNFR utilise différentes voies de signalisation intracellulaire

incluant TRADD, TRAF, JNK et la P38-MAPK jusqu'à l'activation du NFκB (275, 480, 513, 665). Si la fonction attribuée au TNF-α est de médier la réponse pro-inflammatoire ou d'induire l'apoptose dans des conditions physiopathologiques, il est un médiateur important de la progression des maladies auto-immunes comme la maladie de Crohn ou l'arthrite rhumatoïde (665). Médiateur de l'immunité innée, le TNF-α est également capable de moduler le système immunitaire lors d'une réponse correctement effectuée, indépendamment de son rôle dans les défenses de l'hôte (328).

2.2.1.4 Les lymphocytes T régulateurs CD4+CD25+

Les T_{Reg}, lymphocytes T CD4+ exprimant constitutivement le CD25, sont produits sous forme mature et fonctionnelle par le thymus et forment une population cellulaire (5-10% des lymphocytes circulants) endogène et distincte des lymphocytes T CD4+ naïfs activés après exposition aux Ag exogènes (543). Exprimant *Foxp3*, gène codant pour un facteur de transcription contrôlant leur développement et leur fonction (202), les lymphocytes T_{Reg} CD25+CD4+Foxp3+ s'engageraient dans un contrôle négatif des réponses immunes pathologiques et physiologiques et pourraient être exploités pour augmenter les défenses immunitaires anti-microbiennes de l'hôte (541, 542). Dépendant de l'IL-2 sécrétée par les lymphocytes T CD4+ activés qui maintient leur expression de CD25, les lymphocytes T_{Reg} expriment constitutivement le CTLA-4 (385, 508).

Les DCs $CD86^{hi}$ matures présentant un Ag exogène activent les lymphocytes T CD4+ naïfs et atténuent la suppression médiée par les lymphocytes T_{Reg}, provoquant ainsi une réponse immune effectrice face aux pathogènes microbiens (190). En effet, le CD86 possède une plus haute affinité pour le CD28, alors que le CD80 interagit préférentiellement avec le CTLA-4 (122). De plus, l'IL-6 sécrétée par les DCs via l'activation du TLR rend les lymphocytes T CD4+ naïfs résistants à la suppression par les lymphocytes T_{Reg}, suggérant l'existance d'un mécanisme de blocage de la suppression via les TLRs (476, 477). Le TGF-β, produit par les cellules Th3, et l'IL-10, produite par les

lymphocytes régulateurs Tr1 CD25- et les lymphocytes T_{Reg} CD4+CD25+, participent à l'homéostasie et au contrôle de la prolifération des lymphocytes T (203).

2.2.1.5 Les lymphocytes T mémoires

Les lymphocytes T à mémoire centrale (T_{CM}) et à mémoire effectrice (T_{EM}), générés par l'accumulation des expositions aux Ag exogènes des individus durant leur vie, se distinguent par la présence ou l'absence de fonctions effectrices immédiates et l'expression de récepteurs leur permettant de migrer vers les ganglions lymphatiques ou les tissus inflammés (548). Les lymphocytes T_{CM} CD45RO+ humains, exprimant constitutivement le CCR7 et le CD62L nécessaires au processus trans-migratoire des cellules vers les ganglions lymphatiques, sont plus sensibles à la stimulation par les Ag que les lymphocytes T naïfs (347, 547). Après stimulation du TCR, ces cellules vont produire de l'IL-2, proliférer, devenir effectrices (CCR7-) et sécréter de large taux d'IL-4 et d'IFN-γ (547). Les lymphocytes T_{EM} humains, CCR7- et hétérogènes pour l'expression du CD62L, constituent une réserve de lymphocytes Th1, Th2 et CTL et expriment les récepteurs de chemokines et les molécules d'adhésion nécessaires pour leur migration vers les tissus inflammés (547). Les lymphocytes T_{EM} CD8+ sécrètent la perforine, et les lymphocytes T_{EM} CD8+ et CD4+ de l'IFN-γ, de l'IL-4 et de l'IL-5 cinq heures après stimulation par un Ag in vitro. Les lymphocytes T_{CM} sont enrichis dans les ganglions lymphatiques et les amygdales, alors que les poumons, le foie, et l'intestin contiennent une proportion plus grande de T_{EM} (92). Bien que le système T_{CM}/T_{EM} semble s'étendre à la souris, les quelques différences de fonction du CCR7 murin et l'utilisation du CD44 comme marqueur des lymphocytes T CD4+ et CD8+ mémoires ont généré des résultats encore controversés (83, 117, 409, 484, 547, 593). Dans les ganglions lymphatiques, les lymphocytes T_{CM} expriment le CXCR5, récepteur du CXCL13 produit par les follicules B (547). ILs expriment également le CD28 et le CD40L leur conférant un rôle d'aide aux lymphocytes B et produisent de l'IL-2 et de l'IL-10 après activation sans polarisation Th

(547). Les lymphocytes T_{EM} peuvent être divisés en Th1 et Th2, le CCR5 et le CXCR6 discriminant les lymphocytes CD4+ Th1 et les CTL, et le CCR3 et le CRTh2 identifiant les lymphocytes CD4+ Th2 (547). Cependant, dans des conditions normales de stimulation, les lymphocytes T_{EM} conservent leur phénotype Th1 ou Th2 mais sont capables d'alterner de phénotype dans des conditions de stimulation opposée à leur profil Th (409). La diminution du potentiel d'expansion des lymphocytes T_{CM} aux T_{EM} est corrélée avec la diminution de la longueur des télomères et l'augmentation de la susceptibilité à l'apoptose, pouvant néanmoins être interrompue par des molécules anti-apoptotiques synthétisées lors de la co-stimulation (547, 587).

2.2.1.6 Les lymphocytes T CD8

Les lymphocytes T CD8+ reconnaissent et répondent aux peptides étrangers présentés par le CMH I en passant d'un stade quiescent à une forme effectrice, au potentiel cytotoxique, spécifique d'un Ag, et pénètrent dans les tissus pour détruire les cellules cibles. Capables de se diviser toutes les 4-8 h suivant le stade de l'infection (53, 86), ces cellules possèdent également la capacité de devenir mémoires pour répondre plus efficacement à la réapparition du pathogène.

2.2.1.6.1 La réponse T CD8

Lors d'une activation non-spécifique ou de signaux de danger (cellules en apoptose ou en nécrose) provenant des APCs, l'activation de la réponse in vivo des lymphocytes T CD8+ est dépendante de la collaboration et de l'activité des lymphocytes T CD4+ (99, 278, 320). Bien que cette collaboration à trois soit définie par la reconnaissance des Ag des APCs par les lymphocytes T CD4+ *helper* qui se lient par interaction de leurs épitopes avec les lymphocytes T CD8+ cytotoxiques (CTL) (53, 278, 417), à l'image de la collaboration lymphocytes T CD4+/lymphocytes B, la fonction d'aide (via l'IL-2 ou l'interaction directe

CD4/CD8) est encore discutée. Des expériences in vivo ont montré que les lymphocytes T CD4+ et T CD8+ doivent reconnaître les épitopes antigéniques sur la même APC pour l'activation en CTL (49). De plus, la fonction des lymphocytes T CD4+ spécifique dans la reconnaissance d'un Ag peut être remplacée en activant les APCs par un anticorps anti-CD40 et la réponse des CTL dépendant des lymphocytes T CD4+ peut être inactivée en bloquant l'interaction CD40/CD40L (48, 515, 565). Les APCs activés par les lymphocytes T CD4+ seraient alors capables de recevoir l'information et de la transmettre aux lymphocytes T CD8+ dans un modèle de collaboration deux à deux (53).

Lors d'une infection, les CTL génèrent une forte réponse primaire indépendante des lymphocytes T CD4+, et l'utilisation de souris KO pour le CMH II ou le co-récepteur CD4 a permis de mesurer cette réponse à l'égard de nombreux agents infectieux (82, 507, 688). L'activation directe des APCs par les agents infectieux via les TLRs et l'induction de la production de TNF-α ou d'IFN de type I pourraient expliquer l'activation directe des CTL sans nécessiter l'aide des lymphocytes T CD4+ (53). Cependant, une action plus tardive des lymphocytes T CD4+, impliquant leur participation à l'étape finale de différenciation en cellules mémoires des CTL a été observée (575, 616). La faible réponse effectrice des CTL à une deuxième infection en leur absence (69, 322) permettrait de leurs attribuer un rôle de programmation des CTLs mémoires (396) et de maintenance des lymphocytes T CD8+ mémoires après l'activation de la réponse primaire (53).

2.2.1.6.2 Activité cytotoxique

L'activité cytotoxique des lymphocytes T CD8+ a été initialement décrite en deux voies distinctes, via les perforines/granzymes ou l'activation de Fas (301). Fas est un récepteur physiologiquement important qui initie la mort des cellules en recrutant les molécules intracellulaires FADD et la caspase 8 (334, 538). Bien que l'apoptose des cellules peut survenir par plusieurs voies intrinsèques, l'activation de Fas et le recrutement de FADD dirigent une voie extrinsèque d'apoptose uniquement via les caspases (jusqu'à

l'activation de la caspase 3) (538). La voie des perforines est dominante chez les lymphocytes T CD8+ (302), mais les cellules naïves, sans activité cytotoxique, doivent être activées via le TCR leur permettant d'exprimer les récepteurs de l'IL-2 et l'IL-6 et d'initier la formation de granules contenant les perforines et les granzymes (538). Lors de la formation de la synapse immunologique entre la cellule cible et les CTLs initiée par le TCR et maintenue par l'interaction LFA-1/ICAM-1 (495), les CTL réorientent les granules avant de les excréter dans la région de contact (338, 538). Les perforines créeraient un canal par polymérisation (Ca2+ dépendant) permettant le passage des molécules cytotoxiques mais seraient incapables d'initier l'apoptose des cellules cibles sans l'action des granzymes (166, 301, 538, 577). De plus, la granzyme B serait nécessaire à l'induction rapide de l'apoptose par les CTL (262, 580) et pourrait activer les caspases des cellules cibles entraînant la fragmentation de l'ADN et leur apoptose (538).

2.2.1.6.3 Les CTL: Tc1 versus Tc2

Les lymphocytes T CD8+ effecteurs expriment en majeur partie l'FN-γ, le TNF-α et la LT de façon similaire aux lymphocytes CD4+ Th1, permettant d'activer les fonctions cytotoxiques des macrophages et des granulocytes (201). A l'inverse des lymphocytes T CD4+ pouvant se différencier en Th1 ou Th2 dépendamment de leur activation, les lymphocytes T CD8+ se différencient préférentiellement en lymphocytes Tc1 (producteurs d'FN-γ) (425). Capables de synthétiser de l'IL-2 en moins grande quantité que les lymphocytes CD4+ Th1, les lymphocytes CD8+ Tc1 se différencient également sous l'action de l'FN-γ et de l'IL-12 (130, 425, 540). Cependant, sous l'action de fortes concentrations d'IL-4, les lymphocytes T CD8+ sont capables de se différencier en lymphocytes Tc2 producteurs d'IL-4 et d'IL-5 (540). Bien que l'IL-6 et l'IL-10 soient sécrétées en grande quantité par les lymphocytes Tc2, les lymphocytes Tc1 en produisent faiblement (425). Les lymphocytes CD8+ Tc1 et Tc2 tuent les cellules cibles en utilisant les deux voies de cytotoxicité (425, 539), mais avec une capacité réduite chez les lymphocytes

T CD8+ sécrétant de l'IL-4 (378).

2.2.2 Les lymphocytes T γ/δ

Les lymphocytes T γ/δ constituent 5 à 10% des lymphocytes T CD3+ circulants et peuvent atteindre jusqu'à 50% des lymphocytes T dans les tissus épithéliaux de la peau, de l'intestin ou du tractus génital (95, 256, 299). Leurs fonctions diffèrent suivant leur distribution dans les tissus, la structure de leurs récepteurs d'Ag, l'environnement local, et le stade de la réponse immunitaire au cours duquel ils sont activés. Ils sont dotés de fonctions de surveillance immune et tumorale, et d'immunoprotection avant la génération et la maturation des lymphocytes T α/β (256). Ils sont CD4- et en majeur partie CD8-, ce qui ôte la restriction au CMH pour la reconnaissance des Ag (256). Le répertoire du TCR des lymphocytes T γ/δ est plus restreint que celui des lymphocytes α/β. 50 à 95% des lymphocytes T γ/δ circulants présentent un TCR Vγ9Vδ2 alors que la majorité des TCR des lymphocytes T γ/δ intra-épithéliaux est constituée de Vδ1 et de Vγ variables (149, 266). Ces lymphocytes T Vγ Vδ1 s'activent au contact du *MHC class I polypetide-related sequence A* (MICA), protéine du CMH humain de classe IB exprimée à la surface des cellules épithéliales en situation de stress (239). Les lymphocytes T γ/δ murins sont activés au contact de certaines glycoprotéines virales, de hsp60, ou des Ag de stress présentés par les cellules épithéliales (256). Les lymphocytes T γ/δ humains reconnaissent des Ag lipidiques présentés par le CD1c, des glycoprotéines du CMV, ou encore l'enterotoxine B de Staphylococcus (SEB) (143, 375, 590). Les capacités fonctionnelles des lymphocytes T γ/δ incluent la production de cytokines (IFN-γ, TNF-α, IL-2, IL-3, IL-4, IL-5, IL-10) (6, 482, 676), de *keratinocyte growth factor-1* (KGF-1) contribuant à la réparation des tissus (64) et de *fibroblast growth factor-9* (684), et une activité effectrice cytotoxique. Les lymphocytes T γ/δ activés utilisent la perforine et les granzymes pour tuer les macrophages infectés par les mycobactéries (299), la granulysine pour tuer les micro-organismes (156), et la voie FasL-Fas pour induire l'apoptose des cellules cibles exprimant

Fas (299). Bien qu'une réponse mémoire protectrice des lymphocytes T γ/δ à l'égard des infections mycobactériennes a été observée chez le macaque (576), les travaux convergent vers une contribution de ces cellules à la réponse innée immédiate réalisée par des cellules ne nécessitant aucune expansion clonale substantielle (65). Les lymphocytes T γ/δ seraient alors nombreux à partager la capacité de répondre aux mêmes Ag, sans la spécificité étroite que possède les lymphocytes T α/β, et ne nécessiteraient aucun passage dans les ganglions lymphatiques ou dans la rate pour être activés (255).

2.2.3 Localisation et activité anti-Candida

2.2.3.1 Les lymphocytes

Le système immunitaire des muqueuses buccales partage avec le système immunitaire de la peau une absence de lymphocytes B (71, 652) mais diffère de celui-ci par une distribution des lymphocytes T sous forme d'agglomérats ou seuls de part et d'autre de la membrane basale, plutôt qu'entourant les veinules post-capillaires du réseau vasculaire superficiel et profond de l'épithélium (71, 652). Rarement observés dans la couche superficielle de l'épithélium, la majorité des lymphocytes T des muqueuses buccales exprime l'Ag mémoire CD45RO+ (120, 489), suggérant que les lymphocytes T naïfs CD45RA+ intra-épithéliaux meurent en absence de présentation des Ag par les LCs (120). Bien que le ratio CD4/CD8 soit de 1 pour 2 dans l'épithélium buccal humain et de 1 pour 4 dans l'épithélium de la peau, suggérant une prépondérance de lymphocytes T CD8+, les lymphocytes T CD4+ sont proportionnellement plus fréquents dans les muqueuses buccales que dans la peau (652). Les lymphocytes T CD4+ et T CD8+ humains sont en proportion égale dans l'épithélium des gencives, indiquant la présence de variations locales de la distribution de ces cellules dans la cavité buccale (120). A l'inverse des observations faites chez l'homme, un ratio de 2 pour 1 a été observé dans les muqueuses buccales de souris normales (66). De plus, la polarisation en Th1 des

lymphocytes T CD4+ est requise dans la réponse protectrice contre la candidose buccale chez la souris, ces cellules exerçant d'importantes fonctions dans les défenses de l'hôte à l'égard de l'OPC (187).

L'épithélium buccal est également un site d'induction de la réponse immune via la génération de lymphocytes T CD8+ cytotoxiques, indépendante de l'aide des lymphocytes T CD4+ et médiée strictement par le CMH I (147). L'IL-8 produit par les kératinocytes semblerait attirer les lymphocytes T CD8 dans l'épithélium (369, 652). Bien que les lymphocytes T CD8+ activés par l'IL-2 possèdent la capacité d'inhiber directement la prolifération de *C. albicans* sous forme d'hyphes (50), les lymphocytes T CD8+ ne semblent pas être en contact directe des hyphes de *C. albicans* qui sont confinés à la couche superficielle de l'épithélium (108, 184, 511). Les lymphocytes T CD8+ pourraient cependant activer les fonctions anti-microbiennes des macrophages et des polymorphonucléaires en produisant des cytokines et/ou répondre aux Ag du CMH I des kératinocytes ayant phagocyté des pathogènes microbiens (663).

2.2.3.2 Les lymphocytes T γ/δ

Représentant environ 2% des lymphocytes T des muqueuses buccales de l'homme (479), les lymphocytes T γ/δ ont été observés à proximité des LCs CD1c+, des LCs CD1a+ et des kératinocytes dans l'épithélium des gencives normales et inflammées (370). De phénotype CD45RA+CD8-CD4- dans les muqueuses normales et CD45RO+CD8+ dans les muqueuses inflammées, les lymphocytes T γ/δ sont constitués en majorité d'un Vδ2 dans le tissu conjonctif sous-jacent à la membrane basale et Vδ1 dans l'épithélium (283, 370, 479). Ces cellules sont capables de produire de l'IFN-γ participant à la réponse immune à l'égard de *C. albicans* (294) et leur nombre augmente dans les muqueuses buccales des souris infectées expérimentalement avec le *C. albicans*, lors de la résolution de l'infection primaire (106).

Les cellules NK sont de larges lymphocytes granuleux représentant 6 à 39% des

populations cellulaires des gencives de l'homme et possèdent une activité anti-microbienne directe à l'égard de *Cryptococcus neoformans* mais aucun effet sur les hyphes de *C. albicans* (50, 370).

2.3 PMNs et macrophages

2.3.1 Les PMNs

Se différenciant à partir de progéniteurs hématopoïétiques myéloblastiques, les PMNs restent exclusivement résidents de la circulation sanguine à l'état normal, mais vont migrer dans les tissus en réponse à l'inflammation. Appartenant aux premières défenses cellulaires de l'immunité innée (404), les PMNs possèdent de multiples mécanismes efficaces d'élimination des pathogènes (571).

2.3.1.1 Les mécanismes dépendant et indépendant de l'Oxygène

L'oxydase NADPH joue un rôle central dans l'élimination des micro-organismes en pompant les électrons du cytoplasme des PMNs vers l'oxygène (O_2) des vacuoles de phagocytose à travers un conduit créé par le flavocytochrome b_{558} (571). Les radicaux oxygénés ($O_2^{\cdot-}$, $HO^{\cdot}$), l'H_2O_2 et les produits de leur réaction (Halogénation médiée par la myéloperoxydase (MPO)), sont générés suite à l'activité de l'oxydase NADPH (571) et possèdent tous une activité contribuant à l'élimination des pathogènes bactériens (219, 531, 532, 677) et fongiques (14, 15).

Produites au stade de promyélocyte et myélocyte (68, 243), les granules des PMNs comprennent les granules primaires azurophiles contenant la MPO, les protéinases neutres (cathepsine G, élastase, protéinase 3) et les défensines (212). Les granules secondaires spécifiques contiennent la lactoferrine (81), les granules tertiaires contiennent la gélatinase (263), et les lysosomes contiennent les hydrolases acides. Les protéines des

granules combleraient 40% du volume vacuolaire (247), et les granules azurophiles et spécifiques fusionneraient avec les vacuoles de phagocytose 20 secondes après l'ingestion des particules (572). Le développement des souris KO pour les protéases des PMNs a permis de montrer que les protéases neutres (élastase, cathepsine G) ainsi que le flux K^+ étaient requis pour l'activité d'élimination et de dégradation des pathogènes phagocytés (43, 510). L'élastase des neutrophiles est nécessaire pour inhiber la croissance de *C. albicans* (510, 571). De plus, de récents travaux ont permis d'émettre l'hypothèse d'une interaction des produits de l'halogénation médiée par MPO et des radicaux de l'oxygène avec les granules des PMNs, permettant la digestion des micro-organismes par déversement des protéases dans les vacuoles et remettant en question l'action directe de ces dérivés oxygénés sur les pathogènes phagocytés (571).

2.3.1.2 Activation et cytokines

Bien que plusieurs cytokines et chemokines (IL-1, IL-8, TNF-α, IL-6, GM-CSF, G-CSF) régulent les fonctions des PMNs (97), l'IFN-γ, sécrété par les lymphocytes T α/β et γ/δ, et les cellules NK, serait l'activateur principal de la fonction des PMNs (179). Les PMNs activés par l'IFN-γ sur-expriment les produits des gènes de l'IL-1β, de l'IL-6, de l'IL-10 et du TNF-α mais sous-expriment ceux de l'IL-8, du MIP-1α et du MIP-1β suggérant que l'IFN-γ arrête les PMNs recrutés au site de l'inflammation (98, 215, 314).

Sous l'action de l'IFN-γ ou de GM-CSF, les PMNs sont capables in vitro d'exprimer le CMH II et les molécules de co-stimulation CD80 et CD86 (228, 281, 505), bien que leurs fonctions restent encore inconnues. Les PMNs expriment les intégrines CD11α, CD11β et CD18 et les récepteurs FcγRII et FcγRIII (FcγRI est seulement inductible) (179). Les Ag membranaires CCR1, CCR3 et CCR6 sont sur-exprimés sous l'action de l'IFN-γ, suggérant que cette cytokine participe au recrutement des PMNs au site d'infection (67, 691).

2.3.2 Les macrophages

2.3.2.1 Ontogénie

Différenciés à partir d'un progéniteur myéloïde commun avec les DCs et les ostéoclastes sous l'influence de l'IL-3, du GM-CSF, du M-CSF et du TNF-α de la moelle osseuse, les monocytes entrent dans la circulation sanguine (227). Ces monocytes circulants pénètrent de façon constitutive dans tous les compartiments tissulaires de l'organisme et se différencient en macrophages résidents (cellules de Kupffer, macrophages alvéolaires, macrophages de la microglie) s'adaptant au microenvironnement local responsable de leur phénotype tissulaire spécifique. Les intégrines β1 et β2, les molécules de la super-famille des immunoglobulines (tel le CD31), les sélectines ou encore le F4/80 participent à la migration à travers l'endothélium et les épithéliums (227). Les cytokines (TGF-β), les chemokines, et les facteurs de croissance influencent l'expression des gènes des macrophages résidents qui cessent de proliférer et activent leur synthèse de protéines. Les macrophages résidents sont activés en réponse à l'inflammation et aux différentes molécules stimulatrices de l'immunité, mais peuvent être épaulés par l'infiltration de macrophages recrutés au site de l'infection (227). Bien qu'il soit difficile de distinguer les macrophages résidents de ceux recrutés, le phénotype altéré des macrophages recrutés a permis de les distinguer en trois classes: (i) les *Antigen-non-specific elicited macrophages* répondant aux corps étrangers ou aux substances inflammatoires stériles et (ii) les *alternatively antigen activated macrophages* et (iii) les *classically antigen activated macrophages* participant à la réponse immune spécifique aux Ag exogènes (227).

2.3.2.2 La réponse immune

L'activation innée des macrophages peut être initiée par le contact des produits des micro-organismes avec les TLRs, le CD14 (interaction avec le LPS) et les récepteurs non-

opsonisants (304). Cette activation entraîne la production de cytokines pro-inflammatoires (IFN-α, IFN-β), des dérivés oxygénés et de NO suivi d'une réponse anti-inflammatoire régulée (227, 304). Les macrophages expriment le MMR et le *scavenger receptor-A* (SR-A) permettant la phagocytose des produits exogènes (également de l'hôte) induisant la sur-expression du CD80 et du CD86 qui favorisera la présentation des Ag (634). L'opsonisation et la phagocytose des macrophages peuvent être médiées par les récepteurs Fc et les récepteurs du complément, entraînant la synthèse de cytokines pro-inflammatoires et l'augmentation de l'activité cytolytique (227, 634). L'IFN-γ active les macrophages qui produiront de l'IL-6, de l'IL-1 et du TNF-α, ainsi que du NO (601). Cette activation est accompagnée d'une augmentation de la flambée oxydative et de la sur-expression du CMH II (227) participant à la réponse cellulaire adaptée. Le TNF-α produit est capable d'activer les PMNs permettant de renforcer la réponse immunitaire innée (21). Bien que l'activation via l'IFN-γ soit considérée comme la voie classique d'activation des macrophages, l'IL-4 et/ou L'IL-13 sont capables d'activer également les macrophages, entraînant la sur-expression du CMH II et du MMR et leur participation dans les réponses allergiques et anti-parasitaires (227). De plus, l'activité cellulaire des macrophages peut être modulée par l'interaction avec les lymphocytes T, l'interaction des récepteurs avec les cytokines (IL-10, TGF-β, IFN-α, IFN-β, M-CSF) et les glucocorticostéroïdes, ou par les pathogènes eux-mêmes. Ces interactions pourraient mener à une activité d'immunosupression des macrophages qui sécrèteraient de l'IL-10, du TGF-β et des PGE2 et à la diminution de l'expression du CMH II (164, 227, 420, 634).

2.3.3 Localisation et activité anti-Candida

Dans les muqueuses buccales de l'homme, les macrophages se situent en grande majorité dans la lamina propria (120), tandis que les PMNs se retrouvent dans la lamina propria et dans l'épithélium seulement en réponse à l'inflammation (370). Les macrophages résidents des muqueuses buccales expriment le CMH II, le CD11b et les

récepteurs Fc (37). L'activation in vitro de la réponse spécifique médiée par les lymphocytes T CD4+ à l'égard de *C. albicans* requiert l'expression du CMH II à la surface des macrophages (456). Les macrophages et les PMNs utilisent les deux mécanismes (dépendant et indépendant de l'O_2) pour tuer les blastoconidies et les formes hyphes de *C. albicans* (655). Les macrophages et les PMNs sécrètent des défensines et la calprotectine démontrant la capacité d'utiliser les deux mécanismes pour tuer le *C. albicans* (655). Jusqu'à aujourd'hui, ces cellules sont, avec les LCs, les seules observées ayant une activité anti-*Candida* (142).

L'IFN-γ produit par les lymphocytes T γ/δ active les macrophages et leur production de NO, et contribue à la résistance à la candidose oro-gastrique, indiquant que les lymphocytes T γ/δ participent indirectement à l'activité d'élimination de *C. albicans* par les macrophages (295).

Dans un modèle expérimental d'OPC chez la souris, les PMNs sont recrutés en grand nombre en réponse à l'inflammation, et migrent de la lamina propria vers les couches superficielles de l'épithélium 24 à 48h après l'infection (343). Cinq à six jours après l'infection, les PMNs sont remplacés par un recrutement important de macrophages dans la lamina propria (107). Ces résultats s'ajoutent aux observations faites chez les patients infectés au VIH présentant une OPC et suggèrent un rôle majeur des macrophages et des PMNs dans la capacité de circonscrire *C. albicans* à la surface de l'épithélium des muqueuses buccales.

2.4 Les cellules épithéliales

Les kératinocytes constituent une barrière physique à la pénétration de la surface de l'épithélium buccal par *C. albicans.* Ces cellules fonctionnent également comme des immunocytes immobiles capables de produire des facteurs solubles et d'exprimer des récepteurs impliqués dans la régulation de la réponse immunitaire (240, 602, 648) ***(Figure 2.1).*** Les facteurs de croissance majeurs, produits par les kératinocytes, comprennent: les

facteurs basiques de croissance des fibroblastes, les facteurs de croissance dérivés des plaquettes, le TGF-α et β, et le TNF-α. Ils produisent également des cytokines (IL-1, IL-3, IL-6, IL-7, IL-8, IL-10, IL-12, IL-15, IL-20) et des facteurs de stimulation de colonies (GM-CSF, G-CSF et M-CSF) (13, 60, 240, 648, 663). Ces médiateurs sont produits de façon non-constitutive dans des conditions normales, mais l'expression de leurs gènes et la sécrétion de ces protéines peuvent être augmentées durant l'inflammation en réponse à l'IFN-γ, le TNF-α et l'IL-17 sécrétées par les leucocytes, les LCs et les kératinocytes eux-mêmes (13, 339, 355, 398, 635, 663). Dans l'infection à *C. albicans* d'un épiderme humain reconstitué, l'expression des ARNm de l'IL-1α, de l'IL-1β, de l'IL-8, du GM-CSF et du TNF-α est augmentée, suggérant que le champignon induit une réponse, médiée par les cytokines, par les kératinocytes de l'hôte (561). Non seulement *C. albicans* active la production d'IL-1α et de TNF-α par les cellules épithéliales in vitro (597), mais les Saps qu'il sécrète ont une action protéolytique sur les précurseurs de l'IL-1β (42) suggérant que ces protéinases pourraient contribuer à l'activation et au maintien de la réponse inflammatoire à la surface de l'épithélium buccal. L'IL-1, l'IL-8 et l'IL-12 possèdent des effets attractifs sur les PMNs, les macrophages et les lymphocytes (663). L'IL-1 et le TNF-α produits par les kératinocytes peuvent promouvoir la maturation des DCs et la capacité des LCs à répondre aux Ag (663). L'IL-7 et l'IL-15 sont impliquées dans le trafic des lymphocytes (240, 663).

Les kératinocytes expriment le CD54 et le CD58 et l'expression du CD54 est augmentée par l'IFN-γ (170, 663). Le CMH I est exprimé constitutivement et pourrait être la cible des lymphocytes T CD8+ (663). Bien que le CMH II ne soit pas exprimé constitutivement à la surface des kératinocytes, son expression peut être induite par l'IFN-γ produit par les lymphocytes T infiltrés dans l'épithélium (663). Les kératinocytes pourraient fonctionner comme des cellules accessoires dans la présentation des Ag et interagir avec les lymphocytes pour produire une réponse Th2 (663).

L'IFN-γ peut induire l'expression de la desquamine, une glycoprotéine impliquée dans l'adhésion cellulaire dans la couche cornée de l'épiderme humain, possédant des

propriétés de lectine pour les sucres aminés (79), et des activités protéinase à serine *trypsin-like* (78) et RNase (574). La desquamine pourrait jouer un rôle crucial dans la desquamation et la perte de *C. albicans* de la partie superficielle de l'épithélium buccal.

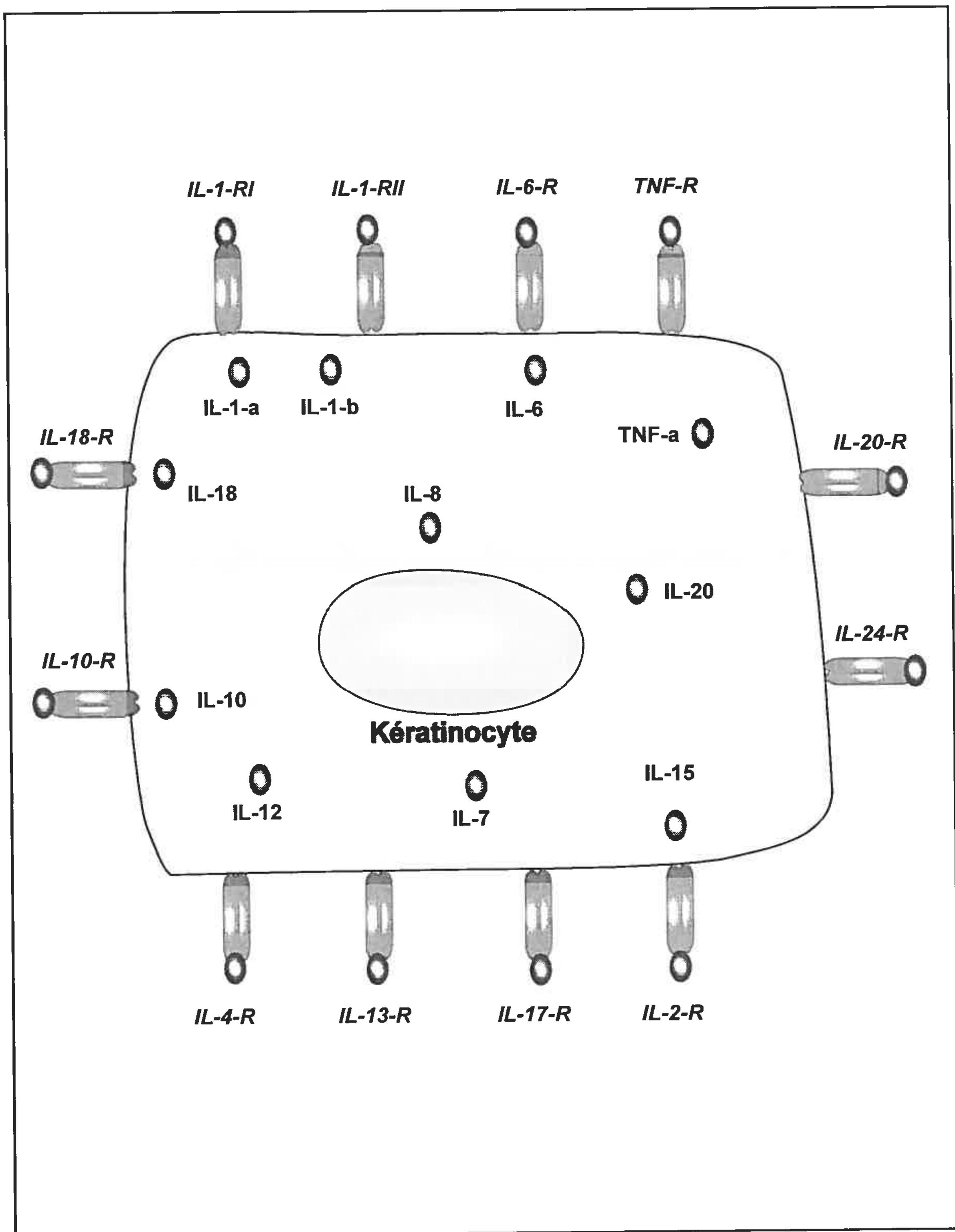

Figure 2.1 : Les kératinocytes comme source de production et cibles des cytokines. Traduite de *Gröne A, Veterinary Immunology and Immunopathology (2002).*

3. Immunité protectrice à l'égard de l'OPC

Depuis une quinzaine d'années, l'utilisation de souris atteintes d'immunodéficiences congénitales (32, 93, 94, 289, 290, 437, 655), de souris irradiées (26, 408) et de souris KO (34, 46, 444, 447, 529) a permis d'étudier les mécanismes de défense des muqueuses de l'hôte contre les infections à *C. albicans*. Certes, le manque de modèles expérimentaux d'OPC (*C. albicans* n'appartenant pas à la flore commensale chez la souris) et la rapidité d'élimination du champignon des muqueuses buccales chez l'hôte (5 à 7 jours) ont certainement favorisé les chercheurs à conduire des études plus ciblées sur les candidoses gastro-intestinales et vaginales. Ces études ont mis en évidence le rôle central de la réponse des lymphocytes CD4 Th1 dans la protection de l'hôte à l'égard des candidoses des muqueuses (56, 104, 195, 196, 291, 591). De plus, dans un modèle d'infection gastro-intestinale, l'activation de la réponse Th1 apparaît chez les animaux présentant une hypersensibilité de type retardée et les protège d'une seconde infection gastro-intestinale à *C. albicans* (104). Les études effectuées sur plusieurs modèles de souris immunodéficientes ont démontré non seulement que les lymphocytes T fonctionnels étaient déterminants dans la résistance aux infections et à la colonisation à *C. albicans* des surfaces des muqueuses, mais qu'une déficience additionnelle des phagocytes entraîne la dissémination du champignon du tractus gastro-intestinal vers les organes profonds (93, 289). L'étude chez des souris KO pour les lymphocytes B n'a démontré aucun rôle protecteur des anticorps (Ac) contre les candidoses des muqueuses ou la dissémination du champignon à partir du tractus gastro-intestinal (664). Bien que ces observations conduisent à l'hypothèse d'un lien entre un profil Th1 spécifique et l'élimination du champignon, l'absence de contact direct entre ces cellules dans les muqueuses oblige l'investigation plus précise des mécanismes des défenses de l'hôte à l'égard de *C. albicans*. Très peu d'études ont été réalisées sur les muqueuses buccales (194), mais les protéines salivaires possédant une activité anti-*Candida*, l'inhibition de la prolifération de *C.*

albicans par les kératinocytes buccaux et la présence d'une hypersensibilité de type retardée à l'égard de *C. albicans* pourraient concourir aux défenses de l'hôte à l'égard de l'OPC. Malgré l'attention toute particulière portée sur l'étude des mécanismes de la réponse immunitaire acquise à l'égard de *C. albicans*, des études récentes sur les TLRs pourraient contribuer à la compréhension des mécanismes des défenses de l'hôte contre les OPC en établissant le lien entre l'immunité cellulaire innée et l'immunité cellulaire acquise à l'égard de ce champignon (46, 444).

L'immunité de la muqueuse buccale à l'égard des pathogènes exogènes et endogènes, tel que *C. albicans*, se compose en une coopération de trois systèmes de défenses comprenant: Les défenses innées (protéines sécrétées, salive), les défenses immunitaires innées (les celles résidentes de la muqueuse) et les défenses immunitaires innées et acquises (cellules mobilisées à la muqueuse) ***(Figure 3.1)***

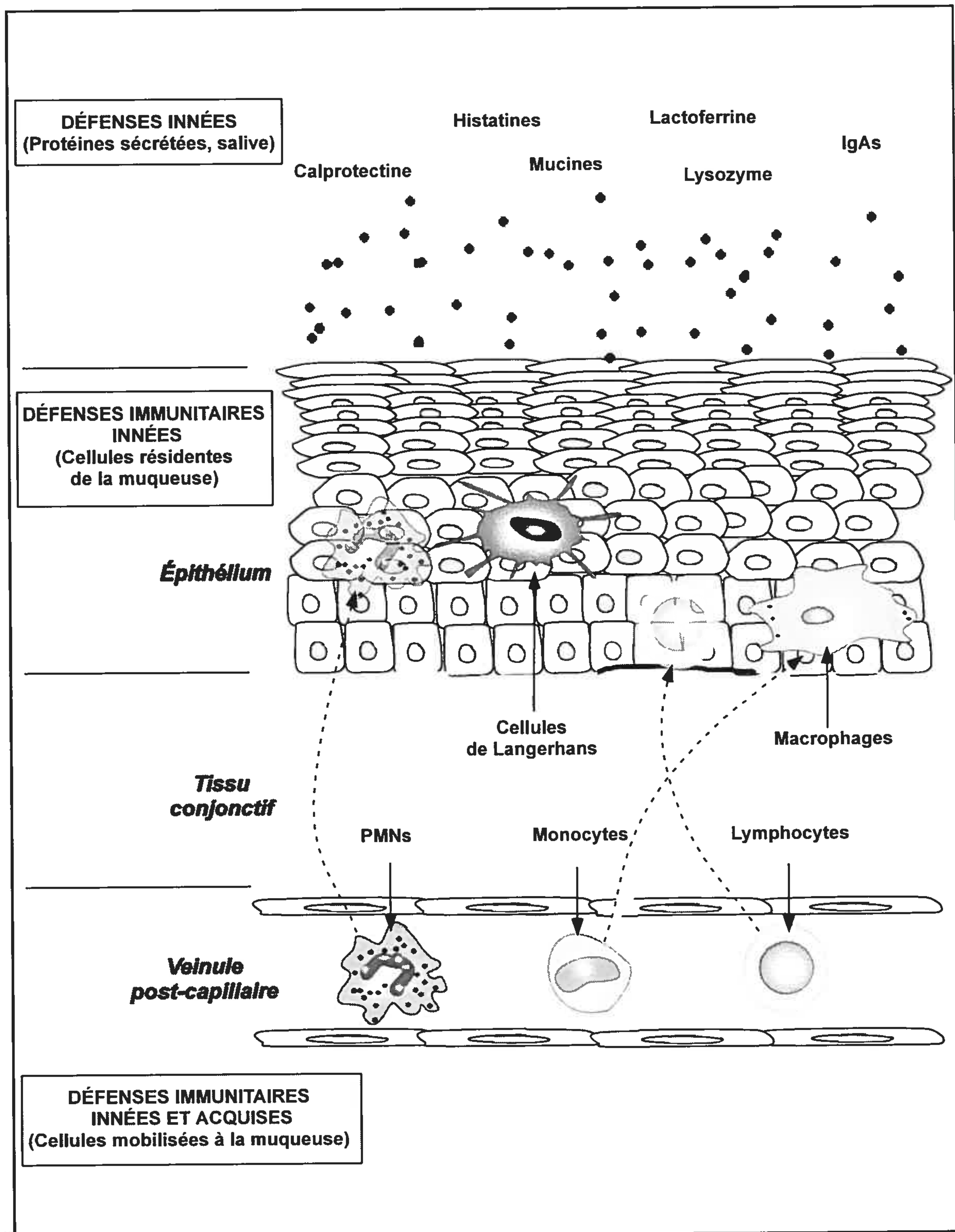

Figure 3.1 : Représentation schématique des trois systèmes de défense des muqueuses buccales de l'hôte.

3.1 Immunité innée

3.1.1 La réponse innée

Chez les mammifères, le flux salivaire et ses composants protéiques représentent le premier système de défense innée non-immunologique de l'hôte à l'égard des pathogènes buccaux. Les protéines salivaires préviennent la prolifération et l'attachement des micro-organismes à l'épithélium buccal et établissent un équilibre dynamique entre les différents membres de la flore commensale de la cavité buccale (41). Parmi celles-ci, certaines protéines (protéines riches en proline, cystatines, stathérines et amylases) protègent des infections par les pathogènes à travers un mécanisme indirect de lubrification des muqueuses buccales (625), tandis que d'autres, telle que la mucine, exercent une seconde activité anti-microbienne plus spécifique (61). Les mucines salivaires présentent des domaines de liaison aux adhésines des micro-organismes permettant d'inhiber leur attachement aux surfaces de l'épithélium buccale de l'hôte (61, 625). L'adhérence de *C. albicans* à l'épithélium des muqueuses, incluant celui de la cavité buccale, est un phénomène complexe impliquant plusieurs types d'adhésines exprimées à la surface des levures et la multiplicité des sites potentiels de liaison de l'hôte (268). Néanmoins, la capacité des mucines salivaires (173, 174) et du protéoglycan (268, 269) de se lier au *C. albicans* faciliterait son ingestion par l'activité de déglutition, prévenant ainsi l'établissement d'une OPC chez l'hôte normal (41). Ce phénomène de protection passive est renforcé par la capacité des mucines à former des complexes hétérotypiques via des interactions non-covalentes avec les Ig A sécrétées (54, 363) et le lysozyme (578) présents dans la salive. De plus, des études in vitro menées sur des dérivés peptidiques de la région N-terminale de la mucine 7 (MUC7), présente dans la salive humaine, ont démontré leur efficacité d'inhibition de la prolifération de *C. albicans* (61). Les peptides MUC7 15-mer, 20-mer et 51-mer , composés respectivement de 15, 20 et 51 a.a, inhibent la

prolifération des souches de *C. albicans* résistantes aux anti-fongiques azolés et à l'amphotéricine B (61). Le peptide MUC7 20-mer pénètre par internalisation et à travers les pores des cellules de *C. albicans* et son activité anti-*Candida* est inhibée par la présence d'ions Mg^{2+} et Ca^{2+}, suggérant que la charge positive nette des acides aminés (a.a) favorise la liaison aux membranes de *C. albicans* de polarité négative. Le remplacement d'a.a de polarité négative par des a.a de polarité positive dans la composition des peptides entraîne une diminution de leurs capacités de liaison à *C. albicans* et de leur activité d'inhibition de la prolifération (61). L'observation du développement d'une OPC lors de l'infection buccale à *C. albicans* chez un modèle de rat présentant une hyposalivation (HSR) (par ablation des glandes salivaires mandibulaires et sublinguales et ligature des conduits salivaires parotidiens) suggère un rôle potentiel de la salive dans la prévalence des OPC (405).

En effet, l'incidence des OPC augmente chez les patients présentant une réduction du flux salivaire (Syndrome de Sjögren (7), une infection avancée au VIH (357)) ou une acidification de la salive (137, 551). In vitro, un pH acide entraîne l'augmentation de l'adhérence de *C. albicans* aux cellules épithéliales (553) et induit la sécrétion des Saps dégradant les protéines salivaires telle que la mucine (121, 140, 353, 435, 518, 552, 562). Plusieurs autres protéines salivaires non-immunologiques sont capables d'inhiber la prolifération de *C. albicans* et son attachement à l'épithélium buccal:

(i) Le lysozyme est une protéine cationique, présente chez l'homme dans la peau, les larmes, les sécrétions nasales et la salive (455). Son activité d'hydrolyse des liaisons N-glycosidiques affecte la paroi cellulaire microbienne et l'intégration des composants dans la membrane cellulaire des micro-organismes (393, 455). Retrouvée en plus forte concentration près des plaques dentaires (554), sa concentration dans la salive peut varier de 1,5 à 57 μg/mL (506, 611). In vitro, 50 μg/mL de lysozyme inhibe la prolifération de *C. albicans*, qui présente une accumulation massive de matériel de la paroi cellulaire dans le périplasme (392, 393). L'accumulation potentielle de chitine polysaccharidique et/ou de β-glucane

dans le périplasme pourrait provenir de deux actions complémentaires distinctes: 1) la coupure enzymatique des composants de la paroi cellulaire de *C. albicans*, 2) l'activation d'un système enzymatique d'auto-lyse via des récepteurs au lysozyme sur la paroi cellulaire des champignons (455). De plus, les phagocytes et plus particulièrement les PMNs ont la capacité d'excréter le lysozyme (554) qui, à forte concentration, diminue la concentration extracellulaire de Saps (687). L'activité antifongique in vitro du lysozyme est dépendante de la concentration, du temps et de la souche de *C. albicans* (554, 696). Bien que sa concentration soit augmentée dans la salive des patients infectés au VIH indépendamment de la présence d'une OPC (22, 269, 381, 697), des souches génétiquement identiques de *C. albicans* provenant d'isolats cliniques de patients VIH+ résistent in vitro à l'action antifongique du lysozyme (554). Cette activité paradoxale exclut actuellement la participation du lysozyme à l'inhibition de la prolifération de *C. albicans* chez ces patients.

(ii) La lactoferrine est une glycoprotéine liant le fer, appartenant à la famille des transferrines. Présente à la surface des muqueuses, elle participe aux défenses de l'hôte contre les infections en séquestrant le fer nécessaire à la prolifération des micro-organismes (666). Produite par l'épithélium glandulaire et/ou par excrétion du contenu des granules spécifiques des PMNs, la lactoferrine est présente dans la salive à des concentration variant de 7 à 20 µg/mL (159, 537). Elle pourrait exercer son activité fongicide à l'égard de *C. albicans* en séquestrant les ions ferriques (399), en altérant la structure de la paroi cellulaire fongique (454), ou/et en activant des enzymes intracellulaires autolytiques (344). La stimulation in vitro des PMNs par des LPS ou des fractions de mannoprotéines de *C. albicans* entraîne leur dégranulation et l'excrétion de lactoferrine (474). Cette dégranulation des PMNs peut survenir en réponse à la stimulation par l'IL-2 inhibant la prolifération de *C. albicans* et l'ajout d'Ac anti-lactoferrine inhibe leur activité anti-*Candida* (160). Les souches génétiquement identiques de *C. albicans* provenant d'isolats

cliniques de patients VIH+ ne développent pas de résistance à la lactoferrine in vitro (554). Néanmoins, les concentrations variables de lactoferrine retrouvées chez les patient infectés au VIH (augmentées (22, 357), inchangées (387) ou diminuées (368, 430)) ne permettent pas d'associer la prédisposition à l'OPC chez ces patients avec une altération de la production de lactoferrine. Cependant, de récents travaux ont montré la capacité des peptides dérivés de la partie N-terminale de la lactoferrine d'inhiber la prolifération de *C. albicans* (372, 649). De plus, le développement récent de tablettes de lactoferrine muco-adhésive ayant une activité fongicide à l'égard de *C. albicans* et de *C. glabrata* (336) pourrait constituer une approche thérapeutique dans le traitement de l'OPC.

(iii) Les histatines s'apparentent structurellement aux protéines cationiques riches en histidine, composantes majeures du système de défense non-immunologique de la cavité buccale de l'hôte (171, 172, 469, 645). Présentes dans la salive humaine à des concentrations variant de 50 à 425 µg/mL (345), elles disposent d'une activité fongicide à large spectre à l'égard des champignons pathogènes, incluant *C. albicans*, *Cryptococcus neoformans* et *Aspergillus fumigatus* (172). Elles sont sécrétées par les glandes parotidiennes, sous-mandibulaires et sous-linguales (469, 470). Les protéines majoritairement retrouvées dans la salive sont les histatines 1, 3 et 5 de 38, 32 et 24 résidus respectivement (469). L'activité anti-*Candida* de l'histatine 5 se caractérise par sa liaison à une protéine de levure de 67 kDa (172), suivi d'une translocation intracellulaire et d'un efflux d'ions, incluant le K^+, le Mg^{2+} et l'ATP (245, 331). Bien que la toxicité induite par l'histatine 5 partage des caractéristiques avec l'apoptose, incluant l'arrêt du cycle cellulaire en phase G1 (29) et la formation de ROS (260), de récents travaux ont montré qu'aucun autre marqueur apoptotique (carbonylation des protéines, fragmentation de l'ADN, libération du cytochrome c des mitochondries) accompagne la mort cellulaire induite par l'histatine 5 (245, 331). Ces dernières données suggèrent que les ROS pourraient contribuer à la signalisation

intracellulaire et à rétablir l'homéostasie suite à la fuite importante des ions de la cellule fongique. Le mécanisme d'action des histatines est distinct des autres peptides cationiques tels que les défensines, qui s'insèrent directement et rompent les membranes cellulaires grâce à la nature amphipathique de leur structure en hélice α (172). Bien que chez un sous-groupe de patients infectés au VIH la diminution des concentrations en histatine semble corréler avec l'augmentation de la candidose buccale (381), ces concentrations ont été observées augmentées (22), inchangées (357) ou diminuées (345, 381).

(iv) La calprotectine est une protéine hétérodimérique, anionique, liant le calcium et le zinc. Communément appelée Ag L1 ou calgranuline A et B, elle est composée de deux protéines MRP8/MRP14 de 10 et 14 KD respectivement (74). S'exprimant dans la phase de différentiation terminale des cellules monomyélocytiques (432), elle est produite par les PMNs (45% des protéines cytosoliques (52, 659)), par les monocytes (1% des protéines cytosoliques (659)), les macrophages et les kératinocytes (74, 109, 182, 585). La calprotectine inhibe la prolifération de *C. albicans* in vitro en le privant de fer qui constitue un élément essentiel à la croissance des micro-organismes (172). Les concentrations salivaires de la calprotectine et son expression par les kératinocytes buccaux sont augmentées en réponse à l'OPC chez les patients infectées ou non au VIH (182, 327, 619). Deux équipes ont observé une diminution significative de la concentration de calprotectine chez des patients VIH+ présentant une OPC ou des décomptes élevés de *C. albicans* dans la salive par rapport à des patients en présentant peu ou pas (428). Bien que cela suggère que la diminution de ce facteur anti-microbien pourrait prédisposer à la candidose buccale chez les patients infectés au VIH, la calprotectine est normalement exprimée par les kératinocytes de la couche épineuse de l'épithélium buccal et donc constitue une barrière empêchant la pénétration en profondeur de *C. albicans* (184).

(v) L' antileukoprotéase (642), produite par les kératinocytes (679), est un inhibiteur

de protéases à serine sécrétées par les leukocytes (176, 205). A l'inverse des β-défensines produites par les kératinocytes et excrétées après stimulation des cellules par les micro-organismes, l'antileukoprotéase est constitutivement produite et libérée dans le milieu extracellulaire par les kératinocytes (678). Son activité antiprotéolytique est médiée par le domaine C-terminal, tandis que le domaine N-terminal isolé exerce une activité anti-bactérienne (264). L'antileukoprotéase possède une activité fongicide à l'égard de *C. albicans*, mais son mode d'action est encore inconnu (642). Il constitue le dernier membre de la famille des protéines anti-microbiennes impliquées dans les défenses non-immunologiques des muqueuses à l'égard de *C. albicans*. Il exerce également une activité anti-VIH-1 in vitro et pourrait constituer une activité anti-virale de la salive associée à la baisse de la transmission orale du VIH-1 (403).

3.1.2 La réponse cellulaire innée

3.1.2.1 les kératinocytes

Les activités mécanique (desquamation) et indirecte (production de cytokines) des cellules épithéliales des muqueuses buccales participent au maintien de l'équilibre entre la colonisation et l'infection à *C. albicans*. Néanmoins, les kératinocytes possèdent plusieurs mécanismes anti-microbiens potentiels qui pourraient contribuer directement aux défenses de l'hôte à l'égard de *C. albicans*.

(i) Les kératinocytes ont une activité de synthèse de NO (58), qui est associée à une activité anti-*Candida* et à la résistance aux candidoses des muqueuses (295).

(ii) Les kératinocytes buccaux humains produisent des peptides anti-microbiens comprenant les β-défensines 1 et 3 (168, 169, 253, 360, 397, 566), les cathélicidines (163, 206, 244, 693), l'adrénomédulline (308, 309), la calprotectine (74, 109, 183, 184, 327, 428, 585, 619) et les protéines bactéricides augmentant la

perméabilité des micro-organismes (BPI) (211, 669) qui comme les antibiotiques naturels, contribuent à l'immunité innée de l'épithélium. Les β-défensines exercent une activité anti-microbienne potentielle à l'égard de *Candida* (566) et leur expression (ARNm et protéines) par les kératinocytes est activée par le TNF-α, l'IL-1β, les bactéries et ses dérivés (LPS) (253, 360, 397, 566). Les lésions de l'épithélium ou l'inflammation des tissus augmentent l'expression et la sécrétion de la cathélicidine humaine LL-37 par les kératinocytes (165, 206). Les β-défensines humaines ont des propriétés chimio-attractives pour les DCs immatures et les neutrophiles, et la cathélicidine LL-37 pour les monocytes et les lymphocytes (693). Les kératinocytes buccaux expriment également la calprotectine possédant une activité anti-*Candida*. En réponse à l'infection, l'expression de la calprotectine par les kératinocytes est augmentée in vitro, et serait dépendant de l'IL-1β (535).

(iii) Les kératinocytes buccaux humains inhibent directement la croissance des blastoconidies et/ou des hyphes de *C. albicans* in vitro, requérant un contact cellulaire direct (598). L'inhibition de la croissance pourrait impliquer les polyosides exprimés à la surface des kératinocytes, sans l'implication des mécanismes de phagocytose et de production de NO, de superoxide, de H_2O_2 et de peptides anti-microbiens tels que les défensines et la calprotectine (599). L'inhibition directe de la croissance de *C. albicans* par les kératinocytes pourrait être un mécanisme nouveau et distinct, complémentaire des mécanismes de défense anti-microbiens connus.

Les kératinocytes de l'épithélium buccal possèdent des mécanismes de défense agissant de façon directe ou indirecte contre les micro-organismes contenus à la surface des muqueuses buccales. Le rôle des kératinocytes dans la protection des muqueuses de l'hôte à l'égard de *C. albicans* est probant, puisque les hyphes de *C. albicans* sont confinés à la surface de la couche superficielle de l'épithélium buccal dans l'OPC, à distance des lymphocytes et des LCs localisés dans les couche plus profondes.

3.1.2.2 PMNs et macrophages

Participants à la première ligne de défense contre les pathogènes, les PMNs, les macrophages et les DCs exercent leurs fonctions dans l'immunité innée par leur activité effectrice via le processus de phagocytose et par la sécrétion de médiateurs pro-inflammatoires (cytokines et chemokines) ayant pour rôle d'informer les cellules du système immunitaire adapté. Ces molécules informatives peuvent induire l'activité co-stimulatoire des phagocytes et la présentation des Ag exogènes par les APCs (525).

Bien que les PMNs ne résident pas dans les muqueuses buccales, ces cellules sont recrutées dans l'épithélium buccal en réponse à l'infection à *C. albicans* (343) et exercent in vitro des propriétés anti-*Candida* de phagocytose (560) et de destruction des formes levures et hyphes de *C. albicans* (150, 151, 293) par augmentation de la flambée oxydative (150). La persistance de *C. albicans* à la surface de l'épithélium buccal chez la souris SCID présentant une absence de lymphocytes T et B, et l'observation, lors de la déplétion des PMNs, d'une dissémination du champignon aux organes profonds et une augmentation de l'infection buccale, suggèrent un rôle des PMNs dans la capacité de circonscrire le champignon à la surface de l'épithélium des muqueuses buccales (290). Chez la souris BALB/c susceptible à la candidose buccale, la déplétion des PMNs a entraîné une augmentation de la charge buccale de *C. albicans* (188) et l'augmentation de la production d'IL-4 et d'IL-10 par les lymphocytes T lors d'une infection gastrique (527) suggérant une contribution des PMNs dans la réponse Th1 protectrice à l'égard de *C. albicans*, vraisemblablement par la sécrétion de molécules immuno-modulatrices.

En raison de l'importante hétérogénéité des macrophages tissulaires et à l'incapacité de les isoler ou de les dépléter des muqueuses buccales, très peu d'informations sont disponibles pour établir le rôle des macrophages dans les défenses de l'hôte à l'égard de l'OPC. Dans des études de candidose systémique chez la souris SCID, la déplétion des macrophages à l'aide d'un Ac monoclonal (Acm) anti-Gr1 ou l'inhibition de leurs fonctions

par traitement à la silice ou à la carrageenan a entraîné une candidose oro-gastrique et une dissémination systémique de *C. albicans* (289). L'augmentation de la susceptibilité à la candidose systémique de ces souris a été observée plus directement en éliminant sélectivement les macrophages de la rate à l'aide de liposomes contenant du dichlorométhylène diphosphonate (499). De plus, dans une infection oro-gastrique, le traitement de souris SCID à l'aide de l'acide polyinosinique/polycytidylique (Poly(I-C)) perturbant les fonctions macrophagiques a entraîné une dissémination de *C. albicans* d'origine endogène provenant du tractus gastro-intestinal (288, 290). Cependant, plusieurs observations suggèrent que la collaboration entre les lymphocytes et les phagocytes serait essentielle dans la résistance de l'hôte à la candidose oro-gastrique (93, 290). En effet, les souris présentant une mutation combinée *bg/bg nu/nu* sont très susceptibles à la candidose oro-gastrique (93) et les souris BALB/c traitées avec l'Acm anti-Gr1 sont résistantes à la candidose des muqueuses (290) suggérant un rôle central des lymphocytes dans les défenses des muqueuses de l'hôte à l'égard des candidoses.

Les macrophages ont cependant la capacité in vitro de phagocyter et de tuer le *C. albicans* sous la forme blastoconidie (254, 656) en utilisant des mécanismes dépendant et indépendant de l'oxygène similaires à ceux des PMNs (656). Néanmoins, les macrophages peuvent présenter une activité anti-*Candida* additionnelle en produisant du NO et du peroxynitrite, de façon variable selon leur provenance tissulaire (656). Une des caractéristiques qui différentie l'activité anti-*Candida* des PMNs et des macrophages est l'utilisation de la myélopéroxidase qui, bien qu'elle soit associée à la destruction du *C. albicans* par les PMNs et les monocytes, est inactive chez les macrophages (390, 391). Le NO pourrait être impliqué dans la résistance à la candidose oro-gastrique, puisque les souris SCID exprimant iNOS sont susceptibles à la candidose oro-gastrique lorsque le NO est inhibé (293, 658). De plus, les macrophages produisant le NO ont été associés à la résistance des souris immuno-compétentes à la candidose systémique (105), et la synthèse de NO des macrophages pourrait être inhibée par les cytokines de type Th2 (IL-4 et/ou IL-10) (105) renforçant l'idée du rôle centrale des lymphocyte CD4 Th1 dans les défenses de

l'hôte à l'égard de *C. albicans*.

Bien qu'il soit difficile de déterminer in vivo la provenance des premières cytokines immuno-modulatrices synthétisées suite à l'inflammation, une des principales cytokines qui semblerait initier l'immunité cellulaire des muqueuses est le TNF-α qui peut être synthétisé de façon importante par les kératinocytes, les PMNs, les macrophages, les DCs et les lymphocytes T. Une possibilité serait que, en présence de l'infection à *C. albicans*, les kératinocytes produisent du TNF-α, de l'IL-1α, de l'IL-1β et de l'IL-8 attirant dans un premier temps les PMNs . Les PMNs produiraient à leur tour du TNF-α capable d'activer les macrophages résidants et de recruter de nouveaux macrophages. PMNs et macrophages seraient alors capables de synthétiser de l'IL-12 et de l'IL-18 activant les DCs. Cette activation pourrait entraîner la migration des DCs aux ganglions lymphatiques et initier la réponse des lymphocytes T CD4+. La synthèse d'IFN-γ et de TNF-α par les lymphocytes CD4+ Th1 pourrait maintenir les défenses de l'hôte à l'égard de *C. albicans* en augmentant l'activation des macrophages et l'expression du CMH I et II et de leurs molécules de co-stimulation. La synthèse de TGF-β et d'IL-10 par les lymphocytes T_{Reg} ou par les cellules immunitaires résidentes des muqueuses et les contacts cellulaires pourraient alors maintenir le contrôle par immunosupression/immunorégulation de la réponse inflammatoire. ***(Figure 3.2)***.

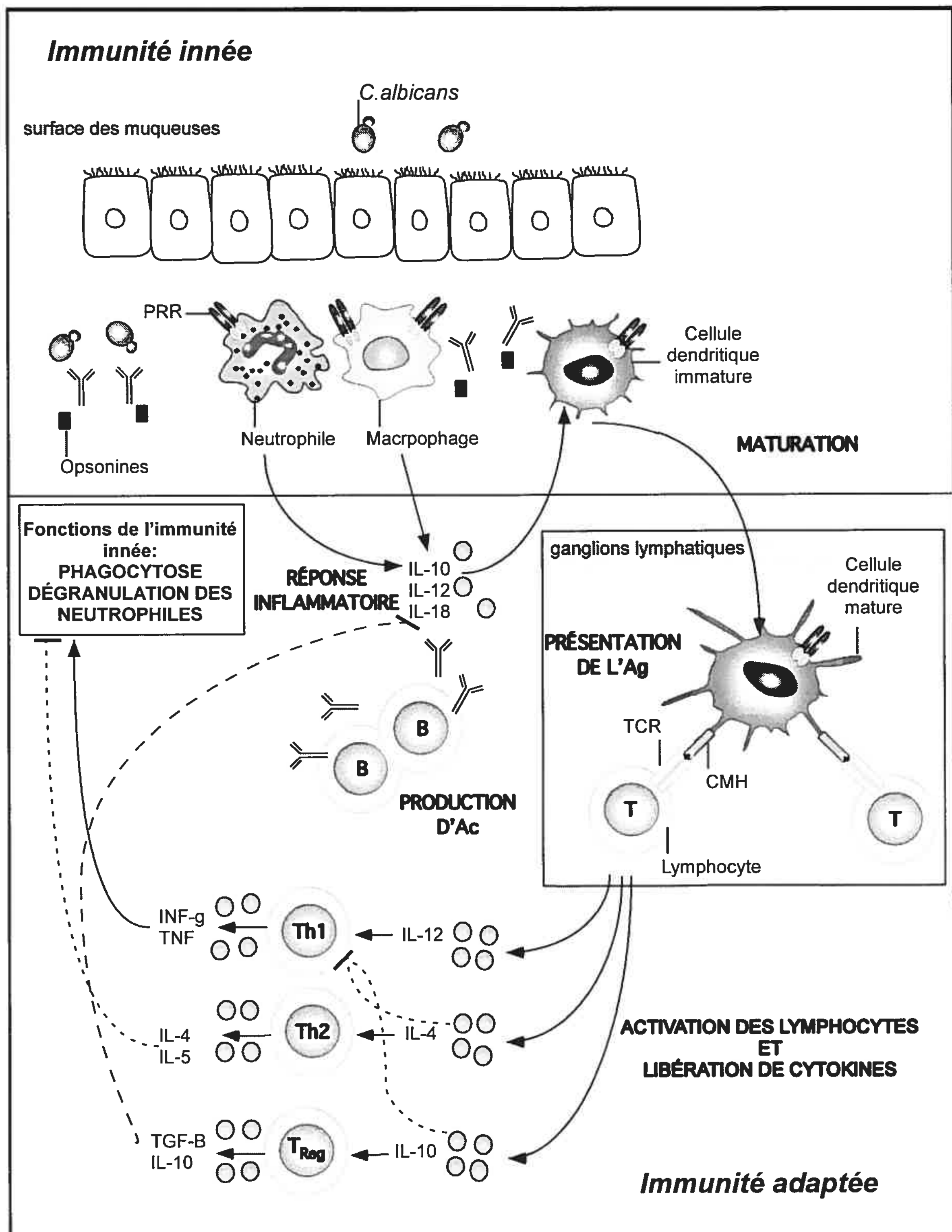

Figure 3.2 : Représentation schématique de la coopération de l'immunité innée et adaptée à l'égard des champignons. Traduite de *Romani L., Nat Rev Immunol. (2004).*

3.2 A l'interface de l'immunité innée et acquise

Depuis les huit dernières années, les équipes du Dr Luigina Romani et du Dr Bart Jan Kullberg se sont intéressées au lien potentiel entre les cellules de l'immunité innée et celles de l'immunité acquise à l'égard de *C. albicans* (47, 91, 406, 441, 442, 445, 448-450, 530, 651). Le rôle des cellules de l'immunité innée dans l'installation d'une immunité cellulaire acquise protectrice à l'égard de *C. albicans* a été exploré chez des souris KO atteintes de candidose systémique. Les souris double KO pour le TNF-α et la LT-α sont susceptibles aux infections gastrointestinales et systémiques à *C. albicans* (406, 450), s'accompagnant d'une réduction de la production d'IL-12 et d'une augmentation des cytokines de type Th2. Ces dernières favorisent la perte des fonctions effectrices des PMNs et la diminution des molécules de co-stimulation à la surface des macrophages (406). De plus, les souris KO IL-6-/- sont également susceptibles à la dissémination de *C. albicans* et les effets de l'IL-6, cytokine pléïotrope impliquée dans la régulation de l'immunité innée, semblent médiés par les PMNs (651). La susceptibilité à l'infection systémique à *C. albicans* est augmentée en l'absence d'interaction CD40/CD40L entraînant la diminution de la capacité des macrophages de tuer le champignon via le NO (442), suggérant que les contacts cellulaires jouent également un rôle important dans l'orchestration des défenses de l'hôte à l'égard des candidoses. En effet, le TNF-α régule l'expression de la molécule CD40 (313) et la réduction de cette molécule chez les souris TNF/LT-α-/- est accompagnée d'une augmentation de l'Ag CD86 et plus modérément de l'Ag CD80 à la surface des macrophages (406). En présence de l'infection à *C. albicans*, seul le CD40 est augmenté lors de l'addition de TNF-α exogène, rétablissant la résistance à la candidose systémique (406), et l'expression du CMH I et II à la surface des macrophages de ces souris est conservée. La perturbation de la voie Th1 protectrice pourrait être la conséquence d'un signal déficient de co-stimulation communiqué aux lymphocytes T CD4+. Le TNF-α et l'IFN-γ sont capables de stimuler la production de NO des macrophages

(442). L'IFN-γ, produit par les lymphocytes T et les cellules NK, stimule la migration, l'adhérence, la phagocytose et la capacité oxydative d'élimination des pathogènes des macrophages et des PMNs et maintient la réponse des lymphocytes CD4+ Th1 en activant les cellules productrices d'IL-12 (525). Par leur production d'IL-12 et d'IL-10, les PMNs et les macrophages pourraient participer au développement de la réponse immune Th1 à l'égard de *C. albicans* (525, 527, 528). Néanmoins, l'IL-10, produit également par les DCs et les lymphocytes T_{Reg}, est capable de supprimer les fonctions anti-*Candida* des phagocytes, la sécrétion des cytokines pro-inflammatoires TNF, IL-1β, IL-6 et IL-12 et l'immunité à médiation cellulaire protectrice (418, 525, 526), suggérant un rôle majeur de cette cytokine dans la régulation de l'inflammation.

Des études récentes sur l'implication des TLRs dans la résistance de l'hôte à l'égard des candidoses proposent de nouvelles perspectives de compréhension des mécanismes de l'immunité à l'égard de *C. albicans.* Les TLRs partagent des homologies avec le récepteur de type I de l'IL-1 (IL-1RI) et une cascade de signalisation similaire se traduisant par l'activation du NF-κB et des MAPKs (167, 628). L'IL-1RI et les TLRs utilisent une voie commune de signalisation intracellulaire impliquant le recrutement du *adapter protein myeloid differentiation primary response gene 88* (MyD88) par l'interaction homophile de leur domaine d'homologie Toll/IL-1R (628). Le MyD88 est capable d'activer les kinases associées au IL-1R impliquées de façon importante dans l'activation de l'immunité innée. De plus, les souris déficientes en MyD88 ne produisent pas de TNF-α en réponse à l'activation du TLR2, TLR3, TLR4 ou TLR9, bien que le TLR4 possède une voie de signalisation connexe indépendante du MyD88 (199, 272). Les souris déficientes pour les kinases associées au IL-1R sont perturbées en réponse au LPS et à l'IL-1 (617). L'activation du TLR2 par le zymosan, polyoside appartenant à la membrane cellulaire des levures, s'effectue en collaboration avec la *dectin-1*, récepteur du β-glucane (210). De plus, le mannane synthétisé par *C. albicans* active le TLR4 et le CD14 des monocytes humains, entraînant la production de TNF-α (626), et suggérant que la reconnaissance de molécules distinctes d'un même micro-organisme s'effectue par la collaboration de récepteurs de

différentes classes.

Bien que la production de TNF-α et d'IL-1β en réponse à *C. albicans* pourrait être indépendante de l'activation du TLR4, les souris déficientes pour ce TLR sont susceptibles à la dissémination de *C. albicans* (446). De plus, les souris TLR4-/- présentent une immunité protectrice Th1 perturbée, et particulièrement en présence de la forme hyphe de *C. albicans* (46).

Le phospholipomannane, glycolipide exprimé à la surface de *C. albicans*, serait capable d'activer le TLR2 des macrophages (298). Les souris TLR2-/- sont résistantes à la dissémination de *C. albicans* et leurs macrophages présentent une augmentation de leur activité fongicide à l'égard de *C. albicans* (444, 446), suggérant un rôle potentiel du TLR2 dans l'évasion de ce pathogène aux défenses immunitaires de l'hôte. Cette hypothèse est renforcée par l'observation d'une diminution du nombre de lymphocytes T_{reg} et de la production d'IL-10 par les macrophages (444), et d'une augmentation de la production de TNF-α par les PMNs (46) en l'absence de TLR2 chez ce modèle murin. L'IL-10 est nécessaire à la génération des lymphocytes T_{reg} au cours de l'infection des muqueuses à *C. albicans* et les lymphocytes T_{reg} participent à l'homéostasie des cellules mémoires à l'égard de *C. albicans* (418).

De récents travaux ont mis en évidence l'implication de la collaboration de l'IL-1R, des TLR et du MyD88 dans la réponse de l'hôte à l'égard de *C. albicans* (46). Les souris IL-1R-/- et MyD88-/- sont susceptibles à la dissémination de *C. albicans* et présentent une immunité protectrice Th1 perturbée (46). Les PMNs des souris IL-1R-/- (46) et les macrophages des souris MyD88-/- (394) ont une activité fongicide perturbée à l'égard de *C. albicans* suggérant l'implication de l'IL-1R et du MyD88 dans les mécanismes d'élimination des pathogènes par les phagocytes. L'IL-R1 et le TLR4 ont la capacité de s'activer au contact des hyphes de *C. albicans* et leur stimulation entraîne l'utilisation de la molécule de signalisation MyD88 et la production du TNF-α et de l'IL-1β (46, 525). De plus, la signalisation via le MyD88 dans les DCs est requise pour la production d'IL-12 et l'activation de la réponse Th1 adaptée à l'égard de *C. albicans* (46, 525).

Toutes ces observations suggèrent que les TLRs pourraient contribuer de façon distincte dans l'apparition de l'immunité inné et de l'immunité Th1 adaptée à l'égard de *C. albicans*. La coopération entre les TLRs ou l'association des TLRs avec des récepteurs tels que l'IL-1R pourraient entraîner une variabilité de signal via le MyD88 dépendant des types morphologiques de *C. albicans* et du site d'infection. Ce pathogène pourrait alors exploiter les TLRs à des fins de colonisation et de survie chez l'hôte.

Bien que l'ensemble de ces récentes observations avancent la compréhension des mécanismes de l'immunité à l'égard de *C. albicans*, cela n'a pas été démontré directement dans un modèle expérimental d'OPC. Néanmoins, d'intéressantes observations ont été effectuées au cours d'une étude sur l'expression des TLRs à la surface des kératinocytes humains et de leur induction en présence de *C. albicans* (487, 588). Les kératinocytes humains expriment les récepteurs fonctionnels TLR4 et CD14 (588), le TLR2 au niveau moléculaire (487), et le MyD88 (487). L'activation des kératinocytes par *C. albicans* en présence d'IFN-γ entraîne l'activation et la translocation du NF-κB au noyau et l'inhibition du NF-κB bloque la capacité des kératinocytes humains de tuer le champignon (487). Non seulement les extraits de *C. albicans* induisent l'augmentation de la synthèse d'ARNm de l'IL-8 des kératinocytes, mais cette synthèse peut être inhibée par le traitement des cellules avec des Ac neutralisants anti-TLR2 et ant-TLR4, suggérant que les TLRs pourraient être impliqués dans l'expression des cytokines pro-inflammatoires des kératinocytes induite par les pathogènes (487).

3.3 Immunité cellulaire acquise

3.3.1 Kératinocytes et macrophages

Les kératinocytes et les macrophages pourraient être impliqués dans la présentation des Ag de *C. albicans* aux lymphocytes T CD4+ et participer à l'initiation de la réponse immunitaire adaptée à l'égard de *C. albicans* dans la cavité buccale. En effet, les

kératinocytes du tractus génital expriment le CMH II et ont la capacité de fonctionner comme des APCs (681). De plus, l'expression du CMH II des kératinocytes épithéliaux est augmentée chez les patients présentant une cheilite commissurale candidosique (465) ou une OPC (296), vraisemblablement en réponse à l'IFN-γ produit par les lymphocytes T infiltrés (20, 663). Néanmoins, les kératinocytes buccaux sont incapables de phagocyter *C. albicans* (599) et les lymphocytes T CD4+, situés sous la membrane basale des muqueuses buccales, sont distants du *C. albicans* localisé à la surface de la couche superficielle de l'épithélium. Les macrophages, de par leur capacité de phagocytose, d'élimination des pathogènes et d'expression du CMH II, pourraient participer à la présentation des Ag de *C. albicans* aux lymphocytes T CD4+ (20, 226, 457). Bien que cette participation ne soit pas formellement démontrée dans les OPC, des études reliées à ce processus ont montré que les monocytes humains étaient capable de phagocyter seulement le *C. albicans* sous la forme levure et d'induire la production d'IL-12, suggérant de nouveau que la forme levure du *C. albicans* est spécialement impliquée dans l'initiation de l'immunité protectrice Th1 (115).

3.3.2 Les cellules de Langerhans

Les LCs, de par leur présence dans les muqueuses buccales, pourraient induire l'initiation de la réponse immunitaire à médiation cellulaire acquise à l'égard de *C. albicans*. Bien que les LCs n'ont jamais fait l'objet d'études dans le cadre d'un modèle expérimental d'OPC, les DCs humaines (451) et murines (134) reconnaissent *C. albicans* par le récepteur au mannose/fucose (MR), phagocytent et dégradent le champignon et peuvent présenter ces Ag aux lymphocytes T. Les DCs humaines cultivées in vitro à partir de monocytes du sang sont capables d'utiliser le DC-SIGN dans la reconnaissance et la phagocytose de *C. albicans* sous la forme blastoconidie (91). De plus, la phagocytose de *C. albicans* sous la forme hyphe par les DCs nécessite la coopération du FcγR et du CR3 (526). *C. albicans* sous la forme blastoconidie est ingéré par les DCs murines via une

phagocytose en anneau, caractérisée par un recouvrement bilatéral de pseudopodes, et sous la forme hyphe via une phagocytose conventionnelle en fermeture éclair (134). Les DCs et les macrophages partageraient la même efficacité dans la capacité d'éliminer le *C. albicans* (451), bien que de récentes observations ont dévoilé une capacité d'élimination du champignon moins importante des DCs comparativement aux macrophages et aux monocytes chez un même sujet (441). Ces dernières observations révéleraient le rôle majeur des DCs dans la présentation des Ag de *C. albicans* aux lymphocytes T CD4+ plutôt que dans l'élimination du micro-organisme, en accord avec leur fonction première (321). Néanmoins, les DCs humaines pourraient utiliser un mécanisme indépendant de l'oxygène pour tuer *C. albicans*, vraisemblablement via les hydrolases lysosomiales (451). La production de NO est corrélée avec l'activité d'élimination de *C. albicans* sous la forme levure ou hyphe par les DCs murines (134).

La phagocytose de *C. albicans* sous la forme levure par les DCs murines entraîne la production d'IL-12 et l'activation des lymphocytes CD4+ Th1 tandis que l'ingestion de *C. albicans* sous la forme hyphe inhibe ces mécanismes et induit la production d'IL-4 in vitro (134). Seul le transfert adoptif, effectué 7 jours auparavant, de DCs de souris BALB/c ayant phagocyté *C. albicans* sous la forme levure confère une immunité protectrice de l'infection systémique à *C. albicans* chez les souris BALB/c (134). La co-culture in vitro de DCs de souris IL-12-/- (ayant phagocyté *C. albicans* sous la forme levure) et de lymphocytes T CD4+ de souris BALB/c entraîne la production d'IL-4 par les lymphocytes. Lorsque le transfert adoptif est effectué de façon identique avec des DCs de souris IL-12-/-, les souris BALB/c transférées présentent une susceptibilité à la candidose systémique indiquant la perte de la réponse Th1 protectrice à l'égard de *C. albicans* (134). L'immunisation, par transfert adoptif de DCs contenant les ARNm de *C. albicans* sous la forme levure, de souris BALB/c irradiées leur confère une résistance à la candidose systémique (26).

L'utilisation de récepteurs distincts pourrait permettre aux champignons de réguler la production de cytokines pro-inflammatoires telle que l'IL-12 pour leur survie

dépendamment du type de morphologie qu'ils présentent. En effet, l'activation du MR conduit à la production de cytokines pro-inflammatoires, incluant l'IL-12, à l'augmentation de l'expression des molécules de co-stimulation et du CMH II, et l'initiation de la réponse Th1 (525). A l'inverse, la co-activation du CR3 et du FcγR induit la production d'IL-4 et/ou d'IL-10, l'augmentation des molécules de co-stimulation et du CMH II, et l'activation des lymphocytes CD4+ Th2 et T_{Reg} (526). Les DCs des plaques de Peyer produisent de l'IL-10 en réponse à l'activation du CR3 induite par le *C. albicans* opsonisé (419). L'IL-10 produite par les DCs active les lymphocytes T_{Reg} qui affectent négativement l'activité antifongique des lymphocytes CD4+ Th1 (525). Bien que l'utilisation du CR3 pourrait favoriser le commensalisme des champignons à la surface des muqueuses de l'hôte, son utilisation intensive dans les interactions cellulaires pourrait constituer une importante stratégie d'évasion du pathogène au système immunitaire protecteur de l'hôte.

L'initiation de la réponse immunitaire des muqueuses en réponse à *C. albicans* dans l'OPC requiert la maturation et la mobilisation des LCs potentiellement induites soit par leur exposition directe au champignon soit par leur exposition aux cytokines produites par les lymphocytes T en réponse à l'infection (36). La maturation des LCs entraîne une augmentation de l'expression du CMH II, des molécules de co-stimulation CD54, CD58 et CD86, et de la sécrétion d'IL-12 (36, 680). L'augmentation de l'expression du CMH II à la surface des APCs a été démontrée par co-culture en présence de *C. albicans* ou par exposition à ses Ag (20). L'IL-1, le GM-CSF, et le TNF-α pourraient contribuer avec les interactions CD40/CD40L à la maturation des LCs dans les candidoses des muqueuses (36). De plus, les alloantigènes de CMH II (226, 456, 636) et les molécules d'adhésion CD54 et CD58 (636) sont directement impliqués dans l'activation des lymphocytes T CD4+ spécifiques à *C. albicans* par les APCs.

3.3.3 Les lymphocytes T

L'immunité acquise à l'égard de *C. albicans*, de par la présence d'une réponse

positive d'hypersensibilité de type retardée, a été démontrée chez les individus adultes immuno-compétents et préviendrait vraisemblablement la progression de la colonisation des muqueuses au cours d'une infection symptomatique. Les lymphocytes T des individus sains prolifèrent en réponse à la stimulation par les Ag de *C. albicans* et produisent des cytokines telles que l'IFN-γ et le TNF-α (443). En raison de l'hétérogénéité de leur répertoire, les lymphocytes T CD4+ et T CD8+ pourraient participer à la multiplicité et la redondance des mécanismes effecteurs permettant le contrôle des infections à *C. albicans*. En effet, la résistance de l'hôte à l'égard des infections à *C. albicans* semble dépendre de l'activation de l'immunité cellulaire médiée par les lymphocytes T, des cytokines, et de l'activité effectrice des phagocytes (525).

Plusieurs observations effectuées chez la souris atteinte d'OPC démontrent le rôle protecteur de l'immunité cellulaire acquise à l'égard de *C. albicans* (177, 187, 188). Infectées oralement à *C. albicans*, les souris DBA/2, plus susceptibles à l'égard de l'OPC, et les souris BALB/c présentent une prolifération des lymphocytes T spécifiques à l'égard des Ag de *C. albicans* et une expression précoce des ARNm de l'IL-4, de l'IFN-γ et de l'IL-12 dans les ganglions lymphatiques (177). Néanmoins, la sécrétion de ces cytokines par les lymphocytes des souris BALB/c stimulés in vitro est corrélée avec l'élimination rapide de *C. albicans* de la surface des muqueuses buccales, tandis que l'élimination retardée du champignon des muqueuses des souris DBA/2 coïncide avec la baisse de la sécrétion d'IL-4 (177). De plus, l'injection répétée d'Ac monoclonal anti-IL-4 chez les souris BALB/c entraîne une augmentation de la charge buccale et un retard dans l'élimination de *C. albicans* des muqueuses buccales, suggérant que la rapidité d'élimination du champignon est dépendante de la réponse des lymphocytes des ganglions lymphatiques cervicaux et de la sécrétion précoce d'IFN-γ et d'IL-4 (177). Toutefois, les expériences ont été effectuées sur la totalité des lymphocytes sans discrimination des cellules responsables de la production des cytokines (177). De plus, l'IL-4 produite par les lymphocytes T Vβ8+ active les fonctions antifongiques des PMNs (524). Bien qu'elle soit associée à un profil Th2, l'IL-4 peut activer ou désactiver les fonctions effectrices des phagocytes et des DCs. Ainsi, son

activité d'inhibition des fonctions antifongiques des phagocytes peut promouvoir la sécrétion d'IL-12 par les DCs (524). Activée par l'IL-4, la résistance à l'égard de l'OPC pourrait être médiée par l'initiation de la réponse protectrice Th1 (407) et par l'augmentation de l'activité anti-*Candida* des PMNs (62) et des macrophages (192).

La déplétion systémique des lymphocytes T CD4+ n'augmente pas l'intensité de l'infection buccale à *C. albicans* chez les souris immuno-compétentes BALB/c et CBA/CaH (187). Cependant, la reconstitution de l'immunité par transfert adoptif de lymphocytes T CD4+ naïfs, mais pas de lymphocytes T CD8+, à des souris immunodéficientes BALB/c *nu/nu* et CBA/CaH *nu/nu* provoque une diminution de la colonisation des muqueuses buccales à *C. albicans* comparativement aux souris contrôles (187). Cette diminution est corrélée à l'augmentation de l'expression de l'IL-12 et de l'IFN-γ dans les ganglions lymphatiques cervicaux (187), démontrant la nécessité pour l'hôte de recourir aux lymphocytes T afin de résister à l'OPC. La déplétion des PMNs et l'inactivation des monocytes/macrophages augmente l'intensité de l'infection chez les souris immunocompétentes BALB/c et CBA/CaH et l'élimination de *C. albicans* des muqueuses buccales est dépendante de l'action des lymphocytes T CD4+ sur les PMNs et les macrophages, présentant une augmentation de leur fonction anti-*Candida* en présence d'IFN-γ et d'IL-12 sécrétées (188). L'augmentation des fonctions des phagocytes pourrait provenir également de l'activation via l'IFN-γ produit par les lymphocytes T γ/δ retrouvés en plus grand nombre dans les ganglions cervicaux des souris infectées oralement à *C. albicans* (177). Les lymphocytes T γ/δ pourraient contribuer indirectement à l'élimination du champignon des muqueuses buccales de l'hôte comme cela a été démontré dans des expériences de candidose gastro-intestinale (294).

4. Pathogenèse de l'OPC dans l'infection au VIH

4.1 Le VIH

Membre de la sous-famille des lentivirus, le VIH est un rétrovirus comprenant deux sous-types (VIH-1 et VIH-2) (333). Découverts entre 1983 et 1986, les VIH-1 et VIH-2 présentent des différences génétiques, d'infectivité et de propagation. Le virus de l'immunodéficience simienne (SIV), observé chez le primate, n'est pas pathogène chez leurs hôtes naturels, suggérant que chez l'homme, le VIH a continué de muter et s'est adapté à son hôte (246). Les analyses phylogénétiques suggèrent que le taux élevé de mutation du VIH-1 a concouru à son établissement au cours du XXème siècle (330). La dissémination progressive du VIH-1 a entraîné une pandémie dévastatrice en Afrique, en Europe et en Amérique dans les années 70, et en Asie dans les années 80. La distribution géographique du VIH-2 est plus limitée (régions de l'Afrique de l'ouest) et il est considéré moins pathogène chez l'homme.

Le VIH est un virus enveloppé, composé d'une capside dense aux électrons, entourée d'une enveloppe riche en cholestérol et en sphyngomyéline dérivés de la membrane plasmique (333). Les glycoprotéines gp120 et gp41 du VIH se joignent aux protéines membranaires des cellules infectées pour former les protéines de l'enveloppe. Un réseau de protéines (protéines de la matrice (MA)) s'organise sous l'enveloppe lipidique protégeant une capside composée d'environ 2000 molécules de protéines de nucléocapside de 24 kD (p24) formant un cylindre effilé ***(Figure 4.1)*** (333).

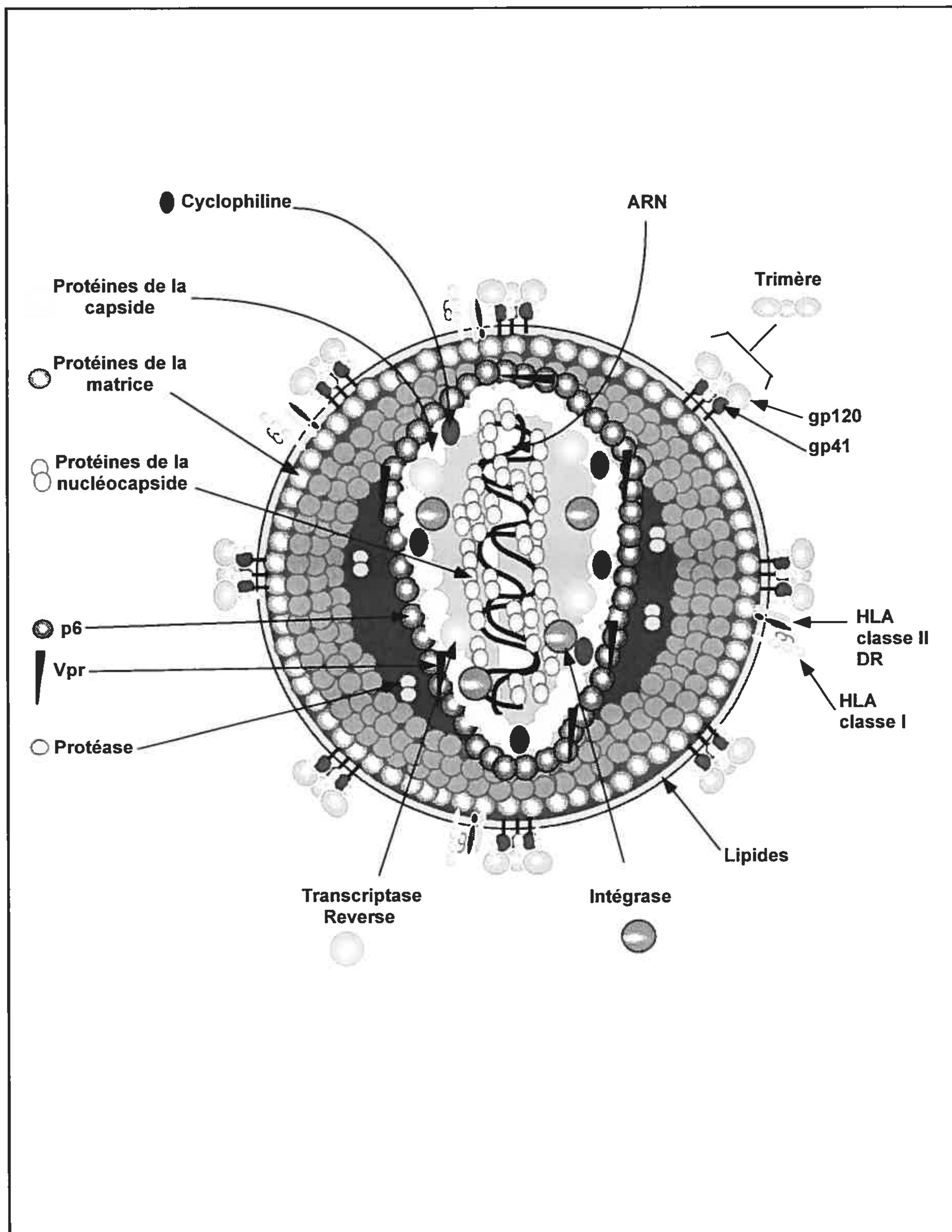

Figure 4.1 : Représentation de la structure du virion et de la position des protéines du VIH le composant. Traduite de *Krogstad P, seminars in Pediatric Infectious Diseases (2003).*

Le génome viral est composé de deux brins d'ARN monocaténaires de polarité positive protégés dans la capside par de multiples copies de p24. La capside virale renferme l'intégrase (IN) et la transcriptase réverse (RT), protéines essentielles aux étapes précoces de la réplication du virus (310). L'intéraction de la gp120 avec l'Ag CD4 constitue le mécanisme majeur de l'attachement du virion à la surface de la cellule. Néanmoins, les polyosides de la région N-terminale de la gp120 ont une forte affinité de liaison au DC-SIGN, suggérant que les DCs exprimant cette lectine livreraient les virions dans les tissus lymphoïdes et les présenteraient aux lymphocytes T CD4+ (342, 416). L'intéraction gp120-CD4 induit un changement de conformation des protéines virales et permet l'engagement dans la liaison des co-récepteurs CCR5 et CXCR4 (333). La liaison de la gp120 avec ces co-récepteurs induit un changement de conformation de la gp41, résultant en la formation d'un complexe stable à 6 hélices entre les domaines de la gp41 et en la fusion de l'enveloppe virale avec la membrane cellulaire. Cette fusion permet l'entrée de la capside virale dans la cellule hôte (333). La structure de la capside se désordonne libérant le complexe de pré-intégration (415) contenant l'ARN génomique, la RT, l'IN, les protéases et les protéines telles que les protéines de nucléocapsides (NC) et Vpr. Ces protéines sont impliquées dans la transcription de l'ARN en ADN, le transfert du complexe protéines-ADN dans le cytoplasme, son entrée dans le noyau et l'insertion de l'ADN viral au génome de la cellule hôte (312). La synthèse d'ADN viral s'effectue grâce à la RT possédant une activité d'ADN polymérase dépendant de l'ARN, de RNase H dégradant l'ARN des hybrides ARN-ADN et d'ADN polymérase dépendant de l'ADN. La synthèse de l'ADN viral débute quelques heures après l'entrée du virus dans la cellule et ne nécessite que 4 à 6 heures pour être complète (700, 701). La molécule d'ADNc comprend des gènes codant pour 9 protéines virales du VIH, flanquée par des «*Long-Terminal Repeat*» (LTR).

Le taux d'erreur de transcription (10^{-4}-10^{-5}), l'absence d'un mécanisme de correction du mauvais appariement des nucléotides et/ou la pression sélective de la réponse immunitaire de l'hôte et des agents antiviraux favoriseraient la multiplicité de variants viraux chez un individu infecté (333). Le transport du complexe de pré-intégration dans le

noyau pourrait être facilité par Vpr, l'IN ou les MA possédant une séquence signale de localisation nucléaire composée d'acides aminés cationiques (80, 660). L'intégration de l'ADN viral dans le génome de la cellule hôte est un mécanisme essentiel à la réplication du virus. Elle est médiée par l'IN, qui catalyse l'attaque nucléophile des groupements hydroxyle en 3' de l'ADN viral sur les ponts phosphodiesters de l'ADN chromosomique de la cellule hôte (608, 703), permettant ainsi à l'ADN viral de devenir un composant du génome de la cellule. Bien que quelques études ont montré que l'ADN viral s'intégrerait préférentiellement près des gènes activement transcrits de la cellule hôte (85), aucune de ces études a pu mettre en évidence des sites hautement préférentiels pour son intégration dans l'ADN chromosomique. Les provirus du VIH peuvent rester silencieux pendant une longue période (481). Néanmoins, l'activation des LTR du génome viral par les facteurs de transcription de la cellule hôte tels que le NFAT et/ou NF-κB pourrait initier la transcription de l'ADN viral (333).

La protéine Tat (*Trans-activator of Transcription*) se lie aux ARNm viraux naissants et à la cycline T1 recrutant la kinase 9 (dépendant de la cycline) qui phosphoryle l'ARN polymérase II permettant l'élongation des ARN viraux et l'accumulation des protéines virales (668). La protéine Rev (Regulator of Viral expression) est constituée d'une séquence d'exportation nucléaire riche en leucine qui interagit directement avec le « *chromosomal region maintenance-1 (CRM-1) complexe exportin* », permettant l'exportation des ARN viraux non-épissés qui composeront les nouveaux virions (333). L'épissage des ARNm codés par le gène ENV conduit à la traduction de deux protéines d'enveloppe, gp120 et gp41, qui s'incorporent dans les virions avec des protéines associées à la membrane plasmique. Deux milles monomères de Gag formeront la capside immature (209). L'ARN viral est reconnu par des domaines de NC présents dans les poly-protéines de Gag et l'association entre les résidus des domaines de la MA et de la gp41 permet l'incorporation des protéines d'enveloppe (209). L'étape finale de l'assemblage du virus s'effectue quand l'enveloppe virale se sépare de la membrane plasmique.

Les protéines Vpr et Nef sont plus spécifiquement impliquées dans le maintien de

l'efficacité de la cellule hôte à produire des particules virales. Vpr empêche la cellule hôte de passer en phase G2/M, inhibant ainsi la division mitotique de la cellule. Les LTR du génome du VIH sont plus actifs en phase G2 et l'arrêt du cycle cellulaire induit probablement l'augmentation de l'expression des gènes viraux (225).

Nef est une protéine myristolée de 27-35 kD. La myristolysation en N-terminal de Nef est requise pour son association à la membrane cellulaire et pour ses activités biologiques (222). Les motifs riches en proline (PxxP) permettent l'intéraction de Nef avec des molécules de signalisation cellulaire, et seraient impliqués dans la capacité de cette protéine à induire l'activation cellulaire pouvant être nécessaire à la réplication du virus (185, 544). L'effet le mieux compris de la protéine Nef est sa capacité à diminuer l'expression membranaire de l'Ag CD4. Nef induit l'endocytose des molécules de CD4 par recouvrement des vésicules de clathrine qui sont redirigées vers les lysosomes pour être dégradées (3, 232). Nef pourrait interagir avec la chaîne μ des adapteurs (AP-1, AP-2) (233, 234), bien que cette interaction soit dispensable pour le CMH I (382, 516). Nef redirige également les molécules du CMH I dans des vésicules à clathrine jusqu'aux endosomes (237), protégeant les cellules infectées de la reconnaissance par les CTL (123). Nef est également capable de diminuer l'expression membranaire du CD28, en accélérant son endocytose (44, 621).

C'est dans sa capacité à manipuler les voies de signalisation cellulaire des lymphocytes T CD4+ et des macrophages, que Nef semble avoir les fonctions les plus importantes. Nef peut aussi bien cibler les voies de signalisation du TcR, de l'IL-2R, et des macrophages entraînant la production de chemockines, que des cascades anti-apoptotiques (237). En modulant l'expression du CD4, Nef dissocie le CD4 du Lck nécessaire lors de l'activation du TcR. Cette tyrosine kinase est responsable de la phosphorylation de la chaîne ζ du TcR, facilitant le recrutement et la liaison de ZAP-70, induisant l'augmentation de l'expression de certains gènes, comme celui de l'IL-2 (670, 671). La protéine Nef est exprimée précocement dans le cycle viral, même à partir du génome du VIH non-intégré (689). L'augmentation de l'expression membranaire de FasL

induite par nef permet à la cellule infectée de piéger les lymphocytes T CD8+ spécifiques du VIH en induisant leur apoptose (237). De plus, Nef serait capable de réprimer la signalisation de mort cellulaire induite par Bad, un membre pro-apoptotique de la famille Bcl-2 (682). Enfin, des études récentes ont montré que Nef pourrait réduire l'expression membranaire du CMH II, suggérant que la présentation des Ag viraux par les APCs aux lymphocytes T CD4+ pourrait être altérée par l'expression de nef (325, 613).

4.2 Perturbation de la réponse immunitaire humorale

Les immunoglobulines A sécrétées (IgAs) participent avec les différentes protéines contenues dans la salive aux premières défenses de l'hôte contre les pathogènes capables de coloniser les surfaces des muqueuses (386). Les IgAs salivaires ont la capacité d'inhiber in vitro l'adhérence de *C. albicans* aux cellules épithéliales des muqueuses buccales (180, 662). Elles se lient à des manoprotéines exprimées sur les cellules et sur les tubes germinatifs de *C. albicans* et de manière plus intense à un pH (5,9-7,5) proche de celui retrouvé dans la salive normale (55). Une augmentation de la concentration en IgAs a été observée chez les patients présentants une OPC (180). Néanmoins, aucune déficience en IgAs spécifiques à *C. albicans* a été démontrée chez ces patients (8). Dans l'infection au VIH, les concentrations salivaires et les quantités totales d'IgAs et de ses sous-classes ont été retrouvées inchangées (381), augmentées (22, 357) ou diminuées (429, 620). Une diminution de l'avidité des IgAs a été observée (109, 127). Certains patients infectés au VIH présentent une augmentation des concentrations en IgAs concomittante d'une diminution de leur activité anti-*Candida* suggérant que l'augmentation des IgAs spécifiques à *C. albicans* chez ces patients serait simplement la conséquence de l'infection (128). En normalisant les données en fonction de la quantité totale de protéines salivaires ou d'une unique classe d'Ig, Wozniak *et al.* (2002) ont quantifié le répertoire complet d'Ig dans la salive en considérant le stade de développement de l'OPC et du décompte cellulaire des lymphocytes T CD4+ des patients infectés au VIH (685). Aucun changement

significatif a été observé dans la salive au niveau de la quantité d'IgA totale et d'IgAs spécifiques à *C. albicans* susceptible de promouvoir l'OPC chez ces patients (685).

4.3 Perturbation de la réponse immunitaire cellulaire

L'impact crée par l'infection au VIH sur les populations cellulaires des muqueuses des patients est certainement central dans la pathogenèse de l'OPC. Détecté par des techniques de RT-PCR et de PCR à ADN (2) dans la salive des individus infectés, le VIH pourrait altérer les mécanismes des défenses immunitaires cellulaires des muqueuses buccales et permettre aux pathogènes buccaux de proliférer.

4.3.1 Les cellules épithéliales

Les kératinocytes buccaux au contact de *C. albicans* à la surface de l'épithélium jouent un rôle important dans la pathogenèse de l'OPC. Le VIH est capable d'infecter les cellules épithéliales de la muqueuse buccale (502), vraisemblablement en utilisant le galactosylceramide et le CXCR4 (361). Les protéines tat, nef et gp120 induiraient l'apoptose des kératinocytes buccaux humains en activant différentes caspases, FasL/Fas et TNF/TNFR (2), suggérant que l'activité anti-*Candida* de ces cellules en serait potentiellement perturbée. Les patients infectés au VIH, atteints d'OPC, ont une diminution in vitro de l'activité d'inhibition de *C. albicans* par leur cellules épithéliales comparativement aux patients ne présentant pas d'OPC. Néanmoins, aucune différence d'activité anti-*Candida* des cellules épithéliales a été observée entre les individus non-infectés et infectés au VIH ne présentant pas d'OPC (598).

4.3.2 Les cellules de Langerhans

Constituant la première cible lors du premier contact avec le virus dans les

muqueuses, les LCs facilitent non seulement le transfert du VIH aux lymphocytes T CD4+ (118, 489, 490), mais expriment les co-récepteurs viraux CD4 et CCR5 requis pour l'entrée du virus dans les cellules (303). De par leur activité d'initiation de la réponse immunitaire cellulaire les rendant mobiles, les LCs répandent l'infection et induisent la réponse immunitaire spécifique au virus dans les ganglions lymphatiques périphériques (303, 489, 490). Bien que ces cellules puissent concourir à l'infection par le VIH des lymphocytes T CD4+ lors des contacts dans les ganglions lymphatiques (489), elles présentent également des altérations causées par l'infection. Le nombre de LCs buccales (118) et oesophagiennes (111) est dramatiquement diminué dans l'infection au VIH, de même que les DCs des ganglions cervicaux (534), de la rate (402) et du sang (39, 162, 231, 374, 473) ***(Figure 4.2)***. La diminution des DCs pourrait être due aux changements cytopathiques entraînés par la production du virus, à la lyse des DCs infectés par les lymphocytes T CD8+ cytotoxiques, ou à la diminution de l'expression de leurs marqueurs de surface (39, 118). La diminution de l'expression du CMH II des LCs des muqueuses (486, 523), pouvant être causée par le blocage de l'expression de son gène par Tat et Nef (306), et pourrait expliquer, avec la perte des granules de Birbeck et le développement limité des dendrites et des organelle, l'altération de la différenciation terminale des LCs infectés au VIH (523). L'expression du CMH I à la surface des APCs est plus modérément perturbée, potentiellement par le blocage de la transcription par Tat (77, 273) et/ou la séquestration intra-cytoplasmique par la protéine Nef (232, 349). L'altération des LCs buccaux contribuerait à la perte progressive de la réponse à médiation cellulaire acquise, protectrice à l'égard des Ag de *C. albicans*, dans l'infection au VIH. La perte de l'expression du CMH II et la diminution de formation de ces complexes antigéniques contribuent à réduire les fonctions des LCs, mais également des monocytes et des macrophages (488), de stimulation et de présentation des Ag aux lymphocytes T CD4+ (366). De plus, l'expression de la gp120 dans les APCs infectées au VIH et la perte de la fonction d'activation du CD40L des lymphocytes T CD4+ contribuent à la diminution de la production d'IL-12 et d'INF-γ, perturbant la réponse protectrice Th1 et la différenciation des lymphocytes T CD8+ en

CTL (483, 614).

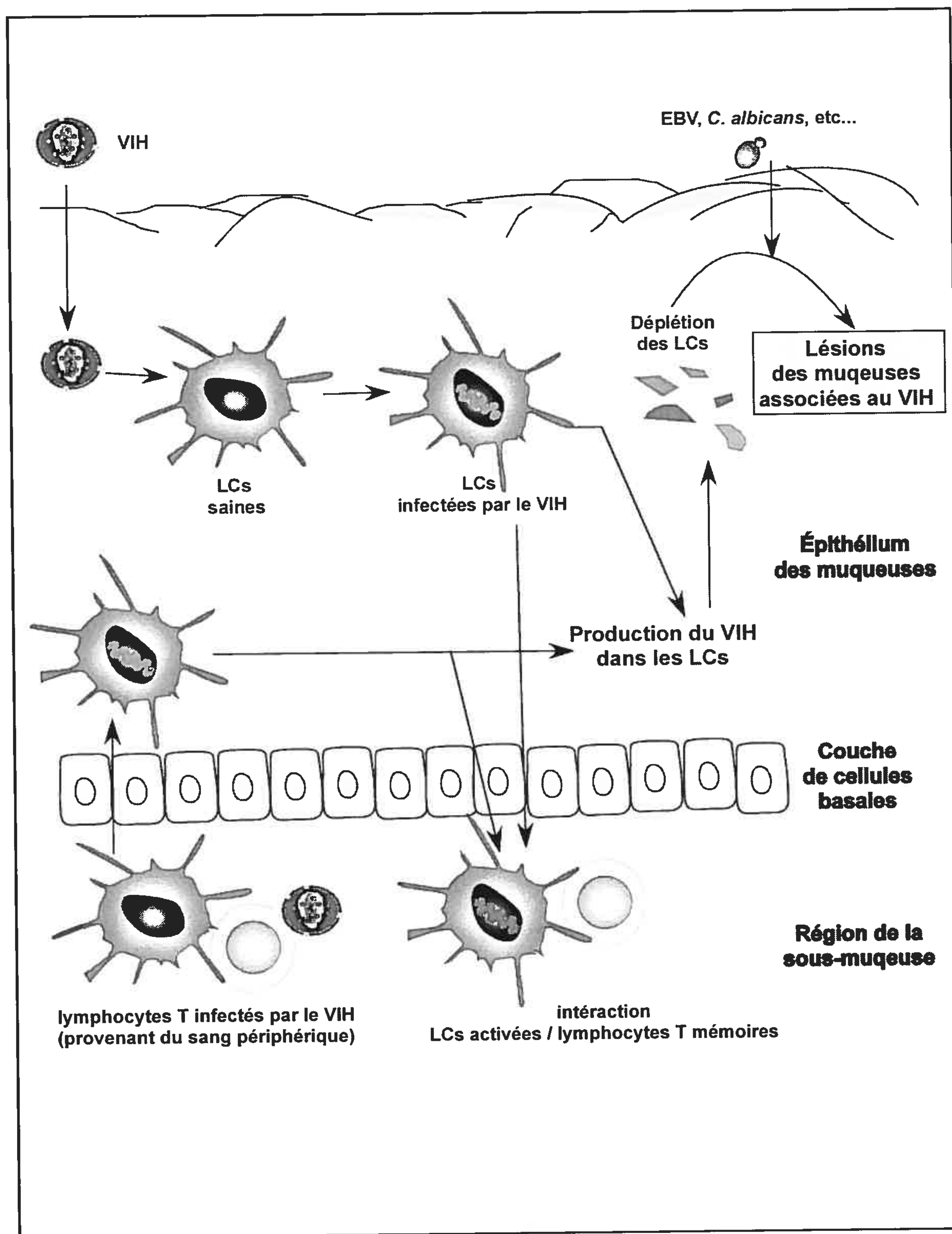

Figure 4.2 : Modèle hypothétique du rôle des cellules de Langerhans des muqueuses buccales dans l'infection au VIH. Traduite de *Chou et al., J oral Pathol. Med. (2000).*

4.3.3 Les lymphocytes T CD8

La diminution progressive du nombre de lymphocytes T CD8+ dans le sang périphérique des individus est une conséquence de la progression de l'infection au VIH (388, 413). Cette déplétion résulte d'une apoptose médiée par les macrophages lors de l'intéraction de la protéine gp120 du VIH avec le CXCR4 (261). L'observation d'une accumulation de lymphocytes T CD8+ à l'interface de la lamina propria et de la couche de cellules basales de l'épithélium buccal chez les patients infectés au VIH présentant une OPC (433) indique que ces cellules peuvent être activement recrutées à la muqueuse en réponse à la candidose (523). Les lymphocytes T CD8+ peuvent être attirés vers l'épithélium par l'IL-8 produit par les kératinocytes (403, 652), et exercent in vitro des fonctions d'inhibition des formes hyphes de *C. albicans* (50). Néanmoins, leur positionnement dans la couche basale ne permettant pas de rentrer en contact avec les hyphes de *C. albicans* situés à la surface de l'épithélium (108, 184, 511), le rôle précis des lymphocytes T CD8+ dans le confinement de *C. albicans* à la muqueuse, soit par action directe ou via un mécanisme indirect (production de cytokines), reste à déterminer (194, 433).

4.3.4 Les lymphocytes T CD4

Perdant l'expression de l'IL-2, de l'IL-2R et de leur capacité de proliférer en réponse aux Ag (318), les lymphocytes T CD4+ des patients infectés au VIH sont non seulement perturbés dans leur fonction, mais également dans leur nombre qui diminue dramatiquement au cours de la progression de l'infection. Cette déplétion apparaît dans le sang, mais également dans les muqueuses buccales des patients, indépendamment de la présence d'une OPC (463, 523, 600). Le nombre de lymphocytes T CD4+ du sang périphérique spécifiques à *C. albicans*, diminue durant la progression de l'infection au VIH

chez les patients présentant une OPC (337). Cependant, l'obtention de prélèvements humains étant difficile pour des raisons éthiques, la déplétion des lymphocytes T CD4+ spécifiques à *C. albicans* dans les ganglions lymphatiques drainant la muqueuse buccale n'a pas été actuellement démontrée. Néanmoins, les patients infectés au VIH présentent un profil Th2 dans la salive (352), corrélé avec la perte de prolifération des lymphocytes T CD4+ en réponse aux Ag de *C. albicans* (352, 503). La perte de l'expression du récepteur CD4 et du CMH II à la surface des lymphocytes et des LCs, induite par les protéines du VIH, ne confèrent pas aux lymphocytes T CD4+ l'entière responsabilité de la susceptibilité de l'OPC chez les patients infectés au VIH. La déplétion et l'immaturité des LCs pourraient interférer avec la présentation des Ag de *C. albicans* aux lymphocytes T CD4+, perturbant ainsi les mécanismes protecteurs à l'égard de l'OPC dans l'infection au VIH.

4.3.5 Les phagocytes

Les phagocytes, considérés comme la première ligne des défenses immunitaires à l'égard des pathogènes, jouent un rôle charnière dans l'orchestration de l'immunité des muqueuses chez l'hôte. Ils sont mobilisés rapidement à la muqueuse buccale en réponse à l'infection à *C. albicans* et leur altération pourrait prédisposer à l'OPC chez les patients infectés au VIH. Les patients atteints d'inflammation chronique des gencives présentent une augmentation des macrophages et des PMNs dans les muqueuses (434), suggérant que la mobilisation des phagocytes en réponse à l'inflammation est conservée. Néanmoins, leur activité anti-*Candida* pourrait être perturbée.

4.3.5.1 Les macrophages

La diminution de l'expression du récepteur CD4 (10, 213) et du CMH I (570) à la surface des macrophages, attribuable à la protéine Nef, et/ou la diminution de la quantité d'INF-γ (412) pourraient perturber l'activité anti-*Candida* de ces phagocytes. En effet, la

phagocytose (319), la flambée oxydative (644), et la production de cytokines (319) sont perturbées in vitro chez les macrophages infectés par le VIH. Néanmoins, les macrophages dérivés de monocytes sanguins de patients infectés au VIH présentent une phagocytose réduite (131), vraisemblablement induit par Nef (498), ou normale (458) à l'égard de *C. albicans*.

4.3.5.2 Les PMNs

Bien que la gp41 du VIH supprime in vitro l'activité de réduction du nitroblue tetrazolium par les PMNs (208), l'activité d'inhibition de la prolifération de *C. albicans* et la production d'IL-1 et d'IL-6 par les PMNs seraient préservées dans l'infection au VIH (100). Toutefois, la phagocytose (178) et l'activité fongicide (509) des PMNs à l'égard de *C. albicans* seraient altérées, mais leur capacité de production de RO serait conservée (673). L'activité anti-*Candida* des PMNs des individus sains ou infectés au VIH est perturbée par les cytokines Th2 IL-4 et IL-10, suggérant un rôle potentiel de ces cytokines dans l'augmentation de la susceptibilité à l'OPC dans l'infection au VIH (631).

L'infection au VIH perturbe les APCs et les lymphocytes T CD4+ et pourrait réduire les fonctions anti-*Candida* des phagocytes dans les muqueuses buccales, entraînant l'apparition de l'OPC. Néanmoins, certains mécanismes de défenses de l'hôte seraient préservés (calprotectine, kératinocytes, lymphocytes T CD8, et une partie de l'activité des phagocytes) et pourraient limiter de façon individuelle ou collective la prolifération de *C. albicans* à la surface des muqueuses buccales et prévenir de la dissémination systémique du champignon chez les patients infectés au VIH.

5. Les modèles animaux

En plus des problèmes éthiques associés à l'utilisation de prélèvements humains, il existe d'importantes différences entre les individus en terme d'alimentation, d'habitudes sociales, de statut immunitaire, et de physiologie de la cavité buccale telle que la fonction salivaire. Ces facteurs, ajoutés aux variations culturelles, ethniques et raciales de l'homme, influencent la pathogenèse de maladie telle que la candidose. Le développement de modèles animaux dans le cadre de l'étude de la candidose buccale fournirait un outil standardisé qui pourrait être contrôlé et manipulé afin d'obtenir de cette infection fongique des données sur l'immunopathogenèse. Le contrôle des facteurs environnementaux (température, humidité, alimentation, luminosité), des facteurs infectieux (durée, souche du pathogène) et des facteurs inter-individus (âge, sexe, lignée) permet à l'expérimentateur d'exercer une maîtrise précise de l'ensemble des paramètres impliqués dans la relation hôte-pathogène.

5.1 Les modèles antérieurs

Plusieurs espèces animales telles que le singe, le rat et la souris ont été utilisées pour étudier, sous différentes conditions expérimentales, la pathogenèse de la candidose.

(i) Primates. Ces animaux ont permis d'étudier les stomatites associées aux prothèses dentaires, prenant avantage de la capacité de fabriquer des appareils en acrylique analogues aux prothèses dentaires humaines. Ainsi, les candidoses érythémateuse et pseudomembraneuse ont été reproduites chez le singe en utilisant des plaques en acrylique (554). Néanmoins, le coût et la difficulté d'obtention de ces animaux limitent l'utilisation de ce modèle.

(ii) Rats. Sprague-Dawley et Winstar ont permis l'étude longitudinale de la pathogenèse de la candidose buccale, grâce à la grandeur de la cavité buccale, leur facilité d'hébergement, et leur disponibilité. La majorité des études faisant

appel à cette espèce animale ont porté sur l'efficacité des antifongiques à l'égard de *C. albicans* (554).

(iii) Souris. La souris, de par son faible coût, sa vitesse de reproduction, la facilité de son hébergement, et la capacité de la modifier génétiquement, est un modèle de choix pour évaluer, en fonction des perturbations immunitaires spécifiques qu'elle présente, les réponses immunitaires humorales et cellulaires en réponse à la candidose buccale. Contrairement au singe et au rat, la souris normale n'est pas colonisée par le *C. albicans*. Ainsi, la candidose doit être induite par l'infection des souris par le *C. albicans*. La compréhension des mécanismes de défense de l'hôte à l'égard de *C. albicans* a été considérablement renforcée par l'utilisation de souris KO ou possédant des immunodéficiences congénitales (551).

Bien que tous ces modèles murins présentent des anormalités immunologiques bien caractérisées, ils ne reproduisent pas les multiples perturbations des cellules immunitaires exprimant le VIH et sont donc perfectibles pour l'étude de la pathogenèse de la candidose dans le contexte de l'infection au VIH. Il existe actuellement aucun modèle animal dans lequel le VIH peut se répliquer activement et induire une maladie apparentée au SIDA. Bien que le macaque infecté au SIV soit un modèle largement utilisé pour étudier le SIDA (145, 146), la faible disponibilité de ces animaux, l'utilisation d'un virus différent du VIH et le manque d'outils pour étudier son système immunitaire limitent l'utilisation de ce modèle pour l'étude de l'OPC. Par contre, une approche différente faisant appel à un modèle chez la souris MAIDS a donné des débuts prometteurs. Les souris infectées au virus de la leucémie murine (Du5H(G6T2)) développent un syndrome désigné « *murine AIDS* » (MAIDS) présentant des perturbations du système immunitaire similaires à celles observées dans l'infection au VIH, incluant: (i) une prolifération polyclonale des lymphocytes B se traduisant par une hypergammaglobulinémie, une lymphodénopathie et une splénomégalie; (ii) une perturbation du profil Th1 des lymphocytes T CD4+ (diminution de la production d'IL-2 et d'IFN-γ) et une activation du profil Th2

(augmentation de la production de l'IL-4, l'IL-5, l'IL-6 et l'IL-10); (iii) la perte progressive de la réponse des CTL; (iv) une susceptibilité accrue aux infections et au développement de lymphomes B. En utilisant ce modèle, Deslauriers et al. (1997) ont étudié la capacité des souris C57BL/6, infectées par ce rétrovirus, à résister au développement d'une candidose buccale. L'infection à *C. albicans* a entraîné l'apparition d'un état de porteur chronique (<100 CFU) d'une durée supérieure à six mois chez les souris contrôles et pour 70% des souris infectées par le virus. Trente pourcents des souris infectées par le virus ont montré des signes de récurrence de candidose se traduisant par une augmentation significative du décompte des CFU buccaux de *C. albicans* suivi d'une courte période de convalescence (144). Néanmoins, aucune perte progressive du nombre de lymphocytes T CD4+ a été observée chez les souris infectées au rétrovirus, suggérant que ce modèle s'appliquerait davantage à l'étude des étapes précoces de l'infection au VIH (424).

5.2 La souris transgénique pour le VIH-1

L'utilisation des techniques de transgénèse et de manipulation des cellules souches embryoniques couplée au développement de recombinaisons homologues efficaces, a permis de générer des souris transgéniques ou/et déficientes pour un gène ciblé. De nombreuses équipes de recherche ont ainsi tenté de faire exprimer les gènes du VIH chez la souris (73, 154, 155, 354, 358, 558, 584, 639). Malheureusement, l'apparition de phénotypes inconnus ou la quasi-absence des phénotypes connus chez les patients infectés au VIH ont initialement empêché de générer un modèle murin satisfaisant. Néanmoins, l'équipe de Jolicoeur et al. (1996) a réussi à développer des lignées de souris transgéniques pour le VIH-1 (CD4C/HIV) reproduisant les manifestations cliniques et biologiques rencontrées dans l'infection au VIH (248, 249).

5.2.1 Le transgène

Afin d'exprimer les gènes du VIH uniquement dans les cellules immunitaires perturbées dans l'infection au VIH chez l'homme, les chercheurs ont construit un transgène dont l'expression est dirigée par les éléments régulateurs du gène de l'Ag CD4 humain (475). À l'inverse de celui de la souris, l'Ag CD4 humain est exprimé à la surface de plusieurs sous-populations de cellules myéloïdes, incluant les macrophages et les DCs (317, 683). L'Ag CD4 humain a été utilisé comme gène rapporteur afin de disséquer la capacité de différents éléments régulateurs (CD4A, CD4B, CD4C) à diriger son expression dans les lymphocytes T CD4+ et les macrophages murins et à la rendre silencieuse dans les lymphocytes B, T CD8+ et dans les cellules non-hématopoïétiques (252).

Les éléments régulateurs CD4C ont dirigé fidèlement l'expression du gène rapporteur dans les cellules cibles. Le CD4C est un ADN chimérique souris/homme de 14,4 kpb comprenant un *enhancer* murin du gène codant pour le CD4 des lymphocytes T (1,9 Kpb) (559) fusionné aux éléments génomiques humains (4,5 kpb) situés en amont du gène CD4 comprenant le 1er exon non-codant (exon 1), le 1er intron (9,9 kpb), l'exon 2 et une partie de l'exon 3 (252) ***(Figure 5.1)***.

L'intron 1 contient un *silencer*, réprimant l'expression du transgène dans les lymphocytes B, T CD8+ et les cellules non-lymphoïdes (629), et les éléments nécessaires pour son expression dans les macrophages (249). Les éléments régulateurs du CD4C ont été utilisés chez la souris transgénique pour exprimer le génome entier du VIH-1 (248) et de mutants géniques (249, 251).

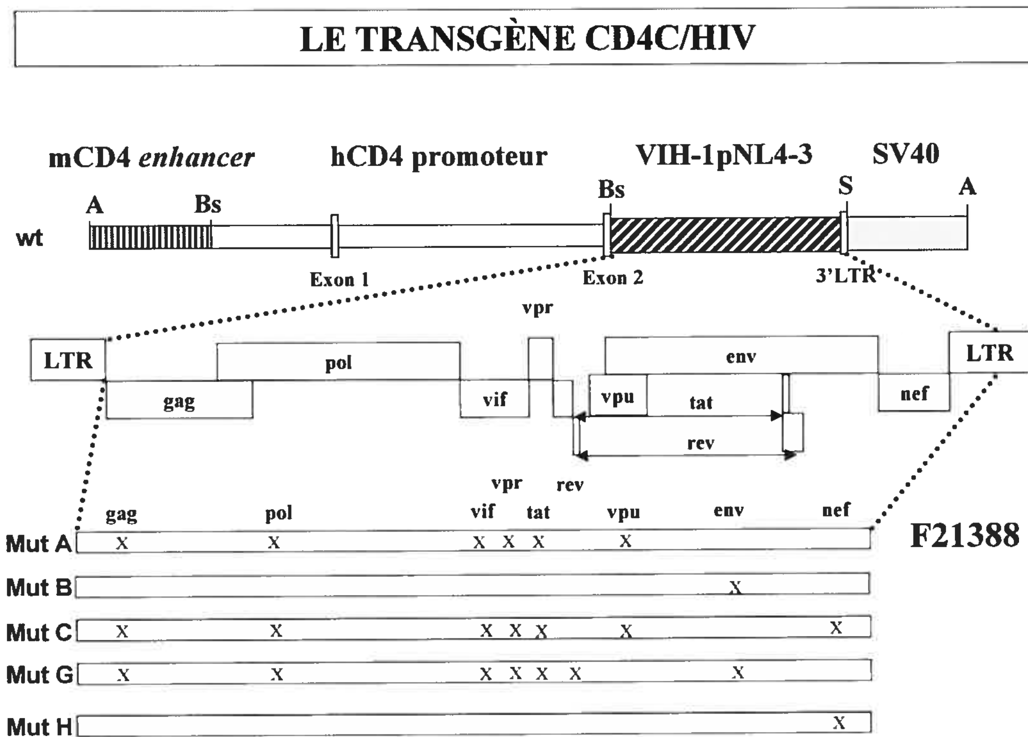

Figure 5.1 : Structure des transgènes CD4C/HIV^{Mut}. (mCD4) le fragment enhancer de souris du gène CD4, (hCD4) le promoteur humain CD4, (Mut A-H) les différentes mutations du génome du VIH-1 du transgène obtenues par l'interruption du cadre de lecture des gènes (X). Traduite de *Hanna et al., Cell (1998).*

5.2.2 La souris transgénique CD4C/HIV

La caractérisation des différentes lignées de souris transgéniques a permis d'observer une corrélation entre le niveau d'expression du transgène, le mutant du VIH utilisé pour créer la lignée, et la demi-vie des souris (249). Le transgène est exprimé dans les mêmes populations cellulaires que les cellules humaines exprimant l'Ag CD4, incluant: les lymphocytes thymiques T CD4+CD8+ immatures et T CD4+CD8- matures, les lymphocytes T CD4+ périphériques, et les cellules de la lignée myéloïde (macrophages périphériques, cellules de Kupffer, DCs et cellules de la microglie) (248). Chez les souris Tg CD4C/HIV, les produits des gènes du VIH sont exprimés dans les mêmes populations cellulaires que celles infectées au VIH chez l'homme. Cependant, le développement de la maladie apparentée au SIDA observée chez ces souris est hautement corrélé au niveau d'expression du transgène (249). Les souris présentant une forte expression du transgène meurent prématurément (quelques semaines après la naissance), celles exprimant des taux modérés du transgène survivent 4 à 6 mois, tandis que les souris présentant de faibles taux d'expression de la protéine Nef survivent jusqu'à un an, mais en présentant les mêmes atteintes associées à l'expression du transgène (249). L'analyse des différentes lignées de souris Tg, créées à partir de points de mutation dans le gènes transgène, a démontré que le gène *NEF* était nécessaire et suffisant pour l'établissement de la maladie (249). Ces souris présentent les manifestations cliniques et pathologiques (248, 249) suivantes:

- un amaigrissement, observé également chez les patients atteints du SIDA (126)
- des difficultés à avaler
- une mort prématurée et soudaine
- une atrophie thymique; la déplétion des cellules thymiques est observée chez les patients atteints du SIDA (218)
- une diminution dramatique du nombre de lymphocytes T CD4+

- une pneumonie et une néphrite interstitielles
- une cardiomyopathie
- quelquefois de la diarrhée et des oedèmes

Le nombre de lymphocytes T CD8+ des souris Tg CD4C/HIV est proportionnellement augmenté au début et diminue significativement à des stades plus avancés de la maladie (248, 249) de façon similaire à ce qui a été observé chez les patients infectés au VIH (189, 485).

5.2.3 La candidose buccale chez la souris Tg CD4C/HIV

Les études longitudinales de la candidose buccale effectuées chez les souris Tg CD4C/HIVMutA, exprimant uniquement les gènes *REV*, *ENV* et *NEF*, ont révélé trois phases suite à l'infection par le *C. albicans* (141):

- une phase de primo-infection (0-10 jours)
- une phase de porteur chronique où apparaissent de fréquentes récurrences de la candidose buccale (10-70 jours)
- une phase de forte remontée finale du nombre de CFU buccaux de *C. albicans*, précédent la mort des souris

Les manifestations cliniques et pathologiques de la candidose buccale ont été retrouvées identiques à celles observées chez les patients infectés au VIH, incluant:

- une augmentation de la charge buccale de *C. albicans* qui se maintient et devient plus importante au stade plus tardif de la maladie (SIDA) (200, 647, 697), (141)
- une pénétration des hyphes de *C. albicans* dans la couche superficielle de l'épithélium pavimenteux stratifié de la cavité buccale et de l'oesophage (184, 511, 523), (141)
- une infiltration de cellules mononuclées dans la muqueuse buccale (307, 421, 646), (141)

6. Hypothèse

Des études effectuées chez des patients infectés au VIH atteints d'OPC ont révélé l'apparition d'une diminution progressive du nombre de lymphocytes T CD4+ et une perte de l'hypersensibilité de type retardée à l'égard de *C. albicans* (523). Ces patients présentent également une altération de la différentiation terminale des LCs des muqueuses buccales caractérisée par une diminution de l'expression du CMH II, la présence de dendrites atténuées, un développement limité des organelles, et la perte des granules de Birbeck (523). Enfin, des études immuno-histochimiques et de microscopie électronique sur les biopsies de muqueuses buccales provenant de ces patients ont révélé la présence d'une infiltration de cellules mononuclées CD8+ et CD14+ dans la couche basale de l'épithélium des muqueuses (523).

Ces observations suggèrent que l'altération des LCs pourrait interférer avec leur développement normal d'apprêtement et de présentation des Ag de *C. albicans* aux lymphocytes T CD4+, eux-mêmes déplétés chez les patients infectés au VIH. Les lymphocytes T CD8+, les monocytes/macrophages ou encore les PMNs pourraient jouer un rôle en limitant l'infection à *C. albicans* et en confinant le champignon dans la couche superficielle de l'épithélium des muqueuses buccales. Nous avons pris avantage d'un nouveau modèle de souris transgénique CD4C/HIV pour déterminer l'implication et le rôle des DCs, des lymphocytes T CD4+, des PMNs et des lymphocytes T CD8+ dans l'immunopathogenèse de la candidose oropharyngée.

Chapitre 2

Les articles scientifiques

Article 1

Altered dendritic cell and CD4+ T-cell phenotype and function determine the susceptibility to mucosal candidiasis in transgenic mice expressing human immunodeficiency virus type 1.

article soumis au *Journal of Immunology*

Contributions

Daniel Lewandowski: Table 2, Figure 1, 3, 4, 5, 6, 7, 8

Miriam Marquis: Table 1, Figure 2

Francine Aumont: maintien de la colonie de souris

Résumé

Les altérations précises qui favorisent la croissance de *Candida* à la surface des muqueuses dans le développement séquentiel de l'infection au VIH n'ont pas été clairement définies. Nous avons pris avantage d'un nouveau modèle de candidose oropharyngée (OPC) chez les souris transgéniques (Tg) CD4C/HIV pour déterminer le rôle des cellules dendritiques (DCs) et des lymphocytes T CD4+, qui expriment le transgène, dans la perturbation de l'immunité protectrice des muqueuses buccales à l'égard de *C. albicans* et la persistance d'un état de porteur chronique de *C. albicans*. Le maintien de la charge buccale de *C. albicans* nécessite l'expression du transgène CD4C/HIV dans les DCs et dans les lymphocytes T CD4+, mais l'expression additionnelle du transgène dans les macrophages a été trouvée dispensable pour produire le phénotype de candidose. Les DCs provenant des souris Tg sont réduites en nombre et présentent un phénotype immature, caractérisé par une diminution de l'expression du CMH de type II et de l'IL-12, et une capacité conservée d'endocyter les blastoconidies de *C. albicans* comparativement à celle des DCs provenant de souris non-Tg. Le nombre des lymphocytes T CD4+ est nettement réduit dans les muqueuses buccales, dans les ganglions lymphatiques cervicaux (CLNs) et dans le sang périphérique chez la souris Tg. Les lymphocytes T CD4+ des CLNs de souris Tg ont progressé in vitro vers un profil Th2 non-protecteur à l'égard de *C. albicans*. Les lymphocytes T CD4+ des CLNs provenant des souris Tg infectées sont anergiques à l'égard des antigènes (Ag) de *C. albicans* in vitro et n'acquièrent pas le phénotype effecteur observé chez les souris non-Tg infectées. La co-culture des lymphocytes T CD4+ en présence des DCs ayant phagocyté le *C. albicans* a révélé que l'expression du transgène dans une seule ou les deux populations cellulaires réduit nettement la prolifération des lymphocytes T CD4+ et la production d'IL-2. Ces résultats ont démontré que les altérations fonctionnelles de ces deux populations cellulaires empêchent la réponse immunitaire à l'égard de *C. albicans* chez la souris Tg. Finalement, le transfert adoptif de lymphocytes T CD4+ naïfs provenant de souris non-Tg chez la souris Tg CD4C/HIV^{MutA},

exprimant les gènes *NEF*, *REV* et *ENV* du VIH, a restauré transitoirement la prolifération des lymphocytes T CD4+ à l'égard des Ag de *C. albicans* et a nettement réduit la charge buccale de *C. albicans*. Ces résultats ont suggéré que la capacité fonctionnelle des cellules présentatrices d'Ag est partiellement maintenue chez la souris Tg. Ces résultats indiquent que l'altération des DCs et des lymphocytes T CD4+ détermine la susceptibilité à la candidose buccale chez ces souris Tg, et suggèrent que des perturbations similaires pourraient induire la susceptibilité à l'OPC chez les patients infectés au VIH.

Altered dendritic cell and CD4+ T-cell phenotype and function determine the susceptibility to mucosal candidiasis in transgenic mice expressing human immunodeficiency virus type 1.

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Condensed title: Mechanisms of defective mucosal immunity against *C. albicans* in HIV-1 transgenic mice.

Key words: Mucosal immunity; candidiasis; HIV; AIDS; transgenic mice

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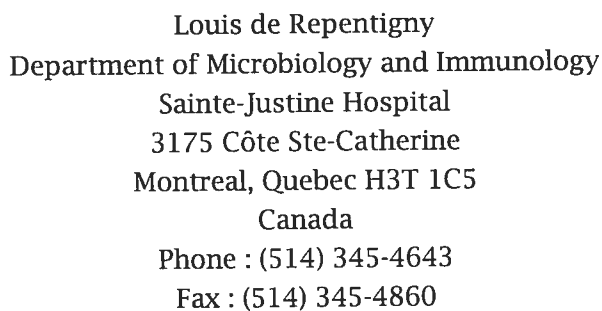

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Abstract

The precise impairments that favour *Candida* outgrowth on mucosal surfaces in the sequential development of HIV-infection have not been defined. Here, we have taken advantage of a novel model of oropharyngeal candidiasis (OPC) in CD4C/HIV transgenic (Tg) mice, to investigate the role of transgene expressing dendritic cells (DCs) and CD4+ T-cells in impaired induction of protective mucosal immunity and maintenance of a chronic carrier state of *C. albicans*. Chronic oral carriage of *C. albicans* required HIV-1 transgene expression in both DCs and CD4+ T-cells, but added expression in macrophages was found to be dispensable to produce this phenotype. DCs from the Tg mice were reduced in number and had an immature phenotype, with low expression of MHC class II and IL-12, and unaltered endocytosis of *C. albicans* yeast cells compared to DCs from non-Tg mice. CD4+ T-cells were sharply reduced in number in the oral mucosa, cervical lymph nodes (CLNs) and peripheral blood of the Tg mice, and displayed a partial shift towards a nonprotective Th2 response. Proliferation of CLN CD4+ T-cells from infected Tg mice in response to *C. albicans* antigen in vitro was almost completely abrogated and the cells also failed to acquire the effector phenotype observed in the infected non-Tg mice. Coculture of *C. albicans*-pulsed DCs with CD4+ T-cells from Tg and non-Tg mice in vitro showed that Tg expression in either or both of these cell populations sharply reduced the proliferation of CD4+ T-cells and their production of IL-2, demonstrating that both cell populations are functionally defective in priming an immune response to *C. albicans* in the Tg mice. Finally, adoptive transfer of naïve non-Tg CD4+ T-cells into CD4C/HIV^{MutA} Tg mice which express rev, env and nef of HIV-1 restored proliferation to *C. albicans* antigen and sharply reduced oral burdens of *C. albicans*, suggesting that the functional capacity of antigen-presenting cells is partially maintained in the Tg mice. Taken together, these results indicate that defects of both DCs and CD4+ T-cells determine the susceptibility to chronic oral carriage of *C. albicans* in these Tg mice, and suggest that similar defects may underlie the susceptibility to OPC in human HIV-infection.

Introduction

Oropharyngeal candidiasis (OPC) is the most frequent opportunistic fungal infection among human immunodeficiency virus (HIV)-infected patients, and it has been estimated that more than 90% of HIV-infected patients develop this often debilitating infection at some time during progression of their disease.[1;2] Although the incidence of OPC in HIV-infection has been significantly reduced since the introduction of highly active antiretroviral therapy (HAART)[3-6], it remains a common opportunistic infection in HIV-infected patients worldwide.

Although an intact adaptive cell-mediated immune response to *Candida albicans* is protective against OPC[7;8], the critical impairments of innate[9-16] and adaptive[17;18] immunity which are responsible for the onset and maintenance of mucosal candidiasis in HIV-infection have not as yet been precisely defined[19;20] (reviewed in[21]). Colonization of oral mucosal surfaces and symptomatic disease are closely correlated with the development and progression of the cellular immunodeficiency of HIV-infection.[22-24] Although deficiencies in *Candida*-specific systemic cell-mediated immunity do not solely account for susceptibility to OPC in HIV-infected patients[25], the devastating impact of HIV-infection on mucosal Langerhans' cells[26-28] and CD4+ T-cell[28;29] populations is most likely central to the pathogenesis of mucosal candidiasis in HIV-infected patients (reviewed in[21]).

Controlled studies on the immunopathogenesis of mucosal candidiasis in HIV-infection have been hampered by the lack of a relevant animal model[30] (reviewed in[21]). The availability of CD4C/HIV transgenic (Tg) mice expressing gene products of HIV-1 in immune cells and developing an AIDS-like disease has provided an opportunity to devise a novel model of mucosal candidiasis that closely mimics the clinical and pathological features of candidal infection in human HIV-infection[31;32] (reviewed in[21]). With the recognition that a cause-and-effect analysis of the immunopathogenesis of mucosal

candidiasis in HIV-infection can now be achieved under controlled conditions in these Tg mice, the present study was undertaken to determine the role of defective dendritic cells (DCs) and CD4+ T-cells in impaired induction of protective immunity and in the phenotype of chronic oral carriage of *C. albicans*. We have taken advantage of novel mouse constructs selectively expressing the transgene either solely in CD4+ T-cells (Hanna Z et al, submitted for publication), in both CD4+ T-cells and DCs[33], or in CD4+ T-cells, DCs and macrophages combined.[34] Here we show that chronic carriage of *C. albicans* requires transgene expression in at least DCs and CD4+ T-cells, that depletion and functional impairment of these cell populations present in Tg mice abrogates *Candida*-specific adaptive immunity in vivo and in vitro, and that protective immunity to *C. albicans* can be reconstituted by adoptively transferring intact CD4+ T-cells into Tg mice. Therefore, altered DCs and CD4+ T-cells are central to the immunopathogenesis of mucosal candidiasis in these Tg mice expressing HIV-1.

Materials and Methods

Generation of Tg mice expressing HIV-1 gene products

The CD4C/HIV^{MutA} Tg mice which express rev, env and nef of HIV-1 have been described elsewhere.[34] CD4C/HIV^{MutA} mutant DNA harbors mouse CD4 enhancer and human CD4 promoter elements to drive the expression of HIV-1 genes in CD4+ CD8+ and CD4+ thymocytes, in peripheral CD4+ T-cells, and in macrophages and DCs. Founder mouse F21388 was bred on the C3H background, and progeny mice were genotyped and routinely examined for signs of disease. Animals from this line express moderate levels of the transgene, with 50% survival at 3 months.[34] The generation of CD4C/HIV^{MutG} mice revealed that selective expression of the nef gene is required and sufficient to elicit an AIDS-like disease in these Tg mice. This disease is characterized by failure to thrive, wasting, severe atrophy and fibrosis of lymphoid organs, loss of CD4+ T-cells, interstitial pneumonitis, and tubulointerstitial nephritis.[34] These changes are not observed in control non-Tg littermates.[34]

CD4E constructs, generated from the CD4C DNA by replacing the 2.6-kilobase pair (kbp) Sac I fragment with the mouse promoter[33], were ligated to HIV^{MutG} DNA[34] to produce CD4E/HIV^{MutG} Tg mice (Hanna Z et al, Manuscript in preparation). These Tg mice selectively express the nef gene in double-positive CD4+ CD8+ thymocytes, single-positive peripheral CD4+ T-cells, a low number of CD8+ cells, and in DCs, but not in macrophages.[33]

mCD4C/HIV^{MutG} DNA was constructed by ligating the mouse CD4 promoter (mCD4c) to HIV^{MutG} DNA (Hanna Z et al, Manuscript in preparation). Because the mouse CD4 promoter specifically directs the expression of genes mainly in CD4+ T-cells[35-37], expression of the nef gene in mCD4C/HIV^{MutG} Tg mice is restricted to CD4+ T-cells.

Specific pathogen-free male and female Tg mice and non-Tg littermates were housed in a protective environment at the University of Montreal Animal Care Unit, in sterilized individual cages equipped with filter hoods. The animals were supplied with sterile water and were fed with sterile mouse chow. In selected experiments, male and female 5-6 week-old C3H mice (17-18g), obtained from Charles River Canada, were used as immunocompetent controls.

Animal model of mucosal candidiasis

Oral inoculation with *C. albicans* LAM-1, assessments for signs of morbidity, quantification of *C. albicans* in the oral cavities of individual mice, and determination of burdens of *C. albicans* in the gastrointestinal tract and internal organs were conducted as described previously.[31]

Candida antigen

C. albicans strain LAM-138 was grown to late-log phase in Sabouraud dextrose broth (Difco Laboratories, Detroit, MI) for 18h at 30°C with rotary agitation. To prepare a crude cytoplasmic extract[7], yeast cells were washed twice in sterile 0.01 M PBS (pH 7.4), resuspended in PBS at 1.2 x 10^{10} cells/ml, and disrupted with 20 successive 20-s runs and intermittent cooling in a Vibra Cell instrument (Sonics and Materials, Inc., Danbury, CT) set at an amplitude of 20%. The cell lysate was centrifuged for 10 min at 2000 x g, and the supernatant was dialyzed against PBS. The crude cytoplasmic extract (8.5 mg of protein/ml) was stored in aliquots at -20°C.

Single-cell suspensions of spleen, cervical lymph nodes and oral mucosal tissue

Groups of 6 to 10 CD4C/HIV^{MutA} and non-Tg littermates (45 to 55 days old) were orally

infected or not with 10^8 CFU of *C. albicans* LAM-1 blastoconidia[31], and assessed at 7, 45 or 70 days post-infection. Independent experiments were conducted by pooling cells from all mice within each group. Heparinized blood was collected by cardiac puncture under anesthesia with Hypnorm (Janssen Pharmaceutica) and Versed (Sabex, Boucherville, Quebec)[31], and the mice were exsanguinated with PBS.[31] Spleens and cervical lymph nodes (CLN) were removed and mechanically disrupted by pressing through a nylon mesh (pore size, 70 μm) and deposited in 25-mm-diameter dishes containing 2 ml of Hanks' balanced salt solution (HBSS; Gibco, Grand Island, NY). Cell suspensions were twice washed in HBSS and resuspended in complete tissue culture medium consisting of RPMI 1640 medium (Gibco) supplemented with 10% of heat-inactivated fetal bovine serum (Gibco), 20 mM HEPES buffer, 2 mM L-glutamine, 5×10^{-5} M β-mercaptoethanol, 100 U/ml penicillin and streptomycin, 0.25 μg/ml amphotericin B, and 50 μg/ml of gentamicin. Cells were then filtered through a sterile nylon mesh (pore size, 70 μm) to obtain a homogeneous cell suspension.

The oral mucosa, including the cheeks, the hard and the soft palate were dissected free of the underlying muscle layer and washed for 5 min in cleaning buffer solution (20 mM TRIS, 20 mM NaCl, 40 mM EDTA, and 1 mM DTT) in order to disperse surface mucus. Tissues were washed in HBSS, cut longitudinally, and finely minced into 1 mm^2 fragments with a sterile scalpel in complete medium. Minced tissues containing epithelium and lamina propria were digested by incubating with 0.25% collagenase type IV (Sigma) in complete medium at 37°C for 30 min with gentle agitation, replacing the medium, and incubating for a further 30 min. Tissue debris were excluded by twice filtering oral mucosal cell suspensions through a 70- μm nylon mesh and washing in HBSS.

Contamination of these cell suspensions by peripheral blood cells was estimated to be 1% according to the red cell: nucleated cell ratio. Spleens, CLN and oral mucosae were pooled for each group of mice. Cell suspensions were resuspended in complete medium and

adjusted to 1 x 10^6 cells/ml, and cell viability was 90% by trypan blue exclusion.

Flow cytometry

Cell surface marker analysis of peripheral blood lymphocytes was conducted as described previously[31], on a FACSCalibur flow cytometer (BD Biosciences) equipped with CellQuest software. CLN and oral mucosal cells were directly stained with fluorescent monoclonal antibodies specific for mouse CD45 (30-F11), CD11b (M1/70), I-A^k (MHC class II alloantigen; 11-5.2), CD11c (HL3), CD3(145-2C11), CD4 (RM4-5), CD8 (53-6.7), and their respective isotype controls (hamster lgG1,l, rat lgG2b,k, mouse lgG2b,k, mouse lgG2a,k, rat lgG1, rat lgG2b) (BD Biosciences) at 1 µg/10^6 cells for 45 min at 4°C. Cells were twice washed in cold PBS and fixed with 2% paraformaldehyde. For spleen cell suspensions, 1 ml of red cell lysing buffer (BD Biosciences) was added to 100 µl of each cell suspension for 5 min before washing and fixation.

Spleen cells were used as a control cell population for comparison of SSC and FSC flow cytometry profiles with the oral mucosal cell population. Acquisition of data was counted on 10,000 events by gating on CD45+ cells, which comprised 6-8% of mucosal cell suspensions. Data were expressed in biparametric diagrams and monoparametric histograms according to the various combinations of antibodies (Fig. 1). In preliminary experiments, collagenase treatment of splenocytes from non-Tg mice decreased the percentage of CD3+ CD8+ T-cells by an average of 28%, but did not alter the percentage of CD3+ CD4- cells which remained equivalent to that of CD3+ CD8+ cells untreated with collagenase. Accordingly, CD8+ T-cells were estimated as CD3+ CD4- cells in the oral mucosa. Treatment with collagenase did not change the percentage of CD3+ CD4+ T-cells.

Determination of intracellular cytokines

Staining of intracellular cytokines was performed to compare the Th1/Th2 profiles of CLN CD4+ T-cells and the production of IL-12 by DCs from Tg and non-Tg mice. Briefly, CLN homogeneous cell suspensions were obtained from CD4C/HIV^{MutA} and non-Tg mice infected or uninfected with *C. albicans* for each time point, as described above. Positive selection of CD4+ T-cells was done by magnetic sorting using biotin-conjugated antibody specific for CD4 (RM4-5; BD Biosciences) and streptavidin Captivate™ ferrofluid particles (Molecular Probes, Eugene, OR), yielding >95% CD4+ T-cells. No difference in the generated data was observed by using the Dynal Mouse CD4 Negative Isolation Kit (Dynal Biotech, Oslo, Norway). 10^6 selected CD4+ T-cells/ml were cultured for 72h on anti-CD3 (145-2C11; BD Biosciences; 5 μg/ml) and anti-CD28 (37.51; BD Biosciences; 5 μg/ml) - coated plates in supplemented RPMI 1640 containing 5 μg/ml of anti-CD3 antibody. The cells were restimulated for 4h with ionomycin (500 ng/ml) and phorbol myristate acetate (5 ng/ml) in presence of monensin (GolgiStop; BD Biosciences) or brefeldin A (GolgiPlug; BD Biosciences), according to the manufacturer's instructions. Preliminary experiments demonstrated a greater fluorescence intensity for expression of IFN-γ, IL-2 and IL-10 using monensin, and for TNF-α and IL-4 using brefeldin A. Cells were surface stained with anti-CD69 antibody (H1.2F3; BD Biosciences) specific for activated T-cells for 30 min at 4°C. After washing, intracellular staining was performed with the Cytofix/Cytoperm kit (BD Biosciences) and with anti-mouse IFN-γ-PE (XM61.2), IL-2-FITC (JES6-5H4), IL-4-APC (11B11), IL-10-FITC (JES5-16E3), TNF-α-PE (MP6-XT22) and their respective isotype control antibodies (BD Biosciences). Flow cytometry acquisition was performed on 10,000 events by gating on CD69+ cells, and the data were analyzed to determine the percentage of CD4+ T-cells expressing each of the cytokines.

DCs were generated by culturing bone marrow cells of Tg and non-Tg mice according to a modification of methods previously described.[39;40] Briefly, after flushing the bone marrow from the femurs of mice, cell suspensions were filtered through a 70-μm nylon mesh and red blood cells were disrupted with lysing buffer. Cells were washed with HBSS and

cultured with GM-CSF (1000 U/ml) (Cedarlane) and IL-4 (500 U/ml) (Cedarlane) in supplemented RPMI 1640 at 10^6 cells/ml in flat bottom 6-well plates. At days 2 and 3, non-adherent cells and medium were removed and fresh medium supplemented with GM-CSF and IL-4 was added. Non-adherent cells obtained at day 7 were determined to be DCs by morphology and FACS analysis (>90% CD11b+CD11c+I-A^k+). 10^6 bone marrow-derived DCs were stimulated with LPS (Sigma; 100 ng/ml or 1 μg/ml) or pulsed with live *C. albicans* blastoconidia at ratios of 2:1, 1:1, or 1:5 in flat bottom 24-well plates for 2h before addition of brefeldin A and 2.5 μg/ml of amphotericin B to prevent *Candida* overgrowth, and the plates were then incubated for a further 16h. Amphotericin B was reported to not modify cytokine production by DCs.[41] Intracellular staining was performed with anti-IL-12-PE (C15.6; BD Biosciences) as described above. Flow cytometry acquisition was performed on 10,000 events by gating on CD11c+ DCs and the data were analyzed to determine the percentage of CD11c+ DCs expressing IL-12.

Proliferative response of CLN CD4+ T-cells to Candida antigen and concanavalin A

In order to assess CD4+ T-cell clonal expansion, we measured the proliferation of 5(6)-carboxyfluorescein diacetate succinimidyl ester (CFSE)-stained CLN cells in response to concanavalin A (ConA) and *C. albicans* antigen. CLN cell suspensions were obtained from Tg and non-Tg mice, either uninfected or 7 days after oral infection with *C. albicans*, as described above. Briefly, cells (1 x 10^7/ml) were stained with 1 μM of CFSE dye (Molecular Probes) in PBS and incubated in the dark with gentle mixing at room temperature for 8 min. Cells were washed 3 times with PBS containing 5% fetal bovine serum and cultured in triplicate for 96h in flat bottom 96-well plates at 2 x 10^5 cells/well, containing ConA (5 μg/ml; Sigma), C. albicans antigen (66 μg/ml) or cells alone. These concentrations produced maximal proliferation of CLN cells from C3H mice, seven days after oral infection with *C. albicans*. Then, the cells were stained with anti-CD4-PerCP and anti-CD62L-PE antibodies (BD Biosciences) and analyzed by flow cytometry. The optimal CFSE concentration was

defined by staining of all cells at a fluorescence intensity between 10^3 and 10^4 log units (FLI channel). Analysis was performed by gating on CD4+ T-cells, and cell proliferation was estimated as the percentage of CD4+ T-cells with decreased fluorescence intensity.[42] In addition, proliferating cells were phenotyped for CD62L antigen surface expression in order to determine differentiation into CD4+ CD62L- effector cells.

In vitro activation of CLN CD4+ T-cells by Candida-pulsed DCs

Bone marrow-derived DCs were obtained as described above. Purified CLN CD4+ T-cells (>90% CD3+ CD4+ T-cells) were isolated from 7d-infected or -uninfected Tg and non-Tg mice, using the Dynal Mouse CD4 Negative Isolation Kit (Dynal Biotech, Oslo, Norway). Tg and non-Tg DCs (2 x 10^4 cells), pulsed or unpulsed for 18h with live *C. albicans* blastoconidia at a 1:5 ratio as described above, were cultured with 2 x 10^5 CFSE-labeled CD4+ T-cells in 96-well tissue culture plates for 5d in 100 µl of supplemented RPMI medium. Cell proliferation was estimated as the percentage of CD4+ T-cells with decreased CFSE fluorescence intensity.[42] In addition, IL-2 production in coculture supernatants was quantitated using the Mouse Cytokine Bead Array Kit (BD Biosciences).

CLN activated/memory-like T-cells

In order to measure the relative proportions of CLN memory-like T-cells of Tg or non-Tg mice infected or uninfected with *C. albicans*, we phenotyped these cells by using anti-CD4, -CD8, -CD44 and -CD62L antibodies. CLN homogeneous cell suspensions harvested from each group of mice were pooled to 1 x 10^6 cells/ml and stained with 1 µg of CD4-PerCP, CD8-FITC, CD44-biotin and CD62L-PE antibodies (BD Biosciences) for 30 min at 4°C, washed twice with PBS, and stained with streptavidin-APC (BD Biosciences) for 45 min at 4°C. After washing, 10,000 events were acquired in flow cytometry and analysis was performed by gating on CD4+ or CD8+ cells to quantitate the proportion of naïve and

memory-like CD4+ and CD8+ T-cells.[43]

C. albicans phagocytosis assay

Immediately prior to use, 5 x 10^7 *C. albicans* blastoconidia/ml were heated at 95°C for 30 min and labelled with FITC (Sigma;10 μg/mL) in 0.5M carbonate buffer (pH 9.5) for 60 min at 4°C in a light-protected environment. For the phagocytosis assay, 2.5 x 10^5 bone marrow-derived DCs were incubated with heat-inactivated *C. albicans* cells in 100 μL of supplemented RPMI 1640 with a DC to blastoconidia ratio of 2:1, 1:1 or 1:5 for 15, 30, 60, or 120 min at 37°C in independent wells. Phagocytosis was stopped by cooling the samples to 4°C. A control phagocytosis assay was conducted at 4°C (1:5 ratio) in presence of cytochalasin B (Sigma;5 μg/mL).[44] Ethidium bromide was added at a concentration of 10 μg/ml and flow cytometry acquisition of 10,000 DCs was done immediately as described.[45] Phagocytosis of *C. albicans* by DCs was verified by confocal microscopy.

Adoptive transfer of DCs and CD4+ T-cells

Bone marrow-derived DCs and purified CLN CD4+ T-cells were obtained from uninfected non-Tg mice as described above. 5 x 10^5 DCs and/or 2.5 x 10^6 CD4+ T-cells in 100 μl of PBS, or PBS alone, were injected intravenously into *C. albicans*-infected CD4C/HIV^{MutA} Tg mice at days 21 and 27 after oral infection with *C. albicans*, respectively, in the early phase of chronic oral carriage. Oral burdens of *C. albicans* were serially monitored after transfer, and CLN CD4+ T-cell proliferation was measured at the conclusion of the observation period.

Statistical analysis

Differences in oral burdens of *C. albicans* among groups of mice were determined by

using PROC MIXED software (SAS Institute, Cary, NC). Repeated-measurements analysis of variance with Tukey-Kramer contrast analysis when required was conducted with 2 factors, 1 between (group) and 1 within (time). Significant interactions ($p < 0.05$) were further analyzed, and significant differences ($p < 0.05$) between group means at fixed times were determined by use of the 2-sample, 2-tailed Student's t test for independent samples. The Mann-Whitney U test[46] was used to analyze immunophenotypes of peripheral blood and CLN lymphocytes from CD4C/HIV^{MutG}, CD4E/HIV^{MutG} and mCD4C/ HIV^{MutG} Tg mice. Immunophenotypes of cell populations and cytokine expression by CD4C/HIV^{MutA} Tg mice determined on the day of infection were analyzed using Student's t test. Statistical analyses for all other data from the CD4C/HIV^{MutA} mice were performed with SPSS Version 11.5 software (SPSS, Chicago, IL), using an analysis of variance followed by Tukey contrast analysis when necessary. Differences were considered to be significant at $p < 0.05$.

Results

Mucosal candidiasis in Tg mouse constructs expressing HIV-1 gene products in defined cell populations

CD4C/HIV^{MutG} Tg offspring from founder F2736734 and non-Tg littermates were inoculated orally with *C. albicans* LAM-1, and longitudinal assessments of oral burdens were done by sampling the oral cavities. Oral burdens of *C. albicans* were elevated in these Tg mice, compared with control non-Tg animals (Fig. 2A). Beginning 5 days after inoculation, significant ($p < 0.05$) and sustained enhancement of infection in the Tg mice was maintained during recovery from primary infection and a 4.5-month chronic carrier state. In contrast, primary infection in the non-Tg mice was self-limited, with uniform clearance of *C. albicans* from the oral cavities within 9 days after inoculation. The sustained enhancement of infection in these CD4C/HIV^{MutG} mice is comparable to our previous observations in CD4C/HIV^{MutA} mice[31], indicating that the nef gene is necessary and sufficient for persistent candidal infection.

In CD4E/HIV^{MutG} Tg mice originating from founder F104990 (Hanna Z et al, manuscript in preparation), oral burdens were significantly ($p < 0.05$) enhanced compared to non-Tg controls beginning 3 days after inoculation and extending throughout a 3.5-month sustained carriage of *C. albicans* (Fig. 2B). These findings indicate that expression of the nef gene in CD4+ T-cells and DCs, but not in macrophages, is sufficient to establish and maintain chronic carriage of *C. albicans* in these Tg mice.

In striking contrast to the CD4C/HIV^{MutG} and CD4E/HIV^{MutG} Tg mice, however, *C. albicans* was rapidly cleared from the oral cavities and a chronic carrier state failed to be established in mCD4C/HIV^{MutG} mice descending from founder F69457, expressing nef in only CD4+ T-cells (Fig. 2C). In addition, oral burdens of *C. albicans* during primary

infection in these mCD4C/HIV^{MutG} mice were not significantly different ($p > 0.05$) from those in non-Tg mice. These results demonstrate that expression of the nef gene in both CD4+ T-cells and DCs is not only sufficient but also necessary for persistent oral carriage of *C. albicans* in these Tg mice.

High burdens of *C. albicans* were found in stomach and small and large intestine of CD4C/HIV^{MutG} and CD4E/HIV^{MutG} Tg mice, but were uniformly absent in mCD4C/HIV^{MutG} Tg and in the non-Tg animals (Table 1). Dissemination to brain, lungs, liver or kidneys occurred in 3 of 6 CD4C/HIV^{MutG} and in 2 of 6 CD4E/HIV^{MutG} Tg mice. However, dissemination was limited to one or two organs, with the exception of a single CD4C/HIV^{MutG} mouse with widespread dissemination to all four organs. Burdens of *C. albicans* in these deep organs of CD4C/HIV^{MutG} Tg mice remained low, consistent with previous observations in CD4C/HIV^{MutA} Tg mice.[31] The median survival of the CD4C/HIV^{MutG} Tg mice infected with *C. albicans* (10 months) was identical to that previously reported for uninfected offspring of founder F2736734, indicating that candidal infection did not cause premature mortality in this Tg mouse construct.

CD4+ T-cells were severely depleted in both peripheral blood and cervical lymph nodes of CD4C/HIV^{MutG}, CD4E/HIV^{MutG} and mCD4C/HIV^{MutG} Tg mice orally infected with *C. albicans*, relative to non-Tg age-matched controls (Table 2), consistent with expression of the transgene in CD4+ T-cells of each of these mouse constructs and similar to decreases in CD4C/HIV^{MutA} Tg mice (Fig. 3).[31;34] Moreover, the depletion of a CD11b^{lo}, I-A^{k}, CD11c+ mature DC population observed in the oral mucosa of CD4C/HIV^{MutA} Tg mice (see below) was also found in CD4E/HIV^{MutG} but not in mCD4C/HIV^{MutG} Tg mice (data not shown). Interestingly, the percentages of CD8+ T-cells were consistently increased in CLNs of these Tg mice (Table 2), as found previously in mesenteric lymph nodes of CD4C/HIV^{MutA} Tg mice.[34] In contrast, peripheral blood CD8+ cells of CD4C/HIV^{MutG} Tg mice were depleted compared to non-Tg mice (Table 2), as also observed in CD4C/HIV^{MutA} Tg mice (Fig. 3)[31],

while this cell population was augmented in mCD4C/HIV^{MutG} Tg mice (Table 2).

Immunophenotypes of oral mucosal and CLN cell populations

To longitudinally assess the impact of the HIV-1 transgene on DCs, and CD4+ and CD8+ T-cell populations, multiparametric flow cytometry analysis was conducted on CD4C/HIV^{MutA} Tg mice and non-Tg littermates infected or uninfected with *C. albicans*. In comparison to non-Tg littermates, CD4C/HIV^{MutA} Tg mice had a strikingly depleted $CD11b^{lo}$, I-A^{k}+, CD11c+ mature DC population in the oral mucosa early and throughout the course of AIDS-like disease ($p < 0.05$), which did not progress over time ($p > 0.05$) (Figs. 3 and 4). Oral infection with *C. albicans* did not alter the percentage of these mature DCs in the Tg mice ($p > 0.05$), but resulted in a sharp decrease ($p < 0.001$) of this cell population seven days after oral infection in the non-Tg animals (Fig. 3). A similar transient decrease ($p = 0.006$) in mature DCs coinciding with recovery from primary oral infection with *C. albicans* was observed in the oral mucosae of C3H mice (data not shown). In contrast to mature DCs, no depletion of the $CD11b^{hi}$, I-A^{k}+, CD11c+ immature DC population appeared during the course of AIDS-like disease in these Tg mice ($p > 0.05$), and oral infection with *C. albicans* did not alter the percentage of these cells in either Tg or non-Tg animals ($p > 0.05$). A depletion in oral mucosal CD4+ T-cells in uninfected Tg compared to non-Tg mice appeared early ($p = 0.01$) and remained constant throughout AIDS-like disease (Figs. 3 and 4). Interestingly, both Tg and non-Tg mice were able to respond to *C. albicans* infection with a significant increase ($p < 0.05$) in oral mucosal CD4+ T-cells; however, CD4+ T-cells remained significantly lower ($p < 0.001$) in infected Tg compared to infected non-Tg mice (Figs. 3 and 4). Finally, oral mucosal CD8+ T-cells, estimated as CD3+ CD4- cells, were significantly augmented ($p < 0.05$) in infected Tg mice compared to uninfected non-Tg animals both early and late in the course of AIDS-like disease, demonstrating that the Tg mice recruit this cell population to the oral mucosa in response to candidal infection (Fig. 3).

In contrast to the oral mucosa, the proportion of CLN I-A^k+, CD11c+ DCs was significantly augmented ($p < 0.01$) in the Tg compared to the non-Tg mice (Fig. 3), but was not significantly altered by oral infection with *C. albicans* ($p > 0.05$). However, because of the hypocellularity of CLNs (Fig. 3) and of the secondary lymphoid organs in these Tg mice[34], the absolute numbers of CLN DCs were actually markedly decreased in the Tg compared to the non-Tg mice ($p < 0.05$) (Fig. 3). In contrast to the oral mucosa and CLNs, however, the percentage of peripheral blood DCs was unaffected by expression of the transgene or infection with *C. albicans* throughout AIDS-like disease ($p > 0.05$) (data not shown). As in the oral mucosa, CLN CD4+ T-cells were also sharply depleted in the Tg compared to the non-Tg mice ($p < 0.001$) (Fig. 3). In contrast to the oral mucosa, however, oral infection with *C. albicans* did not change the proportion of CD4+ T-cells in the CLNs of the Tg mice ($p > 0.05$), but resulted in a significant ($p < 0.05$), sustained increase of this cell population in the CLNs of the non-Tg mice (Fig. 3). In addition to being depleted in the Tg mice, the mean fluorescence of these CLN CD4+ T-cells was significantly reduced ($p < 0.001$) to only 60% of values measured in cells from non-Tg mice, throughout AIDS-like disease and irrespective of infection with *C. albicans* ($p > 0.05$) (data not shown). The percentage of CD8+ T-cells was augmented in the CLNs of the infected Tg compared to the non-Tg mice prior to and until 7 days after infection ($p < 0.02$), but was comparable in all four groups of mice during the later period of observation ($p > 0.05$) (Fig. 3). Expressed as absolute numbers, however, CLN CD8+ cells were depleted in the Tg compared to the non-Tg mice ($p < 0.05$), but increased significantly ($p < 0.05$) in both groups of mice 7 days after oral infection with *C. albicans* (Fig. 3). The mean fluorescence of these CLN CD8+ T-cells was also significantly decreased ($p < 0.001$) to 67% of values found in cells from the non-Tg mice, both early and late in the course of AIDS-like disease and independently of infection with *C. albicans* ($p > 0.05$) (data not shown).

In addition to their depletion in the Tg mice, oral mucosal DCs from these animals

displayed a significantly lower ($p < 0.05$) expression of MHC class II (I-A^k) molecules compared to non-Tg mice, thus further compounding the immune defect in these Tg mice (Fig. 3). Surface expression of MHC class II was also sharply decreased on CLN I-A^k+, CD11c+ DCs from the Tg mice ($p < 0.05$). However, MHC class II expression on oral mucosal and CLN dendritic cells from Tg or non-Tg mice was unaltered by oral infection with *C. albicans* ($p > 0.05$) (Fig. 3).

Expression of cytokines by CLN CD4+ T-cells and bone-marrow derived DCs

In order to determine the effect of the HIV transgene on Th1 and Th2 responses, the expression of cytokines by CLN CD4+ T-cells was compared in Tg and non-Tg mice infected or uninfected with *C. albicans.* The percentages of CD4+ T-cells producing the Th1 cytokines IL-2 and TNF-α and the Th2 cytokine IL-10 were all significantly augmented ($p < 0.05$) in the Tg compared to the non-Tg mice throughout AIDS-like disease (Fig. 5A). In addition, expression of IL-10 in the Tg but not the non-Tg mice was further enhanced by oral infection with *C. albicans* ($p < 0.05$), in contrast to the expression of IL-2 and TNF-α which was unaltered by infection in both Tg and non-Tg mice ($p > 0.05$). Percentages of CD4+ T-cells producing the Th1 cytokine IFN-γ were also significantly augmented early ($p = 0.003$) and until day 45 in the Tg compared to the non-Tg mice, but fell precipitously by day 70 to a level significantly lower ($p < 0.001$) than at the earlier time points. In contrast, no such change in expression over time was observed in the non-Tg mice ($p > 0.05$), and oral infection with *C. albicans* did not alter expression of IFN-γ in either the Tg or the non-Tg mice ($p > 0.05$). Finally, the percentages of cells producing the Th2 cytokine IL-4 were comparable in the four groups of mice until day 45 ($p > 0.05$), but rose markedly in the Tg mice infected with *C. albicans* compared to infected or uninfected non-Tg mice ($p = 0.025$) on day 70. Expression of IL-4 in these Tg mice infected with *C. albicans* was significantly greater on day 70 compared to earlier time points ($p < 0.01$). Taken together, these results indicate a partial polarization towards a Th2 response during progression of AIDS-like

disease in these Tg mice orally infected with *C. albicans.* Because IL-12 promotes CD4+ T-cell differentiation into CD4+ Th1 cells[47;48], we next measured the production of IL-12 by bone marrow-derived DCs. In comparison to non-Tg mice, DCs generated from the Tg mice with GM-CSF and IL-4 and pulsed with live *C. albicans* blastoconidia had a reduced capacity to produce IL-12 (Fig. 5B), indicating that defective priming of Th1 cells in the Tg mice may at least partially result from defective production of IL-12 by DCs.

Proliferative response of CLN CD4+ T-cells

CLN CD4+ T-cells harvested from non-Tg mice 7 days after infection proliferated in vitro in response to *C. albicans* antigen and acquired an effector phenotype (CD4+ CD62L-), while the cells from the infected Tg mice failed to proliferate and did not acquire an effector phenotype (CD4+ CD62L+) (Fig. 6A). Although infected and uninfected Tg mice maintained proliferative responses to ConA, these were significantly lower ($p < 0.05$) in the Tg compared to the infected and uninfected non-Tg mice, respectively (Fig. 6A). In addition, oral infection with *C. albicans* significantly increased the response of the non-Tg ($p = 0.004$) but not that of the Tg animals ($p > 0.05$) to ConA. Accordingly, the proliferative response to ConA was diminished in the Tg compared to the non-Tg mice independently of infection with *C. albicans.*

In vitro activation of CLN CD4+ T-cells by Candida-pulsed DCs

When cocultured with unpulsed DCs from Tg or non-Tg mice, CLN CD4+ T-cells isolated from infected or uninfected Tg mice had a consistently reduced ability to proliferate compared to CD4+ T-cells from non-Tg mice ($p < 0.01$) (Fig. 6B). The reduced proliferation of CD4+ T-cells from the Tg mice was not significantly altered ($p > 0.05$) by infection with *C. albicans* and was not rescued by co-culture with unpulsed DCs from non-Tg mice ($p > 0.05$). Furthermore, *Candida*-pulsed DCs from Tg mice were uniformly defective in their

ability to activate CD4+ T-cells from infected or uninfected Tg and non-Tg mice ($p < 0.001$) (Fig. 6B). Therefore, defective DCs and CD4+ T-cells from the Tg mice independently contributed to the impaired proliferation of CD4+ T-cells in vitro, which was further demonstrated by a sharply reduced production of IL-2 in coculture supernatants (Fig. 6B). Nevertheless, infection with *C. albicans* significantly enhanced the proliferation of CD4+ T-cells on coculture with *Candida*-pulsed DCs ($p < 0.001$), independently of the source of CD4+ T-cells and DCs from Tg or non-Tg mice, and hastened peak IL-2 production from 120h to 24h of coculture (Fig. 6B).

Phagocytosis of C. albicans by DCs

Phagocytosis of *C. albicans* yeast cells by bone marrow-derived DCs from Tg and non-Tg mice was compared to determine whether defective uptake of the fungus by DCs from the Tg mice contributes to their perturbed activation of CD4+ T-cells. No significant differences ($p > 0.05$) in percent of DCs with ingested *C. albicans* were found between the Tg and non-Tg mice, measured sequentially over 120 min and at three different DC : blastoconidia ratios (Fig. 6C). Perturbations of DCs which impact on the induction of adaptive immunity to *C. albicans* in the Tg mice are therefore likely located further downstream, after phagocytosis of the fungus.

Memory-like phenotype of CLN CD4+ and CD8+ T-cells

In the absence of in vitro antigenic or mitogenic stimulation, the proportion of naïve CLN CD4+ T-cells ($CD44^{-/lo}$, CD62L+) was significantly decreased ($p < 0.05$) and the proportion of activated CD4+ memory-like T-cells ($CD44^{hi}$, CD62L-) increased ($p < 0.01$) in the Tg compared to the non-Tg mice infected or uninfected with *C. albicans*, both early and late during the course of AIDS-like disease (Fig. 7). However, the proportion of resting CD4+ T-memory-like cells ($CD44^{hi}$, CD62L+) was unaltered in these Tg mice ($p > 0.05$). Oral

infection with *C. albicans* did not change the proportion of naïve, resting memory-like or activated memory-like CD4+ T-cells in the Tg or non-Tg mice ($p > 0.05$). Taken together, these results demonstrate a relative decrease of naïve CD4+ T-cells but an increased proportion of CD4+ T-cells with an activated/memory-like phenotype in these Tg mice, both unaffected by oral infection with *C. albicans*. When calculated as absolute numbers, however, CLN naïve, resting and activated memory cells were all depleted in the Tg compared to the non-Tg mice 70 days after infection with *C. albicans* (Fig. 7). Furthermore, candidal infection significantly enhanced the numbers of naïve but not memory-like CLN CD4+ T-cells in both Tg and non-Tg mice both early and late in AIDS-like disease (Fig. 7). Therefore, despite their enhanced proportion, CLN CD4+ T-cells with an activated/ memory-like phenotype were depleted due to the hypocellularity of secondary lymphoid organs in these Tg mice. In contrast to the CD4+ T-cells, the proportions of CLN naïve and memory-like CD8+ T-cells were comparable in the Tg and non-Tg mice infected or uninfected with *C. albicans*, both early and at the later stage of AIDS-like disease ($p > 0.05$) (Fig. 7). However, as for CD4+ T-cells, absolute numbers of naïve and memory-like CLN CD8+ T-cells were sharply decreased in the Tg compared to the non-Tg mice at early and late times in AIDS-like disease, but were significantly enhanced by *C. albicans* infection only in the non-Tg mice (Fig. 7). Altogether, these findings indicate a broad depletion of both naïve and memory-like CD4+ and CD8+ T-cells in these Tg mice.

Adoptive transfer of DCs and CD4+ T-cells

Naïve CD4+ T-cells and DCs isolated from non-Tg mice were transferred either alone or combined into CD4C/HIV^{MutA} Tg mice with persistent oral carriage of *C. albicans*. Adoptive transfer of CD4+ T-cells alone produced a striking reduction of oral burdens of *C. albicans* to levels one-fifth to one-half of those in sham-transferred Tg mice ($p < 0.02$), and this diminution was sustained over 8 days following transfer (Fig. 8A). In contrast, oral burdens of *C. albicans* were not significantly altered ($p > 0.05$) over four weeks following

transfer of DCs alone into these Tg mice (Fig. 8B). Consistent with these findings, adoptive transfer of DCs did not significantly change ($p > 0.05$) oral burdens of *C. albicans* in these Tg mice prior to transfer of CD4+ T-cells six days later (Fig. 8C), but the latter transfer was followed by a sharp and sustained reduction of the oral fungal burdens in comparison to sham-transferred Tg mice ($p < 0.001$) (Fig. 8C), comparable to that observed after transfer of CD4+ T-cells alone (Fig. 8A). Finally, $8.4 \pm 0.3\%$ of CLN CD4+ T-cells proliferated in response to *C. albicans* antigen 13 days after transfer of CD4+ T-cells into these Tg mice with persistent oral candidiasis, demonstrating a substantial restoration of adaptive immunity to *C. albicans* in comparison to infected non-Tg mice (Fig. 6A).

Discussion

The results of this study demonstrate that (i) expression of the HIV-1 transgene in both DCs and CD4+ T-cells is required and sufficient to produce the phenotype of persistent oral and gastrointestinal carriage of *C. albicans* in HIV^{Mut} Tg mice; (ii) quantitative depletion and functional impairements of both of these cell populations prevent the priming of a protective T-cell-mediated adaptive immune response to *C. albicans*; and (iii) adoptive transfer of CD4+ T-cells into these Tg mice reconstitutes protective immunity against chronic oral carriage of *C. albicans*. Perturbations of DCs and CD4+ T-cells are therefore central to the immunopathogenesis of persistent oral candidiasis in these HIV-1 Tg mice.

HIV-1 transgene expression in DCs and CD4+ T-cells is required and sufficient for persistent oral carriage of C. albicans

Chronic oral carriage of *C. albicans* required HIV-1 transgene expression in both DCs and CD4+ T-cells, but added expression in macrophages was found to be dispensable to produce this phenotype. The observation that transgene expression in CD4+ T-cells alone did not result in persistent oral carriage suggested that defective DCs contribute to the perturbed mucosal immune response to *C. albicans* in these Tg mice, and that defects of CD4+ T-cells, if present in the Tg mice, can be overridden by normal DCs to prime protective immunity to *C. albicans*. This interpretation was supported by the preserved in vitro activation of CLN CD4+ T-cells isolated from infected Tg mice, by *Candida*-pulsed DCs from non-Tg mice, in contrast to defective activation when these same CD4+ T-cells were cocultured with DCs from Tg mice (see below). The lack of a requirement to express the HIV-1 transgene in macrophages in addition to DCs and CD4+ T-cells in order to obtain the phenotype of persistent oral carriage could indicate either that macrophages are not affected by expression of the HIV-1 transgene, play a limited role in host defense

against oral candidiasis, or, as is more likely, are indirectly perturbed by transgene expression in DCs and CD4+ T-cells and therefore do not require targeted transgene expression.

DCs and CD4+ T-cells from oral mucosa and CLNs of CD4C/HIV^{MutA} Tg mice with persistent candidiasis are quantitatively and functionally impaired

Expression of the HIV-1 transgene resulted in a profound depletion of an oral mucosal and CLN DC population with a CD11b^{lo}, I-A^{k}+, CD11c+ mature phenotype, and downregulated DC MHC class II expression. These findings demonstrate an impaired maturation of DCs at the site of primary uptake of *Candida* antigens in the oral mucosa, consistent with the previously observed defective maturation of lymph node DCs from these Tg mice.[40] Defective maturation of DCs in the Tg mice could have resulted from a direct effect of HIV-1 gene products, most probably nef, or from indirect effects on DCs from an impaired T-cell environment.[40] The nef gene has been reported to down-modulate surface expression of MHC class II antigens[49;50], to induce intracellular accumulation of MHC class II-peptide complexes[51], and to uncouple cytokine and chemokine production from membrane phenotype maturation in DCs.[52] In other studies, however, HIV-1 nef either did not impair the function of human DCs[53], or triggered phenotypic and functional differentiation of immature DCs including up-regulation of expression of MHC II, CD40, CD80 and CD86[54], by targeting the Vav/Rac signalling pathway leading to cytoskeleton rearrangement and NF-κB activation.[55-57] Despite these apparently discordant effects of nef on DC maturation, impairements of DCs observed in the Tg mice are strikingly similar to those reported in human HIV-infection. Immature peripheral blood DCs have been reported in HIV-infected patients[58], and impairment of terminal differentiation of oral Langerhans' cells was demonstrated in human HIV-infection by decreased expression of MHC class II antigens[27;28], as well as the presence of blunt dendrites, limited development of organelles and lack of Birbeck granules.[28] Furthermore, numbers of both oral[26;59] and

esophageal[60] Langerhans' cells are depleted in HIV-infection, congruent with decreased numbers of cervical[61], splenic[62] and blood[63-68] DCs. Depletion of these DC populations in humans may result from cytopathic changes caused by productive HIV-infection, cytotoxic T-cell responses resulting in lysis of targeted DCs, migration to lymph nodes where active viral replication occurs, or down-regulation of DC surface markers.[26;64]

Tg expression did not alter the ingestion of *C. albicans* yeast cells by bone marrow-derived DCs, a critical first step required to activate DCs for IL-12 production needed to prime Th1 cells and acquired protective immunity.[41] However, the CD4C/HIV^{MutA} transgene caused cellular defects further downstream, by downregulating MHC class II expression and by reducing the percentage of bone marrow-derived DCs expressing IL-12. The maturation of bone marrow-derived DCs from CD4C/HIV^{MutA} Tg mice is impaired[40], consistent with this defective production of IL-12.[69] Of added importance, the CD11b^{lo}, CD11c+ DC population which was found to be depleted in these Tg mice is known to induce high concentrations of the Th1 cytokines IFN-γ and IL-2 but little or no Th2 cytokines, while the preserved CD11b^{hi}, CD11c+ population induces large amounts of the Th2 cytokines IL-4 and IL-10, in addition to IFN-γ and IL-2.[69] Because both human[70] and murine[41] DCs play a critical role in the initiation of an adaptive cell-mediated immune response to *C. albicans* (reviewed in[21]) by their capacity to recognize, phagocytose and degrade *Candida*, and subsequently present *Candida* antigens to T-cells, these defects of DCs in the Tg mice likely perturb presentation of *Candida* antigens and reduce the differentiation of CD4+ T-cells into a protective Th1 phenotype. Expression of the HIV env gene product gp120 in antigen-presenting cells (APCs) and impaired CD40L induction on CD4+ T-cells activated by antigen have been shown to contribute to impaired IL-12 and IFN-γ production by APCs, thereby preventing a protective Th1 response.[71;72] Interestingly, the CD4C/HIV^{MutA} Tg mice express env, rev and nef of HIV-1, and CD4+ T-cells from these mice express low levels of CD40L.[73]

HIV transgene expression also caused a profound and persistent loss of CD4+ T-cells in the oral mucosa, CLNs and peripheral blood of these Tg mice, strikingly similar to CD4+ T-cell depletion in human HIV disease (reviewed in[74;75]). The expression of nef in CD4C/ HIV^{MutA} and CD4C/HIV^{MutG} mice has been shown to downregulate the CD4 cell surface molecule, enhance apoptosis of peripheral CD4+ T-cells, and induce chronic activation of CD4+ T-cells which also contributes to the low number of CD4+ T-cells observed in these Tg mice.[76] Although vpu, env and nef are independently capable of down-modulation of CD4[77], there is a stronger dependence on nef for reducing cell surface CD4 molecules[77-85] by their rapid endocytosis and lysosomal degradation.[86-88] In addition, nef contributes to apoptosis of CD4+ T-cells in HIV-infection[89-92] by upregulating the Fas/FasL pathway[93-95] or independently from the Fas pathway.[96;97] Finally, the HIV nef gene was found to activate T-cells indirectly by its expression in macrophages[98], or directly by up-regulating kinase activity, transcription factor NF-κB and IL-2 production[99-104], although other studies have provided conflicting results.[105-109] Consistent with nef expression in CD4+ T-cells of CD4C/ HIV^{MutA} Tg mice[34], the proportion of CLN CD4+ T-cells with a naïve (CD44-/^{lo}, CD62L+) phenotype was decreased and the proportion with an activated/memory-like phenotype (CD44^{hi}, CD62L-) was increased in the Tg compared to the non-Tg mice, indicating persistent immune activation that may have contributed to exhaustion of the naïve T-cell pool.[74-76] Moreover, infection with *C. albicans* did not independently alter the proportion of activated/memory-like CD4+ T-cells in these Tg mice, congruent with the observation that upregulation of activation occurs independently of stimulation by antigens through the TCR.[76] These mechanisms of T-cell depletion mediated by nef expression in the Tg mice are strikingly similar to those elucidated in human HIV-infection, in which chronic immune activation of T-cells increases the fraction of these cells prone to apoptosis and leads to accelerated depletion of the T-cell pool.[75]

In addition to being severely depleted, CLN CD4+ T-cells also failed to increase in response to *C. albicans* infection in the Tg mice, although this capacity was maintained in

the oral mucosa. These CLN CD4+ T-cells may have failed to proliferate in response to *C. albicans* antigens, congruent with their defective proliferation in vitro (see below), or to migrate as a result of nef expression which blunts the T-cell response to chemokines.[110]

As predicted by the defective production of IL-12 by immature DCs from the CD4C/HIV^{MutA} Tg mice, chronic oral carriage of *C. albicans* in these animals failed to induce a protective anticandidal Th1 cytokine response but instead led to a polarization towards development of a nonprotective Th2 response by CLN CD4+ T-cells. This functional impairment likely resulted not only from the reduced production of IL-12 by DCs from the Tg mice, but also from impaired IL-12 responsiveness due to a loss of IFN-γ production which is required to override the IL-4-induced inhibition of IL-12 receptor 2 expression.[111] IFN-γ has been shown to be required for IL-12 responsiveness in mice with *C. albicans* infection[112], but production of this cytokine by CD4+ T-cells was sharply decreased in the Tg mice late in the course of AIDS-like disease. The enhanced production of IL-4 and IL-10 found in these Tg mice is closely associated with a nonprotective immune response to *C. albicans.*[113] HIV-1 nef directly induces the expression of IL-10[114] and TNF-α[115] but not IL-4[116] and may thus have modulated cytokine expression by CD4+ T-cells in these Tg mice. HIV-infected patients have a Th2 cytokine profile in saliva[17] consistent with the well-documented switch from Th1 to Th2 in HIV infection.[117]

Impairments of DCs and CD4+ T-cells of CD4C/HIV^{MutA} Tg mice cause defective priming of an adaptive immune response to C. albicans in vivo and in vitro

Proliferation of CLN CD4+ T-cells from infected Tg mice in response to stimulation with *C. albicans* antigen was almost completely abrogated and these cells also failed to acquire the effector phenotype observed in the infected non-Tg mice, indicating that infected CD4C/HIV^{MutA} Tg mice are anergic to *C. albicans.* A more modest but significant decrease in the proliferative response of CD4+ T-cells to the mitogen ConA was also found in these

Tg mice, as previously observed with T-cells from other Tg mice expressing nef.[85] These perturbed proliferative responses were correlated with reduced absolute numbers of CLN CD4+ memory T-cells in the Tg mice, 70 days after infection with *C. albicans*. Coculture of *C. albicans*-pulsed DCs and CD4+ T-cells from Tg and non-Tg mice in vitro showed that expression of the HIV-1 transgene in either or both of these cell populations sharply reduced the proliferation of CD4+ T-cells and their production of IL-2, demonstrating that both cell populations are functionally defective in priming an immune response to *C. albicans* in the Tg mice, even with adjustment of the numbers of cells to equal those in the non-Tg mice. Finally, defective proliferation of CD4+ T-cells from the Tg mice did not result from reduced phagocytosis of *C. albicans* yeasts upon pulsing of DCs, since uptake of the yeasts by DCs was equivalent in Tg and non-Tg mice.

DCs from the CD4C/HIV^{MutA} Tg mice appear to process antigens normally, but show low expression levels of MHC class II and of the costimulatory molecules CD40 and CD86, resulting in impaired delivery of costimulatory signals and of the capacity to present pigeon cytochrome C to AD10 TcR CD4+ T-cells.[40] However, MHC class II[118-120] and costimulatory molecules[120] are both directly involved in *Candida*-specific T-cell activation by APCs. Accordingly, loss of MHC class II and costimulatory molecule expression on DCs from the Tg mice are likely to cause impaired presentation of *C. albicans* antigens and to account at least in part for the impaired proliferation of CD4+ T-cells. In human HIV-infection, expression of MHC class II[121] and costimulatory molecules CD80 and CD86[122], formation of MHC class II-antigen complexes[121], and antigen presentation capacities of APCs and their ability to stimulate CD4+ T-cell proliferation are all impaired[123;124], as observed in these Tg mice.

CD4+ T-cells from the Tg mice had an impaired proliferative response and production of IL-2 in comparison to cells from non-Tg mice, upon coculture with pulsed or unpulsed DCs from non-Tg mice. In addition, proliferation of CD4+ T-cells from the Tg mice was

also reduced in response to the mitogen ConA, which stimulates proliferation of T-cells directly and therefore does not require DCs.[125;126] Taken together, these results indicate that the reduced proliferative response of CD4+ T-cells from CD4C/HIV^{MutA} Tg mice to *C. albicans* involves defects not only of DCs but also of the CD4+ T-cells themselves. The present findings concur with the limited capacity of CD4+ T-cells from CD4C/HIV^{MutG} Tg mice, which express only nef, to divide in response to stimulation with anti-CD3 and anti-CD28 or in allogeneic mixed leukocyte reactions.[76] Among its multiple points of interference with CD4+ cellular mechanisms (reviewed in[89]), the nef protein interacts with several signalling molecules and thereby inhibits activation pathways required for cell proliferation. Nef not only interferes with activation of p56^{lck} and as a consequence signalling via the IL-2 receptor[127], but also hinders cell cycle progression by downregulating cyclins D1 and A.[128] The defective production of IL-2 by CD4+ T-cells from the Tg mice may also have contributed the defective proliferation of these cells in vitro, because T-cells require IL-2 to complete the late portion of the G1 phase and enter the S phase of the cell cycle.[129] Interestingly, CD4+ T-cells from the CD4C/HIV^{MutG} Tg mice are able to proliferate after stimulation with PMA plus ionomycin.[76] Because PMA directly activates protein kinase C[130], the alteration of signal transduction in these Tg mice appears to be at the level of or distal to the TCR and upstream of protein kinase C.[76] The perturbed proliferative response of CD4+ T-cells in the Tg mice correlates closely with similar defects in patients with HIV-infection. Proliferative responses to mitogens[131-133], *C. albicans* antigens[5;18;133;134], and stimulation with anti-CD3[133;135] are all impaired in patients infected with HIV. Defective T-cell proliferation after stimulation with anti-CD3 antibody is accompanied by impaired induction of the early activation marker CD69[136], reduced protein tyrosine phosphorylation[137], and defective expression of cyclins A and D.[138] Finally, as was found in these Tg mice, diminished proliferation of CD4+ T-cells in human HIV-infection is associated with diminished IL-2 production.[139]

Adoptive transfer of CD4+ T-cells into CD4C/HIV^{MutA} Tg mice restores protective immunity against C. albicans

Conclusive evidence demonstrating that these defective CD4+ T-cells determine at least in part the susceptibility to chronic oral candidiasis in these Tg mice was revealed by a significant reversal of this phenotype and restoration of proliferation of CD4+ T-cells, following adoptive transfer of naïve non-Tg CD4+ T-cells. Unexpectedly, however, transfer of naïve DCs failed to significantly decrease oral burdens of *C. albicans.*

By analyzing these results together with those obtained in the Tg mouse constructs expressing HIV-1 in defined cell populations, as well as the activation of CLN CD4+ T-cells by *Candida*-pulsed DCs, the relative contributions of DC and CD4+ cell defects in determining the susceptibility to persistent oral candidiasis in these Tg mice can be more fully assessed.

Rapid clearance of oral *C. albicans* in the mCD4C/HIV^{MutG} Tg mice expressing the transgene only in CD4+ T-cells was correlated with the largely preserved proliferative response of Tg CD4+ T-cells cocultured with DCs from non-Tg mice. Therefore, the defects identified in CD4+ T-cells of these Tg mice are insufficient in and of themselves to significantly impair priming of adaptive immunity against *C. albicans.* In contrast, however, expression of the HIV-1 transgene in both DCs and CD4+ T-cells of CD4C/HIV^{MutA}, CD4C/HIV^{MutG} or CD4E/HIV^{MutG} Tg mice resulted in a phenotype of chronic oral candidiasis and a sharply reduced proliferative response on coculture of Tg DCs and Tg CD4+ T-cells, compared to non-Tg DCs and Tg CD4+ T-cells. These in vivo and in vitro findings suggest that defective Tg DCs potentiate the qualitative and quantitative CD4+ defects in these Tg mice beyond a critical threshold, resulting in anergy of in vivo-harvested CLN CD4+ T-cells to *C. albicans* antigen and persistent candidiasis. Adoptive transfer of naïve non-Tg CD4+ T-cells alone into CD4C/HIV^{MutA} Tg mice had the ability to restore proliferation to *C. albicans* antigen and reduce oral burdens of *C. albicans*, suggesting that Tg APCs maintain a partial functional capacity to present antigens, as

supported by the partially preserved proliferative response of non-Tg CD4+ T-cells cocultured with Tg DCs. However, adoptive transfer of naïve non-Tg DCs alone into these CD4C/HIV^{MutA} Tg mice surprisingly did not reduce oral carriage of *C. albicans*, as would have been predicted from the absence of persistent candidiasis in mCD4C/HIV^{MutG} Tg mice which express the transgene in CD4+ T-cells but not DCs. It is unlikely that this lack of protection resulted from an insufficient number of transferred DCs, since an identical number of DCs protected mice against an i.v. challenge of *C. albicans*.[41] Alternately, transferred DCs may not have successfully migrated to the oral mucosa to endocytose *C. albicans*, or failed to navigate towards CLNs for antigen presentation to CD4+ T-cells. Taken together, however, these combined results nevertheless clearly indicate that defects of both DCs and CD4+ T-cells are involved in the onset and maintenance of chronic oral candidiasis in these Tg mice, and suggest that similar defects may underlie the susceptibility to OPC in human HIV-infection. The results further emphasize the critical role of adaptive T-cell-mediated immunity in protection against OPC (reviewed in[21]), as previously shown by significantly decreased *C. albicans* oral colonization after reconstitution of immunodeficient BALB/C and CBA/CaH nu/nu mice with naïve CD4+ T-cells.[8]

CD8+ T-cells are depleted in CD4C/HIV^{MutA} Tg mice but nevertheless increase in response to persistent candidiasis

Absolute numbers of CD8+ T-cells were sharply reduced in the Tg compared to the non-Tg mice throughout AIDS-like disease, in accordance with their decreased absolute numbers[140;141] and shortened half-life[142] in the peripheral blood of HIV-infected patients. Nef induces caspase-8-mediated apoptosis of CD8+ T-cells by upregulating DC expression of TNF-α and FasL.[143] Apoptosis of CD8+ T-cells is also mediated by macrophages through interaction of HIV gp120 with chemokine receptor CXCR4.[140] Accordingly, the depletion of CD8+ T-cells observed in these CD4C/HIV^{MutA} Tg mice may have indirectly resulted from

nef and env expression in DCs, leading to apoptosis of CD8+ T-cells. In addition to this indirect mechanism, soluble gp120 and nef, both expressed in the CD4C/HIV^{MutA} Tg mice[34], downmodulate expression of CD8 molecules on the surface of CD8+ T-cells[144], as was found in these Tg mice. In contrast to the findings with CD4+ T-cells, however, these Tg mice exhibited a more modest proportional decrease of naïve (CD44-/^{lo}, CD62L+) and proportional increase of CD8+ memory-like (CD44^{hi}, CD62L ^{lo}/-) cells[43] that did not achieve statistical significance, indicating that chronic immune activation likely did not contribute to the depletion of CD8+ T-cells. Naïve CD8+ T-cells progressively decrease while memory CD8+ T-cells increase in peripheral blood of HIV-infected adults[141] and children.[145]

Despite their progressive depletion in the Tg mice, CD8+ T-cells maintained the ability to respond to *C. albicans* infection by a quantitative increase in both oral mucosa and CLNs. CD8+ T-cells accumulate in the basal epithelial layer of the oral mucosa in HIV-infected patients with OPC, demonstrating that these cells can be actively recruited to the mucosa in response to candidiasis.[28;146] However, the precise role of CD8+ T-cells in mucosal containment of *C. albicans* in HIV-infection, either by direct growth inhibition of *Candida*, or more likely, by an indirect mechanism, remains to be determined.[146;147] HIV-1 nef downregulates MHC class I expression on human DCs[143;148] and on lymph node DCs of CD4C/HIV^{MutA} Tg mice[40], impairs presentation of antigens by DCs to CD8+ T-cells[148], and affects the functional competence of CD8+ T-cells.[143;149] Furthermore, absolute numbers of CLN memory-like CD8+ T-cells were sharply decreased in the CD4C/HIV^{MutA} Tg mice, independently of infection with *C. albicans*. Accordingly, further work will be needed to fully assess the role of CD8+ T-cells in the immunopathogenesis of oral candidiasis in these Tg mice.

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Table 1 Viable colony-forming units in organs of transgenic (Tg) mice expressing HIV-1 in defined cell populations and orally infected with *C. albicans*

Variable	Mice[a]			
	CD4C/HIV^{MutG}	CD4E/HIV^{MutG}	mCD4C/HIV^{MutG}	Control non-Tg
No. inoculated	6	6	4	8
Age at inoculation, days	76	78	91	78-91
Age at assessment, mean days (range)	293 (194-357)[b]	189[c]	293[d]	215 (189-293)[e]
Organs culture positive for *C. albicans*				
Brain				
No. of mice	2	1	0	0
C. albicans count, mean cfu/g	1.8×10^3	6.9×10^2	NA	NA
Range	1.5×10^3-2.1×10^3	NA	NA	NA
Lungs				
No. of mice	1	1	0	0
C. albicans count, mean cfu/g	4.5×10^3	1.8×10^3	NA	NA
Range	NA	NA	NA	NA
Liver				
No. of mice	2	0	0	0
C. albicans count, mean cfu/g	4.5×10^2	NA	NA	NA
Range	2.7×10^2-6.3×10^2	NA	NA	NA
Kidneys				
No. of mice	2	1	0	0
C. albicans count, mean cfu/g	2.8×10^2	6.9×10^2	NA	NA
Range	1.1×10^2-4.4×10^2	NA	NA	NA
Stomach				
No. of mice	5	4	0	0
C. albicans count, mean cfu/g	1.0×10^5	2.3×10^5	NA	NA
Range	7.9×10^2-4.0×10^5	4.4×10^4-5.3×10^5	NA	NA

Variable	Mice[a]			
	$CD4C/HIV^{MutG}$	$CD4E/HIV^{MutG}$	$mCD4C/HIV^{MutG}$	Control non Tg
Small intestine				
No. of mice	5	6	0	0
C. albicans count, mean cfu/g	2.0×10^{3}	2.0×10^{4}	NA	NA
Range	5.9×10^{1}-6.5×10^{3}	1.2×10^{4}-3.8×10^{4}	NA	NA
Large intestine				
No. of mice	5	4	0	0
C. albicans count, mean cfu/g	3.4×10^{4}	8.6×10^{4}	NA	NA
Range	8.5×10^{2}-1.2×10^{5}	2.8×10^{4}-1.9×10^{5}	NA	NA

NOTE: NA, not applicable
[a] Mice studied included Tg and control non-Tg offspring derived from founder mouse F27367 ($CD4C/HIV^{MutG}$), F104990 ($CD4E/HIV^{MutG}$), and F69457 ($mCD4C/HIV^{MutG}$).
[b] Four mice were non-survivors (assessments, days 194 to 338); two surviving mice were euthanized (assessment, day 357).
[c] All 6 mice survived and were euthanized at 189 days of age.
[d] All 4 mice survived and were euthanized at 293 days of age.
[e] Control non-Tg littermates were euthanized on the same day as Tg mice

Table 2 Cell surface marker analysis of peripheral blood and cervical lymph node lymphocytes from transgenic (Tg) mice expressing HIV-1 in defined cell populations and orally infected with *C. albicans*.

Mice[a]	No. inoculated	Age at assessment, mean days (range)	Cell population (%)			
			Peripheral blood		Cervical lymph nodes	
			CD4+	CD8+	CD4+	CD8+
CD4C/HIV^{MutG}	6	293 (194-357)	1.2 ± 0.10[b,c]	5.5 ± 2.3[c]	10.1 ± 5.3[c]	35.0 ± 8.8[c]
mCD4C/HIV^{MutG}	4	293	9.1 ± 1.3[c]	18.6 ± 2.2[c]	17.7 ± 1.0[c]	38.1 ± 9.1[c]
Control non-Tg	8	347 (293-365)	17.1 ± 3.2	13.5 ± 3.9	43.2 ± 4.4	25.8 ± 2.5
CD4E/HIV^{MutG}	6	189	2.2[d]	2.4	15.3	26.4
Control non-Tg	6	189	20.7	8.2	42.7	19.9

[a] Mice studied included Tg and control non-Tg offspring derived from founder mouse F27367 (CD4C/HIV^{MutG}), F104990 (CD4E/HIV^{MutG}), and F69457 (mCD4C/HIV^{MutG})

[b] Data are mean ± SD from individual mice.

[c] Significantly different from control non-Tg mice ($p < 0.05$, Mann-Whitney U test)

[d] Analysis was conducted on cell populations pooled from 6 Tg or 6 non-Tg mice.

Figure legends

Fig. 1 Multiparametric flow cytometry analysis of oral mucosal cell populations in CD4C/HIV^{MutA} transgenic (Tg) mice infected or uninfected with *Candida albicans*. Acquisition was gated on CD45+ cells from spleen and oral mucosa, and multicolor analysis was conducted to identify and quantitate specific cell populations. Immature and mature dendritic cells were identified as CD11b^{hi}, I-A^{k}+, CD11c+ and CD11b^{lo}, I-A^{k}+, CD11c+, respectively, while CD4+ T-cells were CD3+, CD4+.

Fig. 2 CD4C/HIV^{MutG}(A), CD4E/HIV^{MutG}(B), and mCD4C/HIV^{MutG}(C) transgenic (Tg) mice and non-Tg littermates were inoculated intra-orally with 10^{8} CFU of *C. albicans* LAM-1, and burdens were assessed longitudinally by sampling the oral cavities. After primary infection and throughout the chronic carrier state, oral burdens remained elevated in the CD4C/HIV^{MutG} and CD4E/HIV^{MutG} Tg mice (A and B, colored lines). In striking contrast, *C. albicans* was rapidly cleared from oral cavities and a chronic carrier state failed to be established in mCD4C/ HIV^{MutG} Tg mice (C, colored lines) and in non-Tg littermates (A-C, black lines). There were 6 (A, B) or 4 (C) mice in each group.

Fig. 3 Longitudinal assessment of immunophenotypes of oral mucosal, cervical lymph node and peripheral blood cell populations in CD4C/HIV^{MutA} transgenic (Tg) mice infected or uninfected with *Candida albicans*. The data are presented as the percentage of CD45+ cells or as absolute numbers of cells. In the oral mucosa, percentages of CD11b^{lo}, I-A^{k}+, CD11c+ mature dendritic cells (DCs) and CD4+ T-cells were both strikingly decreased in the Tg compared to the non-Tg mice. In the cervical lymph nodes (CLN), the percentage of I-A^{k}+,

CD11c+ DCs was increased, but absolute numbers of DCs, CD4+ and CD8+ T-cells were dramatically reduced because of CLN hypocellularity in the Tg mice. MHC II expression on mature $CD11b^{lo}$, I-A^k+, CD11c+ and immature $CD11b^{hi}$, I-A^k+, CD11c+ oral mucosal DCs, and on I-A^k+, CD11c+ CLN DCs, was strikingly reduced in the Tg compared to the non-Tg mice. CD4+ and CD8+ cells were depleted in the peripheral blood of the Tg mice. The data represent the mean ± SD of 3 to 9 independent experiments at each time point. *, significantly different ($p < 0.05$) from non-Tg mice; **, significantly different ($p < 0.05$) from uninfected mice.

Fig. 4 Multicolor analysis of oral mucosal dendritic cells (DCs) and CD4+ T-cells in CD4C/HIV^{MutA} transgenic (Tg) mice infected or uninfected with *Candida albicans*. Representative analyses are shown, demonstrating the early striking depletion of mature $CD11b^{lo}$, I-A^k+, CD11c+ DCs in the Tg compared to the non-Tg mice (top, 7 days after infection with *C. albicans*), and the depletion of CD4+ T-cells in these Tg mice (bottom, 45 days after infection with *C. albicans*).

Fig. 5 **(A)** Expression of cytokines by cervical lymph node CD4+ T-cells in CD4C/ HIV^{MutA} transgenic (Tg) and non-Tg mice infected or uninfected with *Candida albicans*. The data represent the mean ± SD of 3 to 5 independent experiments at each time point. *, significantly different ($p < 0.05$) from non-Tg mice; **, significantly different ($p < 0.05$) from uninfected mice.

(B) Percentage of bone marrow-derived dendritic cells (DCs) from CD4C/ HIV^{MutA} transgenic (Tg) and non-Tg mice expressing IL-12. DCs were stimulated with LPS or live *C. albicans* blastoconidia at the indicated ratios. The data represent the mean ± SD of 3 to 8 independent experiments. *, significantly different ($p < 0.01$) from non-Tg mice.

Fig. 6 **(A)** (Top) Reduced proliferative response of cervical lymph node (CLN) CD4+ T-cells to concanavalin A (ConA) and *Candida albicans* antigen (Ag) in CD4C/HIV^{MutA} transgenic (Tg) compared to non-Tg mice 7 days after infection with *C. albicans*. The data represent the mean ± SD of 3 and 4 to 6 independent experiments, respectively. (Bottom). CFSE-labeled CLN CD4+ T-cells from non-Tg mice harvested 7 days after infection proliferated in vitro in response to *C. albicans* Ag and acquired an effector phenotype (CD4+ CD62L-), while cells from the infected Tg mice failed to proliferate and did not acquire an effector phenotype (CD4+ CD62L+). * significantly different ($p < 0.05$) from non-Tg mice; **, significantly different ($p < 0.05$) from uninfected mice.

(B) (Top) Proliferative responses of isolated cervical lymph node CD4+ T-cells from CD4C/HIV^{MutA} transgenic (Tg) or non-Tg mice infected or uninfected with *Candida albicans*, following coculture with bone marrow-derived dendritic cells (DCs) from Tg or non-Tg mice pulsed or unpulsed with live *C. albicans* yeast cells. In comparison to non-Tg mice, DCs and CD4+ T-cells isolated from the Tg mice independently produced an impaired proliferative response to *C. albicans*. (Bottom) IL-2 production in supernatants of CD4+ T-cell/DC cocultures. HIV-1 transgene expression in either or both of these cell populations sharply reduced production of IL-2. Infection with *C. albicans* augmented but did not fully restore production of the cytokine to levels found in the non-Tg mice. The data represent the mean ± SD of 3 to 12 independent experiments. *, significantly different ($p < 0.01$) from the respective non-Tg CD4 control; **, significantly different ($p < 0.001$) from the respective non-Tg DC control; ***, significantly different ($p < 0.001$) from the respective CD4 control from uninfected mice.

(C) Phagocytosis of *C. albicans* blastoconidia by bone marrow-derived dendritic cells (DCs) from CD4/HIV^{MutA} transgenic (Tg) and non-Tg mice.

Analysis was performed at the indicated DC: blastoconidia ratios. The data represent the mean ± SD of 3 independent experiments.

Fig. 7 Longitudinal assessment of naïve and memory-like CD4+ and CD8+ T-cells in the cervical lymph nodes of CD4C/HIV^{MutA} transgenic (Tg) mice infected or uninfected with *Candida albicans*. The data are presented as the percentage or as the absolute numbers of cells, and represent the mean ± SD of 3 independent experiments at each time point. *, significantly different ($p < 0.05$) from non-Tg mice; **, significantly different ($p < 0.05$) from uninfected mice; ***, significantly different ($p < 0.05$) from the 3 other groups of mice.

Fig. 8 Adoptive transfer of naïve CD4+ T-cells and dendritic cells (DCs) isolated from non-transgenic (Tg) mice, to CD4C/HIV^{MutA} Tg mice orally infected with *C. albicans*. Chronic oral carriage of *C. albicans* was sharply reduced following transfer of CD4+ T-cells alone or with DCs. The data represent the mean ± SD of 4 to 9 mice. *, significantly different ($p < 0.05$) from controls.

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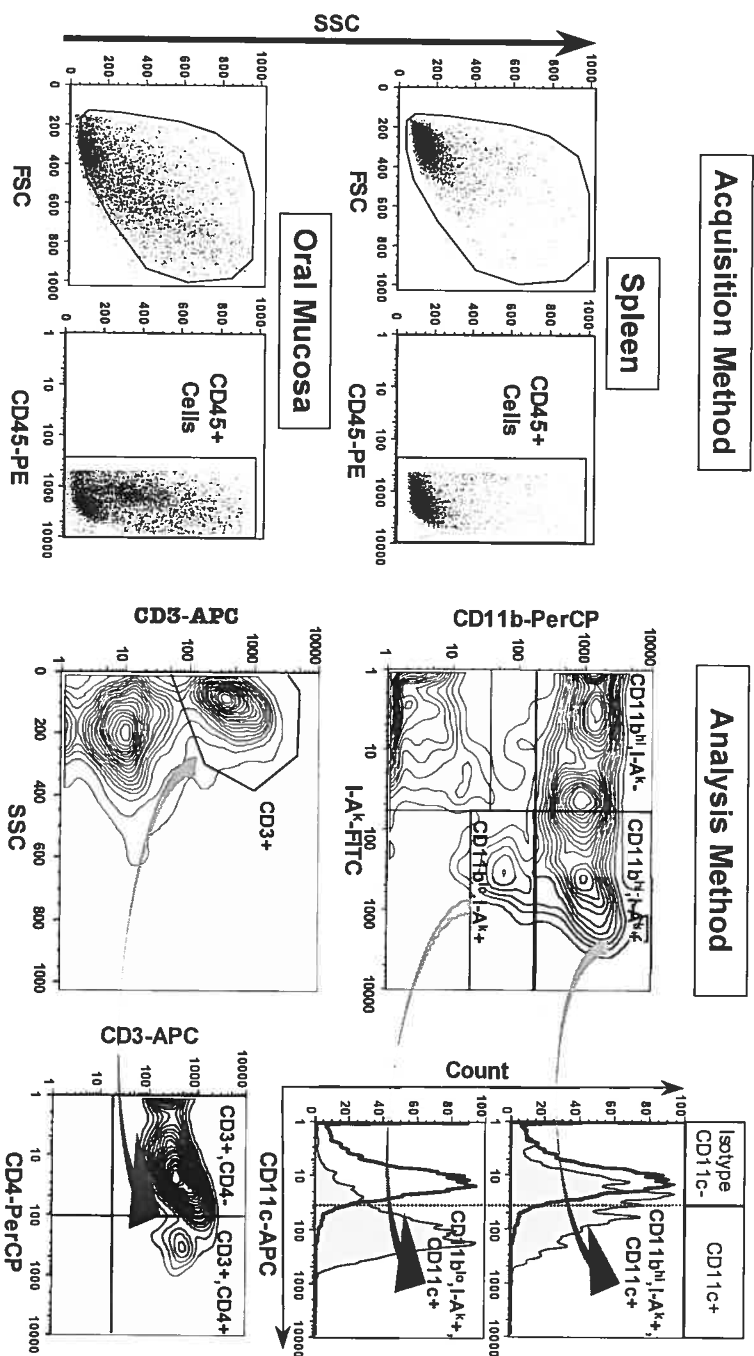


Figure 1

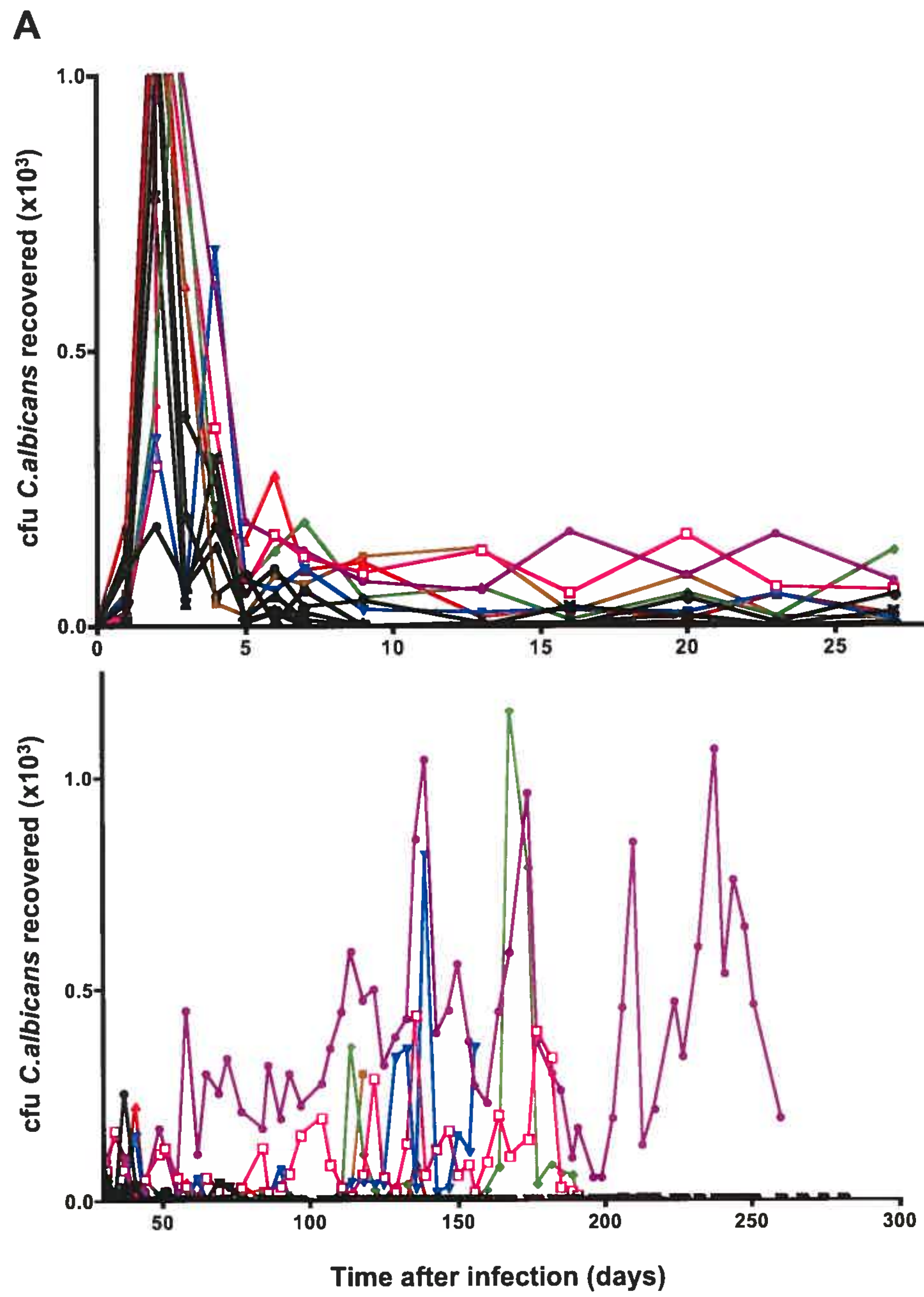
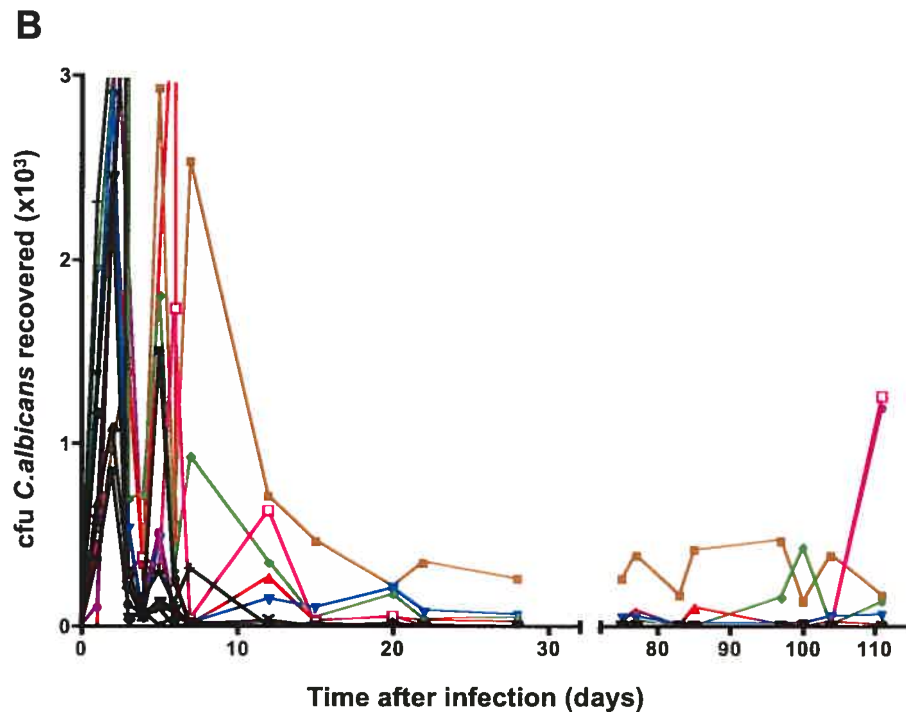
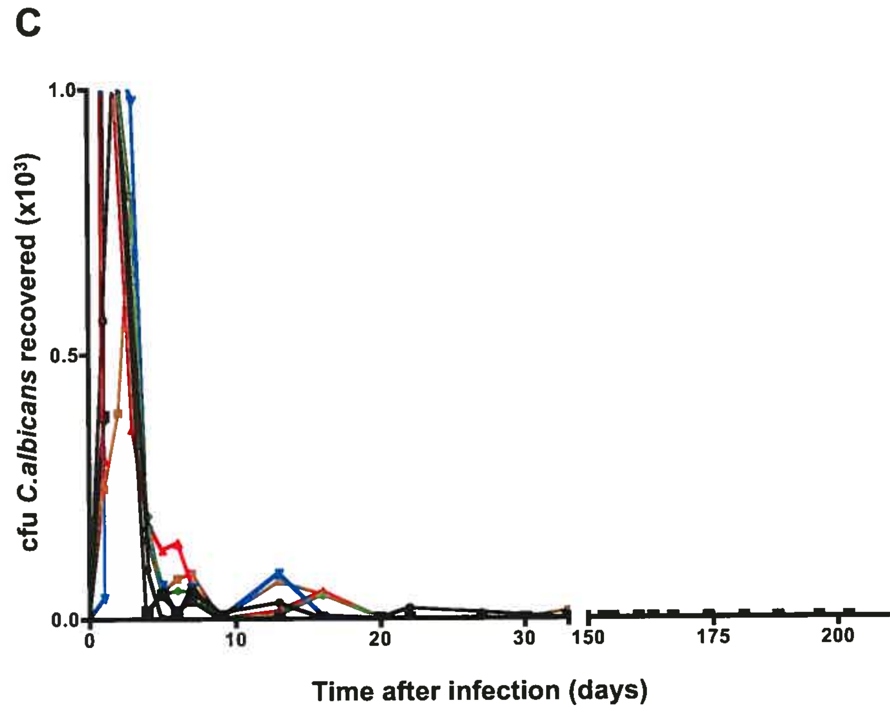


Figure 2


B
cfu C.albicans recovered (x10³)
3
2
1
0
0
10
20
30
80
90
100
110
Time after infection (days)


C
1.0
0.5
0.0
cfu C.albicans recovered (x10³)
0
10
20
30
150
175
200
Time after infection (days)

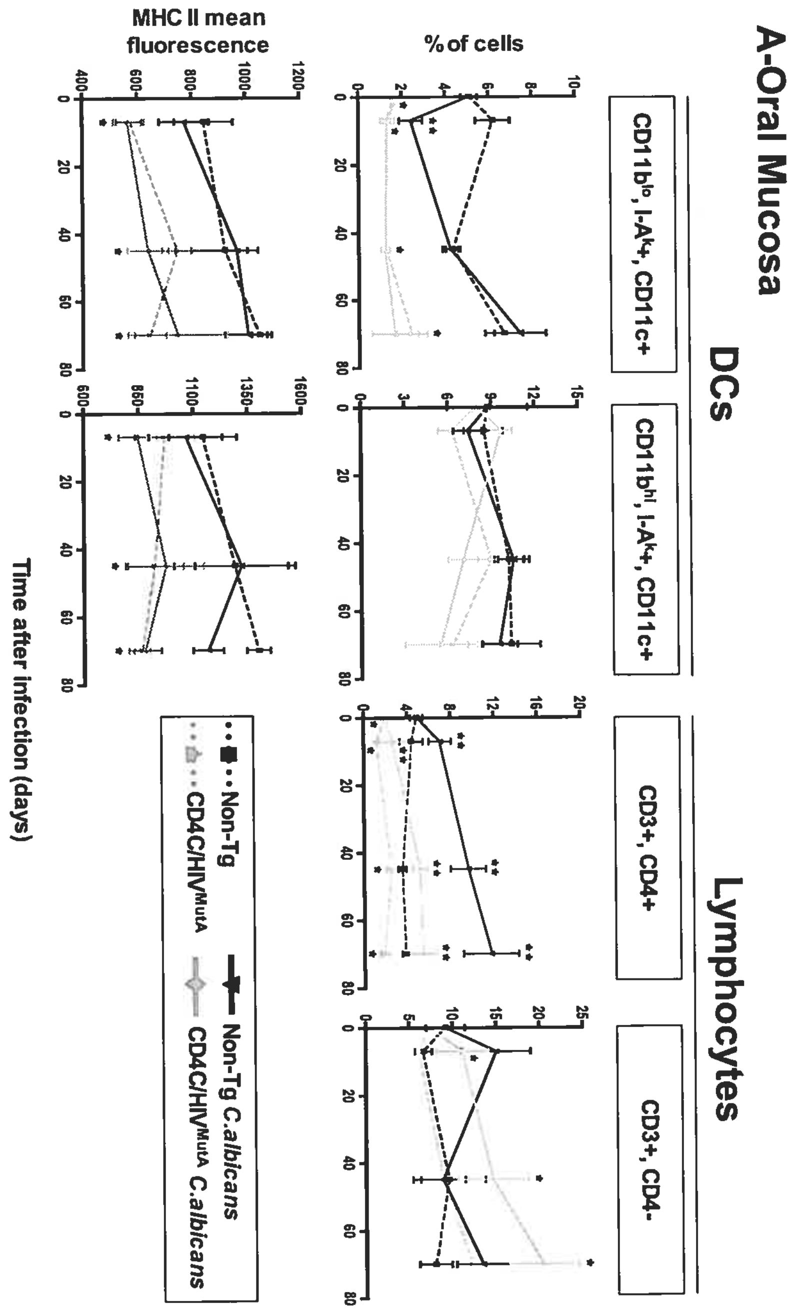
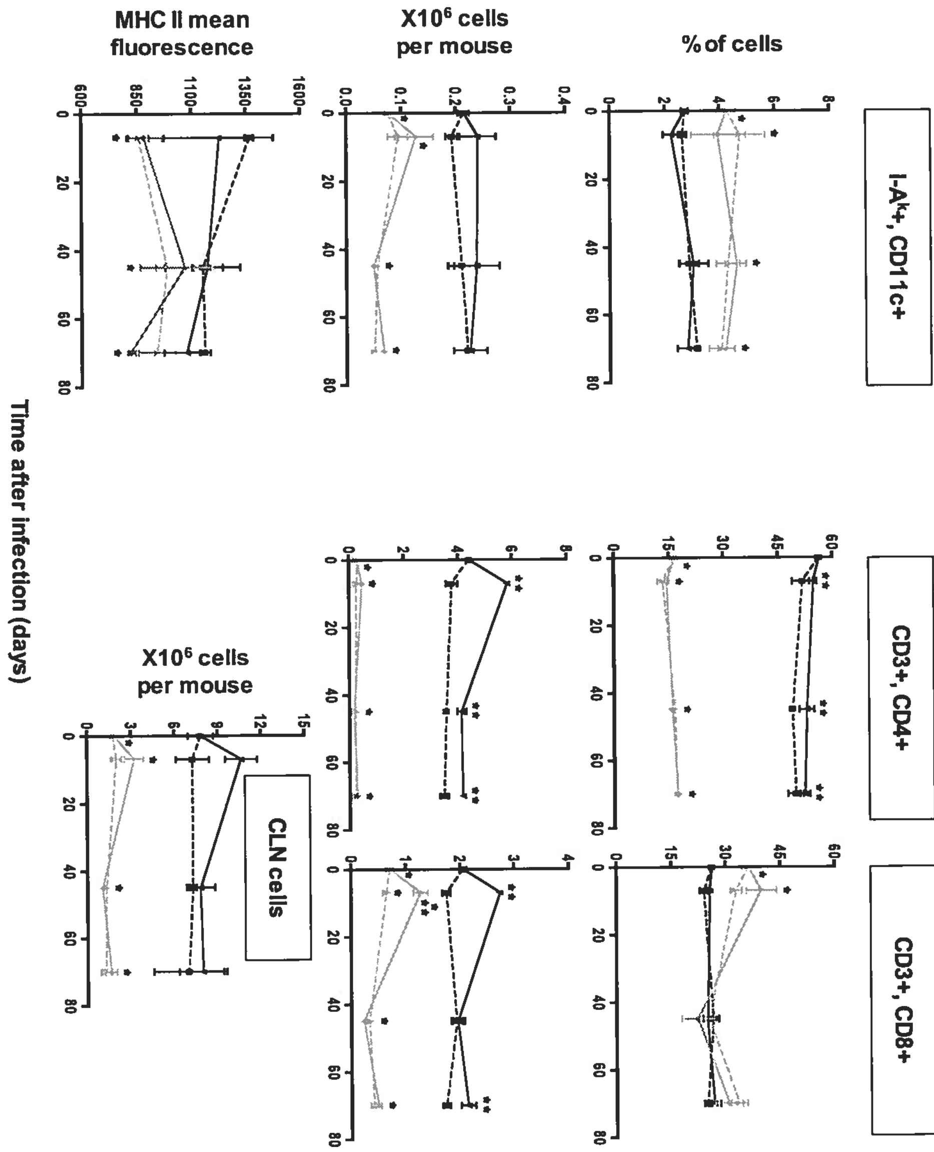
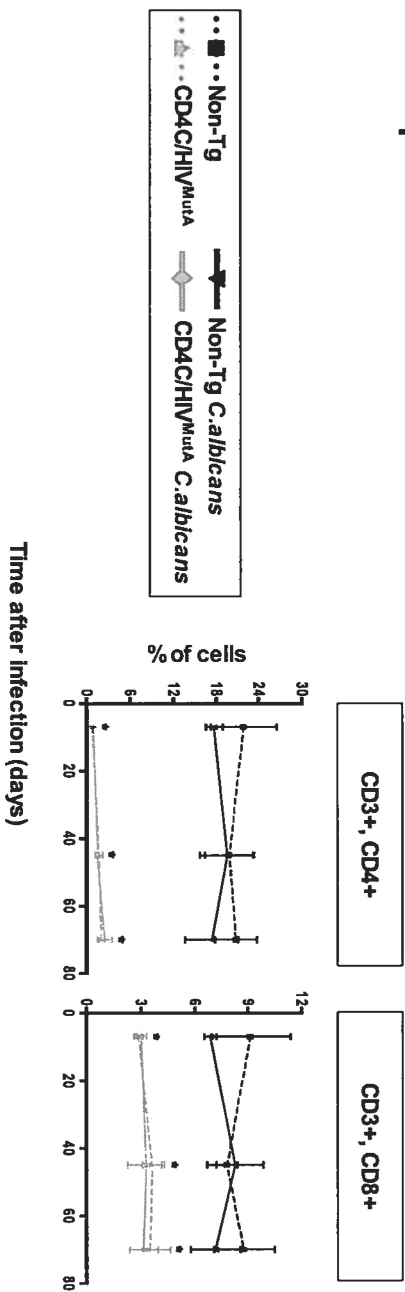


Figure 3


B-CLN
I-A^k+, CD11c+
CD3+, CD4+
CD3+, CD8+
% of cells
X10^6 cells per mouse
MHC II mean fluorescence
CLN cells
Time after infection (days)



C-Peripheral Blood
Non-Tg
Non-Tg C.albicans
CD4C/HIV^{MutA}
CD4C/HIV^{MutA} C.albicans
CD3+, CD4+
CD3+, CD8+
% of cells
Time after infection (days)
0
6
12
18
24
30
3
9
20
40
60
80

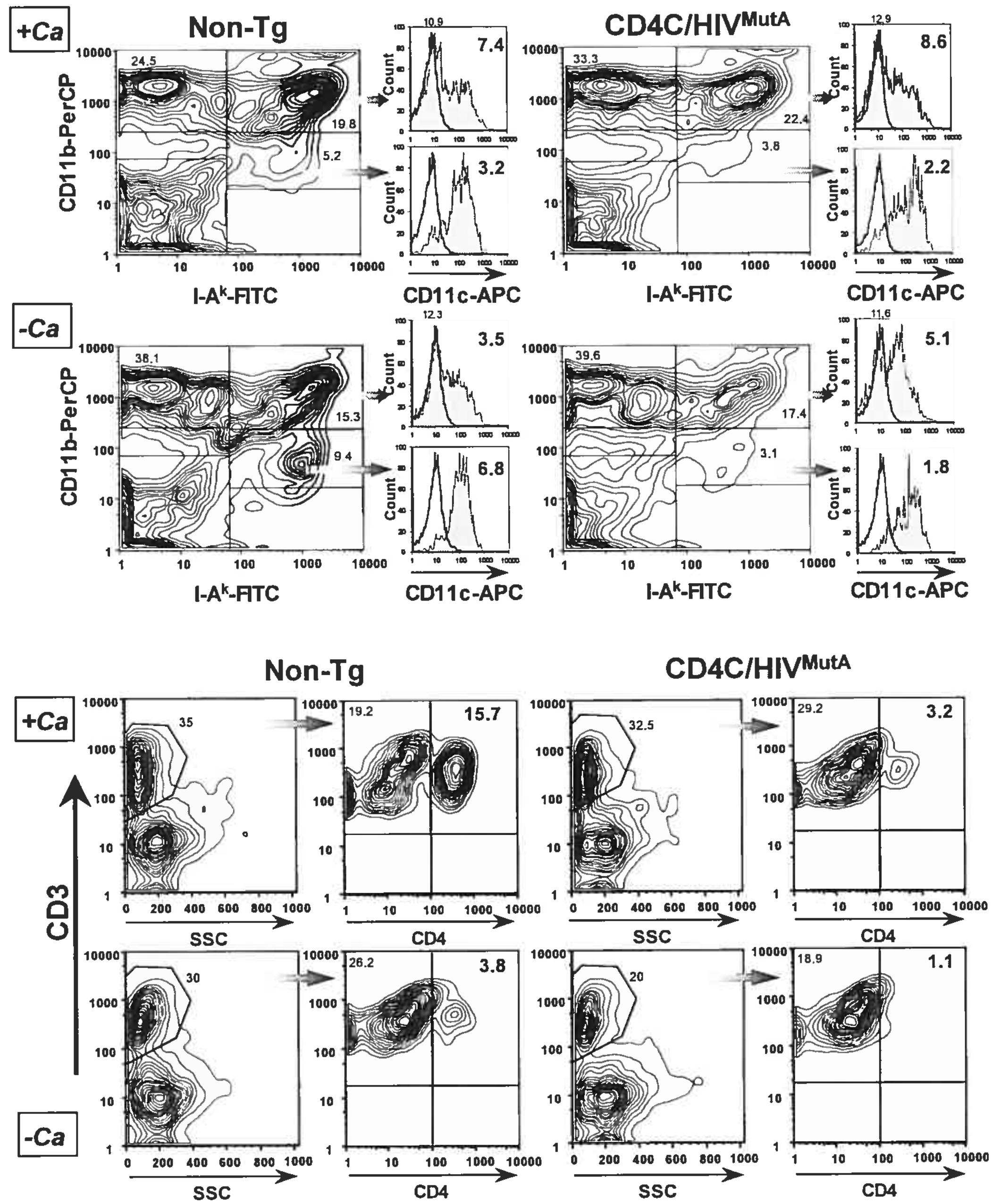


Figure 4

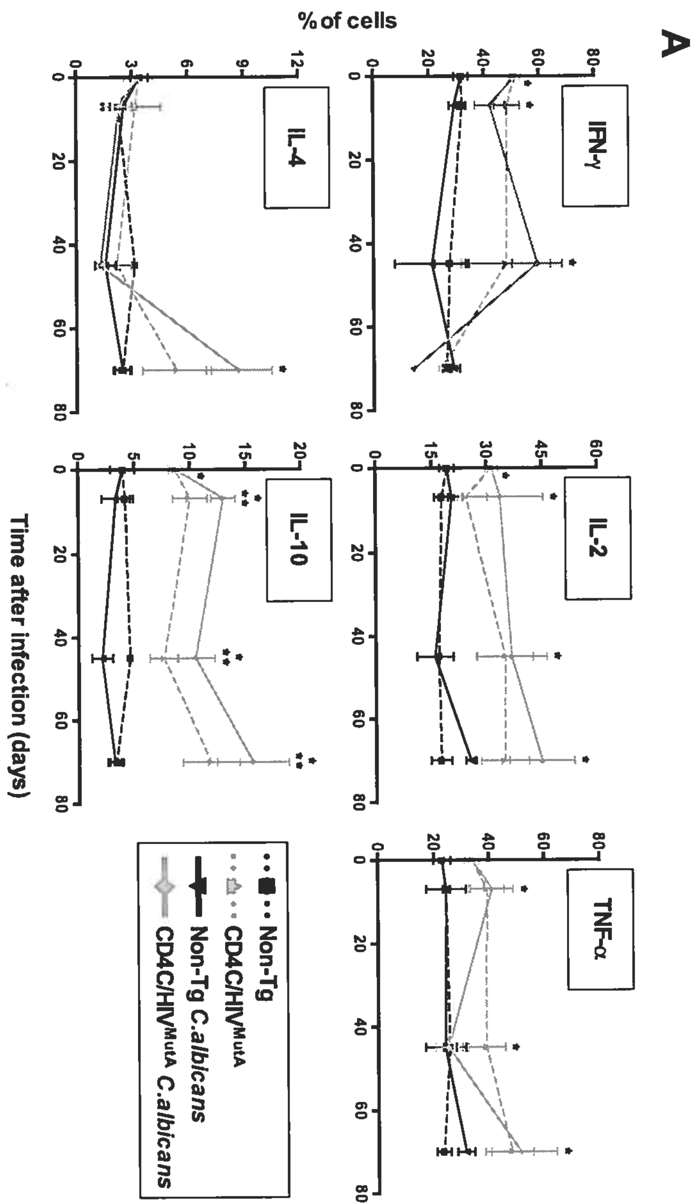
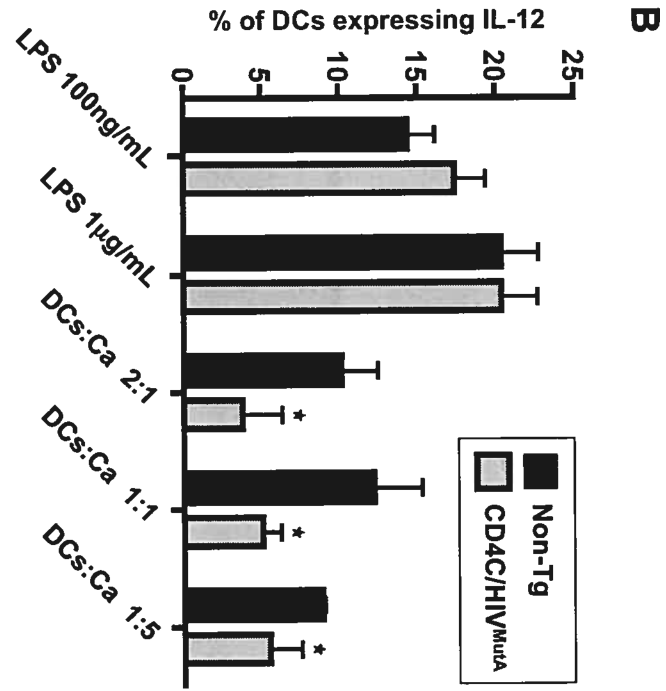


Figure 5


B
% of DCs expressing IL-12
0
5
10
15
20
25
LPS 100ng/mL
LPS 1µg/mL
DCs:Ca 2:1
DCs:Ca 1:1
DCs:Ca 1:5
Non-Tg
CD4C/HIVMutA
*
*
*

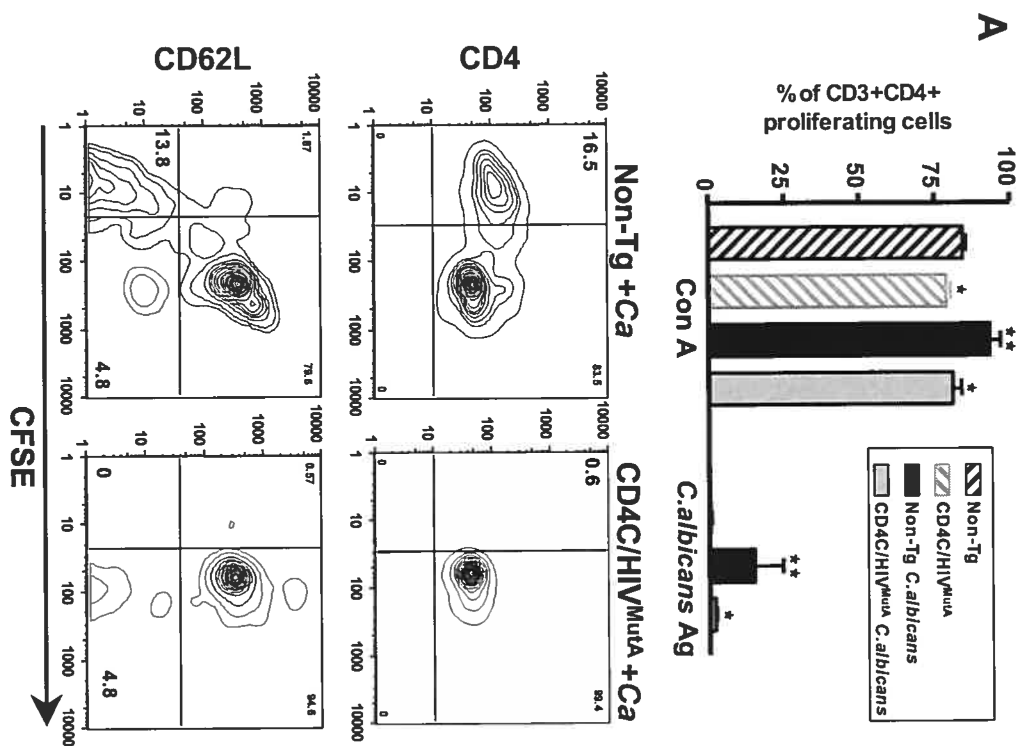
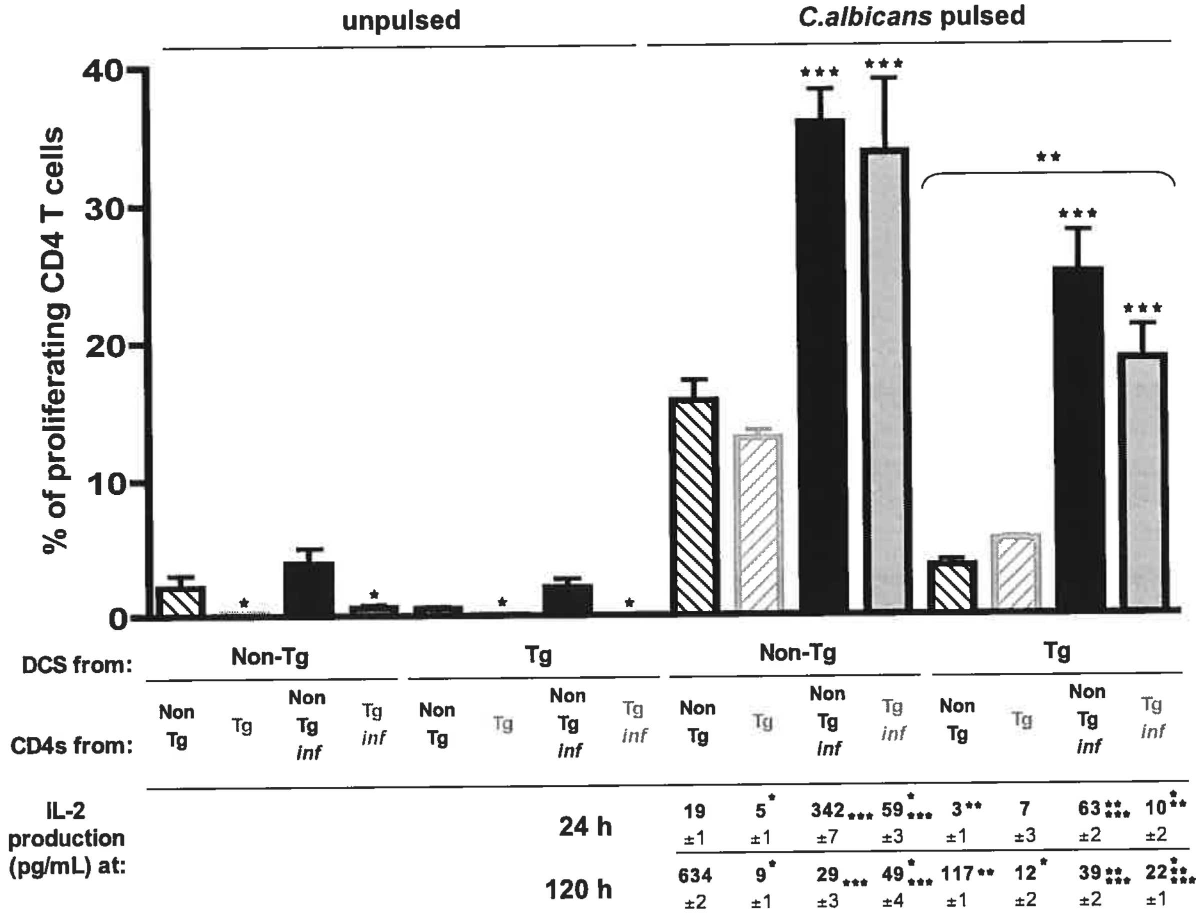
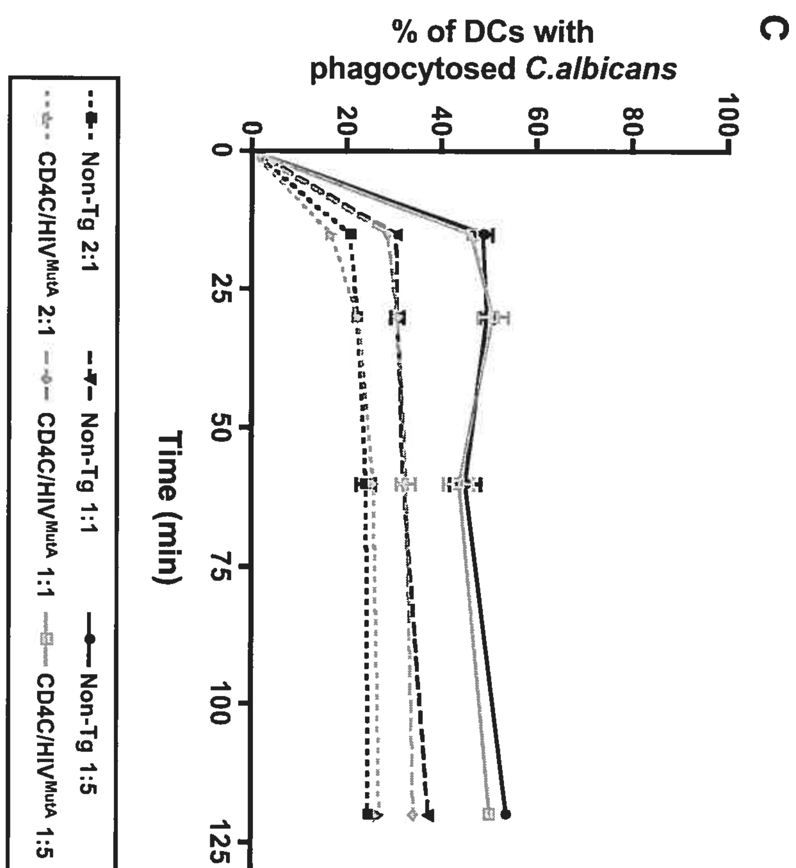


Figure 6


B
DCs-CD4 coculture
unpulsed
C.albicans pulsed
% of proliferating CD4 T cells
0
10
20
30
40
DCS from:
Non-Tg
Tg
Non-Tg
Tg
CD4s from:
Non Tg
Tg
Non Tg inf
Tg inf
IL-2 production (pg/mL) at:
24 h
120 h
19 ±1
5 ±1
342 ±7
59 ±3
3 ±1
7 ±3
63 ±2
10 ±2
634 ±2
9 ±1
29 ±3
49 ±4
117 ±1
12 ±2
39 ±2
22 ±1


C
% of DCs with phagocytosed C.albicans
0
20
40
60
80
100
0
25
50
75
100
125
Time (min)
Non-Tg 2:1
Non-Tg 1:1
Non-Tg 1:5
CD4C/HIV^{MutA} 2:1
CD4C/HIV^{MutA} 1:1
CD4C/HIV^{MutA} 1:5

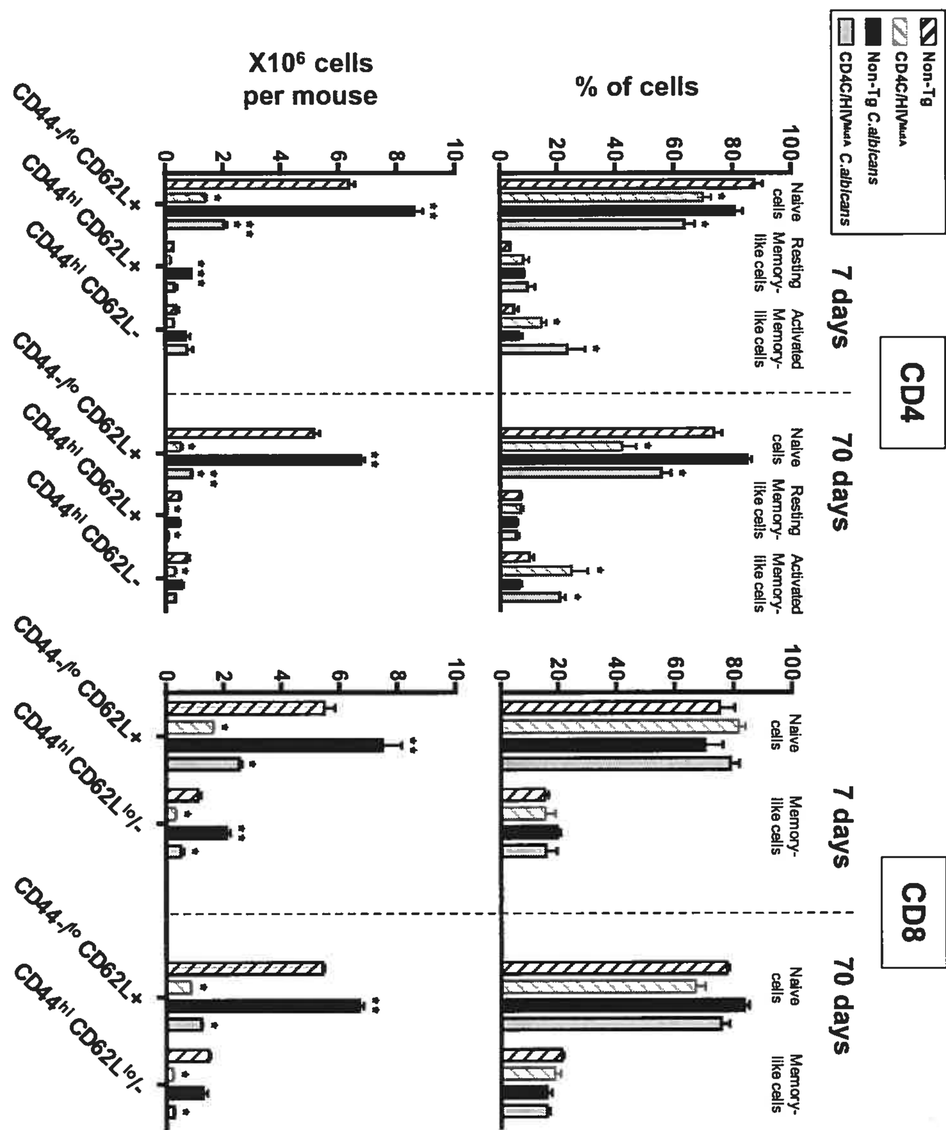
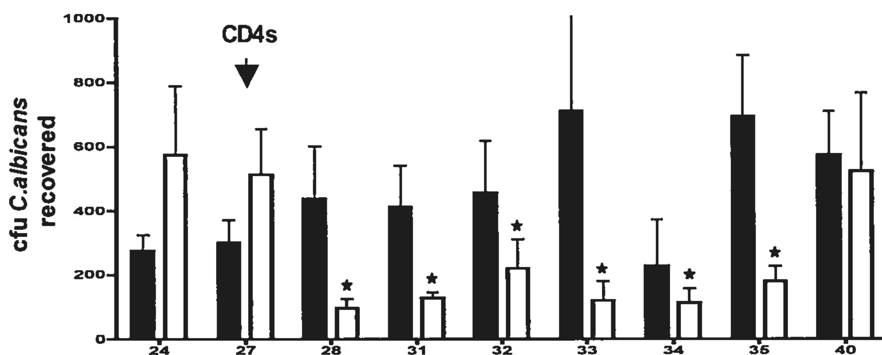
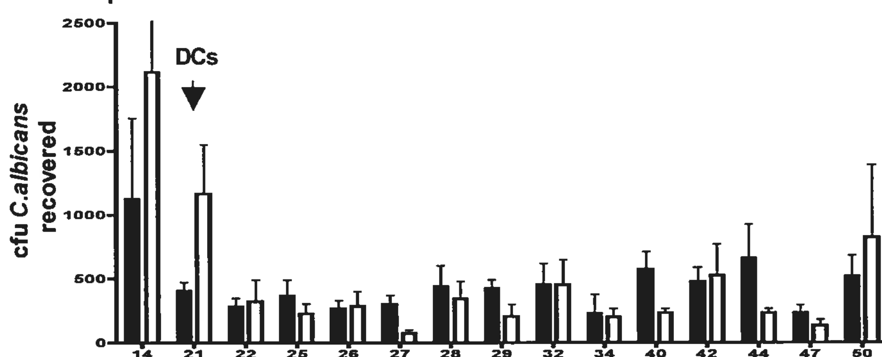


Figure 7

A-CD4+ T cell adoptive transfer



B-DCs adoptive transfer



C-DCs-CD4+ T cell adoptive transfer

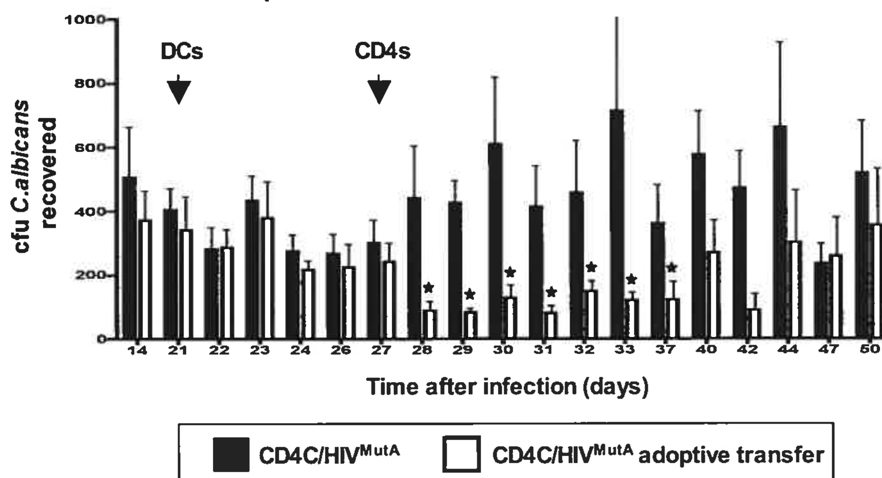


Figure 8

Article 2

CD8+ T-cells but not polymorphonuclear leukocytes are required to limit chronic oral carriage of *Candida albicans* in transgenic mice expressing human immunodeficiency virus type 1

article soumis au *Infection and Immunity*

Contributions

Miriam Marquis: Table 1 et 2, Figure 1, 2, 4, 5

Daniel Lewandowski: Table 3, Figure 3, participation à la table 1 et 2

Véronique Dugas: participation à la Figure 3

Francine Aumont: maintien de la colonie de souris, participation à la table 1 et 2

Résumé

Bien que les lymphocytes T CD4+ et les cellules de Langerhans, altérés dans l'infection au VIH, pourraient jouer un rôle central dans l'immunopathogenèse de la candidose oropharyngée (OPC), des mécanismes compensatoires de défense de l'hôte, potentiellement préservés, pourraient limiter la prolifération de *C. albicans* à la surface des muqueuses et prévenir la dissémination systémique chez les patients infectés au VIH. Nous avons pris avantage d'un nouveau modèle de candidose oropharyngée (OPC) chez les souris transgéniques (Tg) CD4C/HIV pour préciser le rôle des leucocytes polymorphonucléaires (PMNs) et des lymphocytes T CD8+ dans le contrôle de la charge buccale chronique et de la dissémination systémique de *C. albicans* aux organes profonds. Le nombre absolu de PMNs circulants et leur flambée oxydative sont augmentés chez les souris Tg CD4C/HIV^{MutA}, exprimant les gènes *ENV*, *REV* et *NEF* du VIH. Les fonctions de phagocytose et de fongicidie des PMNs de souris Tg à l'égard de *C. albicans* sont conservées comparativement à celles des PMNs de souris non-Tg. La déplétion des PMNs de ces souris Tg CD4C/HIV^{MutA} à l'aide d'un anticorps monoclonal n'a pas altéré les charges buccale et gastro-intestinale de *C. albicans*, ni entraîné de dissémination systémique. Bien que le nombre et la fonction des PMNs soient préservés, ces PMNs sont dispensables pour limiter la prolifération et la dissémination systémique du champignon aux organes profonds chez la souris Tg. Néanmoins, la charge buccale de *C. albicans* est significativement augmentée chez les souris Tg CD4C/HIV^{MutG} exprimant le gène NEF et *knock out* (KO) pour le CD8, comparativement aux souris Tg CD4C/HIV^{MutG}. À l'inverse, Les souris CD8 KO et non-Tg ont éliminé le *C. albicans* de la surface des muqueuses buccales immédiatement après la primo-infection, démontrant que l'augmentation de la charge buccale de *C. albicans* chez la souris CD8 KO apparaît uniquement lorsqu'elle exprime le transgène CD4C/HIV^{MutG}. Ces résultats démontrent pour la première fois que les lymphocytes T CD8+ participent aux défenses de l'hôte contre *C. albicans* in vivo.

CD8+ T-cells but not polymorphonuclear leukocytes are required to limit chronic oral carriage of *Candida albicans* in transgenic mice expressing human immunodeficiency virus type 1

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Condensed title: Host responses against *C. albicans* in HIV-1 transgenic mice

Key words: Mucosal immunity; candidiasis; HIV; AIDS; transgenic mice

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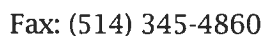

Abstract

Although defective CD4+ T-cells and Langerhans' cells are considered central to the immunopathogenesis of oropharyngeal candidiasis (OPC) in HIV-infection, completely or partly preserved compensatory host defense mechanisms most likely limit *Candida albicans* proliferation to the mucosa and prevent systemic dissemination in HIV-infected patients. Here, we have taken advantage of a novel model of OPC in CD4C/HIV transgenic (Tg) mice to investigate the role of polymorphonuclear leukocytes (PMNs) and CD8+ T-cells in limiting chronic oral carriage and systemic dissemination of *C. albicans* to deep organs. Absolute numbers of circulating PMNs and their oxidative burst were both augmented in CD4C/HIV^{MutA} Tg mice which express env, rev and nef of HIV-1, while phagocytosis and killing of *C. albicans* yeast cells by these PMNs were largely unimpaired compared to non-Tg mice. Monoclonal antibody depletion of PMNs in these CD4C/HIV^{MutA} Tg mice did not alter oral or gastrointestinal burdens of *C. albicans* nor cause systemic dissemination, demonstrating that in addition to being quantitatively and functionally unimpaired, these PMNs are dispensable for limiting mucosal proliferation and systemic spread of the fungus in these Tg mice. However, oral burdens of *C. albicans* were significantly augmented in CD4C/HIV^{MutG} Tg mice expressing the nef gene of HIV-1 and containing a CD8 knockout, compared to CD4C/HIV^{MutG} Tg mice without the CD8 knockout. In contrast, *C. albicans* was rapidly cleared from oral cavities of non-Tg mice with or without a CD8 knockout immediately after primary infection. Accordingly, augmentation of oral burdens of *C. albicans* in CD8 knockout mice occurred in animals which also express the CD4C/HIV^{MutG} transgene, but not in control mice which do not express the transgene. These results represent the first-ever clear evidence indicating that CD8+ T-cells participate in host defense against *C. albicans* in vivo.

Introduction

Oropharyngeal candidiasis (OPC) is the most frequent opportunistic fungal infection among human immunodeficiency virus (HIV)-infected patients, and it has been estimated that more than 90% of HIV-infected patients develop this often debilitating infection at some time during progression of their disease [45]. Although the incidence of OPC in HIV-infection has been significantly reduced since the introduction of highly active antiretroviral therapy (HAART) [31], it remains a common opportunistic infection in HIV-infected patients. The precise mechanisms underlying the predisposition to OPC among HIV-infected patients have not been defined. Although defective CD4+ T-cells and Langerhans' cells are considered central to the immunopathogenesis of OPC in HIV-infection [14], completely or partly preserved compensatory host defense mechanisms most likely limit *Candida albicans* proliferation to the mucosa and prevent systemic dissemination in HIV-infected patients.

CD8+ T-cells accumulate in the basal epithelial layer of the oral mucosa in HIV-infected patients with OPC [34,41], demonstrating that these cells can be actively recruited to the mucosa in response to candidiasis despite their apoptosis and progressive diminution in absolute numbers in HIV-infection [27]. However, the precise role of CD8+ T-cells in mucosal containment of *C. albicans* in HIV-infection, either by direct growth inhibition of *Candida* [5] or by an indirect mechanism, has not been determined. In addition, HIV-infected patients with the erythematous form of OPC have abundant neutrophilic microabscesses in the parakeratin layer of the epithelium [18,39,41], demonstrating that HIV-infection does not prevent a normal innate mucosal immune response by polymorphonuclear leukocytes (PMNs). Although recruitment of PMNs to the oral epithelium does not appear to be perturbed by HIV-infection, investigations comparing production of reactive oxygen intermediates, and phagocytosis and killing of *C. albicans* by PMNs from normal and HIV-infected patients have produced conflicting results

[8,17,24,47,48]. Nevertheless, a role for PMNs in mucosal containment of *C. albicans* in HIV-infection is suggested by a large body of work conducted in congenitally immunodeficient mice. These studies demonstrated that functional T-cells play a role in resistance to *C. albicans* colonizing or infecting mucosal surfaces, but that an added defect of phagocytes is required to produce systemic dissemination of *C. albicans* from the gastrointestinal tract [2,6,28,29]. In addition, the depletion of PMNs increased the severity of experimental OPC in immunocompetent BALB/c and CBA/CaH mice, demonstrating the critical role of these professional phagocytes in the efferent limb of the innate immune response [20].

The availability of CD4C/HIV^{Mut} transgenic (Tg) mice expressing human immunodeficiency virus type 1 in immune cells and developing an AIDS-like disease [26] has provided the opportunity to devise a model of oro-esophageal candidiasis that closely mimics the clinical and pathological features of candidal infection in human AIDS [13]. With the recognition that a cause-and-effect analysis of the immunopathogenesis of mucosal candidiasis in HIV-infection can now be achieved under controlled conditions in these Tg mice, the present study was undertaken to determine the role of PMNs and CD8+ T-cells in limiting chronic oral carriage and systemic dissemination of *C. albicans* to deep organs. Here we show that although PMNs from these Tg mice are quantitatively augmented and nearly intact functionally, they are nevertheless dispensable for limiting chronic oral carriage of *C. albicans* and for preventing systemic dissemination of *C. albicans* in CD4C/ HIV^{MutA} Tg mice which express the env, rev and nef genes of HIV-1. We also demonstrate that augmentation of oral burdens of *C. albicans* in CD8 knockout (KO) mice occurs in CD4C/HIV^{MutG} Tg mice which express the nef gene of HIV-1, but not in control mice which do not express the transgene. These results represent the first-ever clear evidence indicating that CD8+ T-cells participate in host defense against *C. albicans* in vivo.

Materials and Methods

Generation of Tg mice expressing HIV-1

The CD4C/HIV^{MutA} Tg mice have been described elsewhere [26]. CD4C/HIV^{MutA} mutant DNA harbors mouse CD4 enhancer and human CD4 promoter elements to drive the expression of HIV-1 genes in CD4+ CD8+ and CD4+ thymocytes, in peripheral CD4+ T-cells, and in macrophages and dendritic cells. Founder mouse F21388 was bred on the C3H background, and progeny mice were genotyped and routinely examined for signs of disease. Animals from this line express moderate levels of the transgene, with 50% survival at 3 months [26]. Several HIV-1 genes (gag, pol, vif, vpr, tat, and vpu) are mutated in the CD4C/HIV^{MutA} DNA, whereas env, rev, and nef are intact. The generation of CD4C/HIV^{MutG} mice revealed that selective expression of the nef gene is required and sufficient to elicit an AIDS-like disease in these Tg mice [26]. This disease is characterized by failure to thrive, wasting, severe atrophy and fibrosis of lymphoid organs, loss of CD4+ T-cells, interstitial pneumonitis, and tubulointerstitial nephritis [26]. These changes are not observed in control non-Tg littermates [26].

CD8a homozygous KO mice (The Jackson Laboratory, Bar Harbor, ME), containing a targeted mutation of the CD8 receptor alpha chain, were used intact or for the purpose of breeding with CD4C/HIV^{MutG} mice to generate homozygous or heterozygous CD4C/HIV^{MutG} CD8 KO mice.

Specific pathogen-free male and female Tg mice and non-Tg littermates were housed in a protective environment at the University of Montreal Animal Care Unit, in sterilized individual cages equipped with filter hoods. The animals were supplied with sterile water and were fed with sterile mouse chow.

Animal model of mucosal candidiasis

Oral inoculation with *C. albicans* LAM-1, assessments for signs of morbidity, quantification of *C. albicans* in the oral cavities of individual mice, and determination of burdens of *C. albicans* in the gastrointestinal tract and internal organs were conducted as described previously [13].

Quantitation of peripheral blood leukocytes

Peripheral blood was drawn from the saphenous vein using the Unopette microcollection system for determination of total leukocytes (Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ). The differential leukocyte count was performed manually by staining a smear of blood with May-Grunwald-Giemsa, and the absolute leukocyte count was calculated and expressed as number of cells/mm^3.

Polymorphonuclear leukocyte oxidative burst, phagocytosis and killing of C. albicans

To measure PMN oxidative burst, phagocytosis and killing of *C. albicans*, PMNs were isolated from whole peripheral blood by using an HISTOPAQUE®-1119/-1077 double gradient according to the manufacturer's instructions (Sigma-Aldrich, St. Louis, MO). Blood from 4-10 mice was pooled to generate each independent measurement. Briefly, blood was withdrawn from the heart of mice with a heparinized syringe and centrifuged on a pre-built gradient at 500 g for 50 min. Collected PMNs were treated with rel blood cell lysing buffer, washed with PBS and suspended in RPMI 1640 supplemented medium. The purity of PMNs was > 90% by May-Grunwald-Giemsa staining and flow cytometry analysis.

The PMN oxidative burst was assessed by measuring the conversion of nonfluorescent dichlorofluorescein diacetate (DCFH-DA) to the fluorescent compound 2', 7'-

dichlorofluorescein (DCF), using the DCFH. Peroxides CellProbe Reagent (Beckman Coulter, Fullerton, CA) according to the manufacturer's instructions. Blood from two mice was pooled to generate each independent measurement. Mean fluorescence of PMNs was measured on a FACStar flow cytometer (BD Biosciences) equipped with Consort 30 software. Acquisition of data was counted on a mean of 10,000 events by gating on PMNs.

To assay phagocytosis of blastoconidia, 2.5 x 10^5 PMNs were incubated with FITC-labelled, heat-inactivated (90°C, 45 min) *C. albicans* blastoconidia in 100 µl of supplemented RPMI medium, with a PMN to blastoconidia ratio of 2:1, 1:2 or 1:5 for 5, 15 or 30 min at 37°C in independent wells. Phagocytosis was stopped by cooling the samples to 4°C. A control phagocytosis assay was conducted at 4°C (1:5 ratio) in presence of cytochalasin B (Sigma; 5 µg/ml) [21]. Ethidium bromide was added at a concentration of 10 µg/ml and flow cytometry acquisition of 10,000 PMNs was done immediately as described [46]. Phagocytosis of *C. albicans* by PMNs was verified by confocal microscopy.

To assay killing of *C. albicans*, 2.5 x 10^5 PMNs were incubated with FITC-labelled, live *C. albicans* blastoconidia in 100 µl of supplemented RPMI medium at a 1:1 E/T cell ratio for 30, 60 or 90 min at 37°C in independent wells. A control assay was performed at 4°C as described above. After lysing PMNs with 2.5% sodium desoxycholate, killed *C. albicans* cells were identified by ethidium bromide staining and analyzed by flow cytometry.

Monoclonal antibody depletion of polymorphonuclear leukocytes in CD4C/HIV^{MutA} Tg mice

Rat IgG2b monoclonal antibody RB6-8C5 (BD Biosciences), recognizing the Ly-6G (Gr-1) myeloid differentiation antigen on mouse granulocytes [23,35], was used to deplete PMNs in the Tg mice. Three successive 50 µg doses of purified RB6-8C5 antibody administered i.p. every two days resulted in a >90% depletion of circulating PMNs which was sustained for at least 7 days. Control mice were treated with 50 µg of rat IgG2b isotype control

(Clone A95-1; BD Biosciences).

Flow cytometry

Heparinized blood was collected by cardiac puncture under anesthesia with Hypnorm (Janssen Pharmaceutica) and Versed (Sabex, Boucherville, Quebec), and the mice were exsanguinated with PBS [13]. Cell surface marker analysis of peripheral blood lymphocytes was conducted as described previously [13], on a FACSCalibur flow cytometer (BD Biosciences) equipped with CellQuest software.

Cervical lymph nodes (CLN) were removed and mechanically disrupted by pressing through a nylon mesh (pore size, 70 μm) and deposited in 25-mm-diameter dishes containing 2 ml of Hanks' balanced salt solution (HBSS; Gibco, Grand Island, NY). Cell suspensions were twice washed in HBSS and resuspended in complete tissue culture medium consisting of RPMI 1640 medium (Gibco) supplemented with 10% of heat-inactivated fetal bovine serum (Gibco), 20mM HEPES buffer, 2mM L-glutamine, 5×10^{-5} M -mercaptoethanol, 100 U/ml penicillin and streptomycin, 0.25 μg/ml amphotericin B, and 50 μg/ml of gentamicin. Cells were then filtered through a sterile nylon mesh (pore size, 70 μm) to obtain a homogeneous cell suspension, and cell viability was >90% by trypan blue exclusion. CLN cells were directly stained with fluorescent monoclonal antibodies specific for mouse CD45 (30-F11), CD3 (145-2C11), CD4 (RM4-5), CD8 (53-6.7), and their respective isotypes (BD Biosciences) at 1 $\mu g/10^6$ cells for 45 min at 4°C. Cells were twice washed in cold PBS and fixed with 2% paraformaldehyde. Acquisition of data was counted on 10,000 events by gating on CD45+ cells. CLN memory-like CD8+ T-cells were phenotyped as previously described (D. Lewandowski, M. Marquis, F. Aumont, A.-C. Lussier-Morin, M. Raymond, S. Sénéchal, Z. Hanna, P. Jolicoeur, L. de Repentigny, submitted).

Statistical analysis

Differences in oral burdens of *C. albicans* among groups of mice were determined by using PROC MIXED software (SAS Institute, Cary, NC). Repeated-measurements analysis of variance with Tukey-Kramer contrast analysis when required was conducted with 2 factors, 1 between (group) and 1 within (time). Significant interactions ($p < 0.05$) were further analyzed, and significant differences ($p < 0.05$) between group means at fixed times were determined by use of the 2-sample, 2-tailed Student's t test for independent samples. The 2-sample, 2-tailed Student's t test for independent samples was used to determine differences in peripheral blood and CLN CD8+ T-cells. Statistical analyses for all other data were performed with SPSS Version 11.5 software (SPSS, Chicago, IL) using an analysis of variance followed by Tukey contrast analysis when necessary. Differences were considered to be significant at $p < 0.05$.

Results

Enumeration of peripheral blood leukocytes from CD4C/HIV^{MutA} Tg mice

Absolute total leukocyte counts were comparable in Tg and non-Tg mice infected or uninfected with *C. albicans*, and these total counts did not change significantly in each of the four groups of mice over the course of AIDS-like disease ($p > 0.05$) (Fig. 1). However, the Tg mice were lymphopenic compared to the non-Tg mice throughout AIDS-like disease ($p < 0.05$) irrespective of infection with *C. albicans* ($p > 0.05$), but without progression of the lymphopenia over time ($p > 0.05$) (Fig. 1). In the non-Tg mice, absolute lymphocyte counts were significantly lower ($p < 0.05$) after oral infection with *C. albicans* compared to uninfected controls. In uninfected mice, absolute PMN counts were significantly enhanced in the Tg compared to the non-Tg animals both early and late in AIDS-like disease ($p < 0.01$) (Fig. 1). Oral infection with *C. albicans* significantly enhanced PMN counts in both the Tg and non-Tg mice ($p < 0.05$), resulting in significantly greater absolute PMNs in infected Tg compared to infected non-Tg mice ($p < 0.001$) (Fig. 1). Expression of the HIV-1 transgene and oral infection with *C. albicans* also independently contributed to a progressive rise in circulating monocytes ($p < 0.05$) over time, while absolute eosinophils remained unchanged ($p > 0.05$). Taken together, these findings demonstrated that while lymphopenia is present in these Tg mice, chronic carriage of *C. albicans* not only does not result from a quantitative defect in circulating PMNs but in fact stimulates a greater neutrophil response in the Tg than in non-Tg mice.

PMN oxidative burst, phagocytosis and killing of C. albicans

In comparison to uninfected non-Tg control mice, the PMN oxidative burst was modestly but not significantly ($p > 0.05$) increased in infected non-Tg and uninfected Tg mice, demonstrating that *C. albicans* infection and HIV-1 transgene expression do not

independently alter the oxidative burst (Fig. 2). However, the oxidative burst of PMNs from the infected Tg mice was significantly enhanced ($p < 0.05$) at both 7 and 70 days after infection compared to the uninfected non-Tg control animals, indicating that transgene expression and *C. albicans* infection together increase the PMN oxidative burst. Accordingly, chronic oral carriage of *C. albicans* in these CD4C/HIV^{MutA} Tg mice was not associated with a perturbation in the oxidative capacity of PMNs, but on the contrary increased the production of these oxidative intermediates.

Phagocytosis of *C. albicans* blastoconidia by PMNs from infected Tg and non-Tg mice as well as their respective uninfected controls did not differ significantly ($p > 0.05$) 7 days after infection with *C. albicans* (Fig. 3). However, at 70 days after *C. albicans* infection, the percentage of PMNs with phagocytosed *C. albicans* was significantly decreased ($p < 0.01$) in the Tg compared to the non-Tg mice, but only after 30 min of incubation and at a high effector: target ratio (2:1). In contrast, phagocytosis was significantly enhanced ($p < 0.05$) in the Tg compared to the non-Tg mice at a low effector: target ratio (1:5) at this same time of incubation. The mean fluorescence of PMNs with phagocytosed *C. albicans* did not differ significantly ($p > 0.05$) in these infected or uninfected Tg and non-Tg mice, indicating that numbers of endocytosed *C. albicans* per each phagocytosing PMN were comparable in the four groups of mice (data not shown).

Killing of *C. albicans* blastoconidia by PMNs from the Tg mice was modestly reduced ($p < 0.05$) in comparison to the non-Tg mice, 7 days after infection with *C. albicans.* At 70 days after infection, however, a trend towards diminished killing by PMNs from the Tg mice did not reach statistical significance ($p = 0.11$).

Depletion of PMNs in CD4C/HIV^{MutA} Tg mice

In comparison to treatment with an isotype control antibody, monoclonal antibody

depletion of circulating PMNs beginning on days 20, 45 or 63 after oral infection with *C. albicans* did not significantly alter the level of chronic oral carriage of *C. albicans* in these Tg mice over the resulting 7-day period of profound neutropenia ($p > 0.05$) (Fig. 4). In addition, depletion of PMNs in the non-Tg mice did not produce a relapse of oral carriage of *C. albicans*, which was cleared from the oral cavities of these control mice within 10 days after oral inoculation ([13], Fig. 4). Tg and non-Tg mice assessed after 7 days of neutropenia showed an absence of systemic dissemination of *C. albicans* to deep organs, except for minimal spread to lungs and liver in two of eight Tg mice with advanced AIDS-like disease assessed 70 days after oral infection with *C. albicans*, and this depletion of PMNs did not modify the burdens of *C. albicans* in the gastrointestinal tract of the Tg mice (Table 1). All mice survived until final assessment seven days after induction of neutropenia. These results demonstrated that PMNs are dispensable for limiting *C. albicans* proliferation in the oral mucosa and for preventing systemic dissemination of the fungus in these Tg mice.

Oral candidiasis in CD4C/HIV^{MutG} CD8 KO mice

CD4C/HIV^{MutG} CD8 KO Tg mice, CD8 KO mice, CD4C/HIV^{MutG} Tg and non-Tg littermates were infected orally with *C. albicans*, and burdens were assessed longitudinally by sampling the oral cavities. After primary infection and throughout the chronic carrier phase (days 27 to 114 after infection), oral burdens were significantly elevated in CD4C/HIV^{MutG} CD8 KO compared to CD4C/HIV^{MutG} mice ($p = 0.01$) (Fig. 5). In contrast, *C. albicans* was rapidly cleared from oral cavities of both CD8 KO and non-Tg mice immediately after primary infection ($p < 0.05$, compared to CD4C/HIV^{MutG} and CD4C/HIV^{MutG} CD8 KO mice). Augmentation of oral burdens of *C. albicans* in CD8 KO mice therefore occurred in animals which also express the CD4C/HIV^{MutG} transgene, but not in control mice which do not express the transgene. Interestingly, sustained enhancement of infection in these CD4C/HIV^{MutG} mice was comparable to our previous observations in CD4C/HIV^{MutA} mice

[13], indicating that the nef gene is necessary and sufficient for persistent candidal infection.

In contrast to the oral cavity, burdens of *C. albicans* in the gastrointestinal tract were comparable in CD4C/HIV^{MutG} and CD4C/HIV^{MutG} CD8 KO mice, and the limited systemic dissemination of *C. albicans* in these MutG Tg mice was similar to that previously observed during the later stage of infection in CD4C/HIV^{MutA} Tg mice [13] (Table 2). Taken together, these results indicated that CD8+ T-cells directly or indirectly limit chronic oral carriage of *C. albicans* in these Tg mice.

Flow cytometry analysis confirmed the absence of CD8+ T-cells in the peripheral blood and CLNs of CD4C/HIV^{MutG} CD8 KO and CD8 KO mice, and demonstrated a significant depletion of this cell population in the peripheral blood ($p < 0.05$) but its augmentation in CLNs ($p < 0.05$) of CD4C/HIV^{MutG} compared to non-Tg mice (Table 3). Interestingly, CD4C/HIV^{MutG} Tg mice heterozygous for the CD8a KO had unaltered CD8+ T-cell frequencies in peripheral blood (5.1 ± 1.1 [mean ± SD, 5 mice]) and CLNs (33.8 ± 7.8), and no augmentation of oral burdens of *C. albicans* in comparison to CD4C/HIV^{MutG} mice (data not shown). The proportions of CLN naïve (CD44-/^{lo}, CD62L+) and memory-like (CD44^{hi}, CD62^{lo}/-) CD8+ T-cells were comparable in these CD4C/HIV^{MutG} Tg mice and non-Tg animals orally infected with *C. albicans* (data not shown), and were similar to those previously observed in infected CD4C/HIV^{MutA} Tg mice (D. Lewandowski, M. Marquis, F. Aumont, A.-C. Lussier-Morin, M. Raymond, S. Sénéchal, Z. Hanna, P. Jolicoeur, L. de Repentigny, submitted). CD4+ T-cells in the peripheral blood and CLNs of CD4C/HIV^{MutG} and CD4C/HIV^{MutG} CD8 KO Tg mice were severely depleted relative to CD8 KO and non-Tg mice ($p < 0.001$), but were augmented in the CLNs of CD8 KO compared to non-Tg animals ($p < 0.001$) (Table 3).

Discussion

The ability of *C. albicans* to successfully infect the oral and esophageal epithelium but yet to rarely disseminate to deep organs of HIV-infected patients suggest that partially or completely preserved host defense mechanisms effectively restrict proliferation of the fungus to the superficial mucosa in HIV-infection. Studies conducted in congenitally immunodeficient mice with experimental oral or gastrointestinal candidiasis have firmly established that a defect in the protective Th1 CD4+ T-cell response to *C. albicans* results in mucosal candidiasis at these sites, but that an added perturbation of PMNs and/or macrophages is required for systemic dissemination [1,6,7,9,28,29,42]. The role of PMNs in host defense against mucosal and systemic candidiasis was thus investigated in Tg mice expressing HIV-1 and which develop an AIDS-like disease [13,25,26]. Absolute numbers and the oxidative burst of circulating PMNs were augmented in the Tg mice and further amplified by chronic carriage of *C. albicans*, indicating that these properties of the PMN response to candidal infection are quantitatively and functionally unimpaired in these Tg mice. Despite a striking reduction of CD4+ T-cells in the peripheral blood and CLNs of the Tg mice, profound depletion of PMNs sustained over 7 days did not quantitatively alter chronic oral carriage nor lead to systemic dissemination of *C. albicans*, except for minimal spread to lungs and liver in two of eight Tg mice with advanced AIDS-like disease. Accordingly, PMNs are not only essentially unimpaired but are also dispensable for control of mucosal and systemic candidiasis in these Tg mice, despite their well-characterized anti-*Candida* properties in vivo and in vitro [12,20,28,29]. In previous studies, monoclonal antibody depletion of PMNs in both normal [20] and SCID [28,29] mice augmented the severity of oral infection with *C. albicans*, and resulted in disseminated candidiasis in the SCID mice [28,29]. However, monoclonal antibody depletion of PMNs in the normal mice was initiated prior to self-limited primary infection with *C. albicans* [20], characterized by a marked transient influx of neutrophils to the oral mucosa [10,19], and did not examine the role of these cells during chronic carriage in a T-

cell-defective host emulating oral candidiasis in HIV-infection. The T- and B-cell deficient SCID mice, for their part, differ significantly from the Tg mice by the absence of a humoral response, and these animals were monoassociated with *C. albicans* in a germfree environment [28,29] and were therefore devoid of a protective bacterial flora [30]. At the time of euthanasia, the PMN-depleted Tg mice were heavily and uniformly colonized by *C. albicans* throughout the digestive tract, demonstrating that these mice were indeed uniformly challenged by *C. albicans* at this portal of entry for systemic dissemination. Interestingly, a non-protective role of PMNs has also been surmised in women who received an intravaginal *Candida* challenge, in whom protection against infection was noninflammatory while symptomatic infection correlated with a vaginal infiltration of PMNs and a high vaginal *C. albicans* burden [22].

In several investigations producing conflicting results, the oxidative burst of PMNs from HIV-infected patients has been found to be unchanged [48], increased [4,16,44] or decreased [11,33,36,47] compared to patients uninfected with HIV. Likewise, growth inhibition of *C. albicans* by PMNs was found to be preserved in HIV-infection [8], while in other studies phagocytosis and killing of *C. albicans* by PMNs were determined to be either unchanged, impaired or increased [4,17,32,33,44,48]. The present findings in the Tg mice expressing HIV-1 demonstrate an augmentation in absolute numbers and in the oxidative burst of PMNs. Because the HIV-1 transgene is not expressed in PMNs [26], these changes are most likely the indirect result of altered expression of cytokines with activating (IFN-γ/TNF-α/IL-2) or deactivating (IL-4/IL-10) signals to effector phagocytes in the Tg mice [43]. However, the fungicidal activity of PMNs from the Tg mice remained unimpaired 70 days after infection with *C. albicans* despite an enhanced production of the Th2 cytokines IL-4 and IL-10 by CD4+ T-cells from these animals late in AIDS-like disease (D. Lewandowski, M. Marquis, F. Aumont, A.-C. Lussier-Morin, M. Raumond, S. Sénéchal, Z. Hanna, P. Jolicoeur, and L. de Repentigny, submitted). Both of these cytokines have the ability to suppress the candidacidal activity of PMNs from normal and HIV-infected

patients in vitro [47]. The early augmentation of IL-10 in AIDS-like disease may have contributed to the modest decrease in fungicidal activity of PMNs from these Tg mice, 7 days after infection with *C. albicans*. Because the fungicidal activity was decreased while the oxidative burst of PMNs was increased, non-oxidative anticandidal mechanisms of PMNs may have been perturbed in the Tg mice at this early time in AIDS-like disease. Phagocytosis of *C. albicans* yeast cells by PMNs from the Tg mice was unaltered except at 70 days after infection with *C. albicans* and after prolonged incubation at a high E:T ratio, and may also have resulted from the suppressive effect of IL-10.[40] However, PMNs from the Tg mice maintained a normal capacity to kill *C. albicans* at this late stage of AIDS-like disease, demonstrating that chronic oral candidiasis in these Tg mice cannot be explained by a functionally significant defect of PMNs against *C. albicans*.

Several lines of evidence have suggested a protective role of CD8+ T-cells against oral candidiasis in HIV-infection. IL-2 (but not IFN-γ) -activated CD8+ T-cells exert direct growth inhibition against the hyphal form of *C. albicans* in vitro [5]. Progressive depletion of CD8+ T-cells in HIV-infection results from apoptosis mediated by macrophages through interaction of HIV gp120 with chemokine receptor CXCR4 [27]. In addition, nef induces caspase-8-mediated apoptosis of CD8+ T-cells by upregulating DC expression of TNF-α and FasL [37]. Despite a progressive diminution in absolute numbers, remaining CD8+ T-cells nevertheless successfully accumulate in the basal epithelial layer of the oral mucosa of HIV-infected patiens with OPC, demonstrating that these cells can be actively recruited to the mucosa in response to candidiasis [34,41]. However, CD8+ T-cells may not be in proximity to *C. albicans* hyphae which are usually confined to the upper layers of the epithelium [18,38]. Accordingly, the precise role of CD8+ T-cells in mucosal containment of *C. albicans* in HIV-infection, either by direct growth inhibition of *Candida* or more likely by production of cytokines which enhance the antimicrobial activity of macrophages and PMNs against *C. albicans*, has so far remained unclear. Immunohistochemical analysis has revealed an influx of CD8+ T-cells in the basal layer of the oral epithelium of Tg mice

orally infected with *C. albicans*, both immediately after recovery from primary infection (M. Marquis and L. de Repentigny, unpublished data) and late in the chronic carrier phase[13], similar to that observed in HIV-infected patients with OPC. In contrast, CD8+ T-cells were not detected in uninfected Tg or non-Tg mice [13], consistent with their known exceedingly low frequency in normal murine oral mucosa [10,15]. In addition, flow cytometry analysis demonstrated a significant augmentation or oral mucosal CD8+ T-cells in infected Tg compared to uninfected non-Tg animals both early and late in AIDS-like disease, again showing that the Tg mice recruit this cell population to the oral mucosa in response to candidal infection (D. Lewandowski, M. Marquis, F. Aumont, A.-C. Lussier-Morin, M. Raymond, S. Sénéchal, Z. Hanna, P. Jolicoeur, and L. de Repentigny, submitted). This increase of CD8+ T-cells was also demonstrated in the CLNs of the Tg compared to the non-Tg mice orally infected with *C. albicans*. A role for CD8+ T-cells in limiting oral proliferation of *C. albicans* was clearly demonstrated by significantly enhanced oral burdens in CD4C/HIV^{MutG} CD8KO mice compared to CD4C/HIV^{MutG} mice throughout the chronic carrier state, in striking contrast to the rapid clearance of *C. albicans* from the oral cavities of both CD8KO and non-Tg mice immediately after primary infection. Therefore, augmentation of oral burdens of *C. albicans* in CD8KO mice occurs in mice which also express the CD4C/HIV^{MutG} transgene, but not in control mice which do not express the transgene. These results suggest that CD8+ T-cells become critical to host defense against *C. albicans* only when CD4+ and/or antigen-presenting cells are perturbed by expression of the HIV nef gene in CD4C/HIV^{MutG} Tg mice [26]. In previous investigations, depletion of CD8+ T-cells in immunocompetent BALB/C or CBA/CaH mice did not alter the clearance of primary oral infection with *C. albicans* [20], and adoptive transfer of naïve CD8+ T-cells to BALB/C nu/nu only resulted in a modest and transient decrease in oral colonization [19]. Likewise, β_2-microglobulin knockout mice, which lack major histocompatibility complex (MHC) class I expression and are deficient in CD8+ T-cells, were susceptible to systemic candidiasis of endogenous origin but showed only superficial and transient infection of tongues and esophagi after monoassociation with *C. albicans* [3]. The present results thus

clearly demonstrate for the first time that CD8+ T-cells participate in host defense against oral candidiasis in vivo.

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Table 1. Viable colony-forming units in organs of CD4C/HIV^{MutA} transgenic (Tg) mice inoculated orally with *Candida albicans*, and depleted of PMNs beginning on days 20, 45 or 63 after inoculation.

	Mice[a] and treatment[b]								
	Assessment day 27			Assessment day 52			Assessment day 70		
Variable	Tg / RB6-8C5	Tg /IgG	Non-Tg/RB6-8C5	Tg / RB6-8C5	Tg /IgG	Non-Tg/RB6-8C5	Tg / RB6-8C5	Tg /IgG	Non-Tg/RB6-8C5
No. inoculated	6	6	6	7	6	7	8	5	6
Age at inoculation, mean days (range)	50 (43-57)	50 (43-57)	50 (43-57)	57 (49-64)	57 (49-64)	57 (49-64)	55 (52-57)	55 (52-57)	55 (52-57)
Age at assessment, mean days (range)	77 (70-84)	77 (70-84)	77 (70-84	109 (101-116)	109 (101-116)	109 (101-116)	125 (122-127)	125 (122-127)	125 (122-127)
Organs culture positive for *C. albicans*									
Brain									
No. of mice	0	0	0	0	0	0	0	0	0
C. albicans count, mean cfu/g	NA	NA	NA	NA	NA	NA	NA	NA	NA
Range	NA	NA	NA	NA	NA	NA	NA	NA	NA
Lungs									
No. of mice	0	0	0	0	0	0	2	1	0
C. albicans count, mean cfu/g	NA	NA	NA	NA	NA	NA	7.8×10^1	2.8×10^1	NA
Range	NA	NA	NA	NA	NA	NA	$7.2 \times 10^1 - 8.3 \times 10^1$	NA	NA

Variable	Mice[a] and treatment[b]								
	Assessment day 27			Assessment day 52			Assessment day 70		
	Tg / RB6-8C5	Tg /IgG	Non-Tg/RB6-8C5	Tg / RB6-8C5	Tg /IgG	Non-Tg/RB6-8C5	Tg / RB6-8C5	Tg /IgG	Non-Tg/RB6-8C5
Liver									
No. of mice	0	0	0	0	0	0	2	0	1
C. albicans count, mean cfu/g	NA	NA	NA	NA	NA	NA	1.3×10^1	NA	9.3×10^0
Range	NA	NA	NA	NA	NA	NA	$7.2 \times 10^0 - 1.9 \times 10^1$	NA	NA
Kidneys									
No. of mice	0	0	0	0	0	0	0	0	0
C. albicans count, mean cfu/g	NA	NA	NA	NA	NA	NA	NA	NA	NA
Range	NA	NA	NA	NA	NA	NA	NA	NA	NA
Stomach									
No. of mice	6	6	1	6	4	1	8	5	3
C. albicans counts, mean cfu/g	1.8×10^5	2.2×10^5	2.1×10^2	1.1×10^5	2.0×10^5	1.4×10^2	3.5×10^5	3.4×10^5	2.8×10^2
Range	$5.1 \times 10^2 - 3.9 \times 10^5$	$3.4 \times 10^3 - 5.9 \times 10^5$	NA	$2.4 \times 10^4 - 3.5 \times 10^5$	$2.1 \times 10^5 - 2.8 \times 10^5$	NA	$9.8 \times 10^2 - 1.6 \times 10^6$	$6.9 \times 10^4 - 6.3 \times 10^5$	$5.9 \times 10^1 - 5.5 \times 10^2$

	Mice[a] and treatment[b]								
	Assessment day 27			Assessment day 52			Assessment day 70		
Variable	Tg / RB6-8C5	Tg /IgG	Non-Tg/RB6-8C5	Tg / RB6-8C5	Tg /IgG	Non-Tg/RB6-8C5	Tg / RB6-8C5	Tg /IgG	Non-Tg/RB6-8C5
Small intestine									
No. of mice	6	6	3	7	5	3	8	5	3
C. albicans counts, mean cfu/g	2.6×10^4	2.1×10^4	2.2×10^2	1.5×10^4	1.5×10^4	2.9×10^1	1.4×10^4	1.5×10^5	2.1×10^3
Range	$8.7 \times 10^2 – 6.4 \times 10^4$	$5.2 \times 10^2 – 8.1 \times 10^4$	$2.5 \times 10^1 – 5.1 \times 10^2$	$2.1 \times 10^3 – 4.4 \times 10^4$	$1.6 \times 10^3 – 3.9 \times 10^4$	$1.9 \times 10^1 – 4.8 \times 10^1$	$7.4 \times 10^2 – 3.0 \times 10^4$	$1.9 \times 10^3 – 5.8 \times 10^5$	$3.9 \times 10^2 – 3.9 \times 10^3$
Large intestine									
No. of mice	6	6	2	7	6	4	8	5	3
C. albicans counts, mean cfu/g	2.0×10^5	2.7×10^5	5.1×10^2	1.0×10^5	9.0×10^4	1.6×10^2	7.2×10^4	3.2×10^5	1.4×10^4
Range	$1.6 \times 10^4 – 4.4 \times 10^5$	$1.1 \times 10^4 – 4.4 \times 10^5$	$2.6 \times 10^2 – 7.6 \times 10^2$	$3.8 \times 10^3 – 3.0 \times 10^5$	$2.3 \times 10^3 – 3.2 \times 10^5$	$2.7 \times 10^1 – 3.3 \times 10^2$	$8.8 \times 10^3 – 1.4 \times 10^5$	$1.9 \times 10^5 – 5.8 \times 10^5$	$9.4 \times 10^4 – 2.1 \times 10^4$

NOTE : NA, not applicable

[a] Mice studied included Tg and non-Tg offspring derived from founder mouse F21388.

[b] RB6-8C5, anti-granulocyte monoclonal antibody; IgG, isotype control antibody.

Table 2. Viable colony-forming units in organs of CD4C/HIV^{MutG} CD8 KO, CD4C/HIV^{MutG}, CD8 KO, and non-Tg mice inoculated orally with *Candida albicans.*

Variable	Mice[a]			
	Tg CD8 KO	Tg	CD8 KO	Non-Tg
No. inoculated	6	6	6	6
Age at inoculation, mean days (range)	56 (40 - 71)	56 (40 - 71)	56 (40 - 71)	89 (87 - 90)
Age at assessment, mean days (range)	227 (136 - 352)[b]	257 (172 - 352)[c]	337 (321 - 352)[d]	166 (164 - 167)[d]
Organs culture positive for *C. albicans*				
Brain				
No. of mice	4	2	6	0
C. albicans count, mean cfu/g	6.8×10^4	1.8×10^3	1.4×10^3	NA
Range	$3.2 \times 10^2 – 1.2 \times 10^5$	$1.5 \times 10^3 – 2.1 \times 10^3$	$2.2 \times 10^3 – 6.8 \times 10^4$	NA
Lungs				
No. of mice	0	1	0	0
C. albicans count, mean cfu/g	NA	5.0×10^3	NA	NA
Range	NA	NA	NA	NA

Variable	Mice[a]			
	Tg CD8 KO	Tg	CD8 KO	Non-Tg
Liver				
No. of mice	1	2	0	0
C. albicans count, mean cfu/g	9.2×10^3	2.2×10^2	NA	NA
Range	NA	$1.7 \times 10^2 – 2.7 \times 10^2$	NA	NA
Kidneys				
No. of mice	0	2	0	0
C. albicans count, mean cfu/g	NA	2.7×10^2	NA	NA
Range	NA	$1.1 \times 10^2 – 4.4 \times 10^2$	NA	NA
Stomach				
No. of mice	4	4	0	2
C. albicans count, mean cfu/g	1.1×10^6	1.0×10^5	NA	2.4×10^3
Range	$8.1 \times 10^5 – 1.6 \times 10^6$	$7.9 \times 10^2 – 4.0 \times 10^5$	NA	$4.4 \times 10^2 – 4.4 \times 10^3$

Variable	Mice[a]			
	Tg CD8 KO	Tg	CD8 KO	Non-Tg
Small intestine				
No. of mice	4	4	0	4
C. albicans count, mean cfu/g	8.3×10^3	2.1×10^3	NA	1.3×10^3
Range	$3.3 \times 10^3 – 1.5 \times 10^4$	$5.9 \times 10^1 – 6.5 \times 10^3$	NA	$3.4 \times 10^1 – 1.8 \times 10^3$
Large intestine				
No. of mice	5	4	0	4
C. albicans count, mean cfu/g	9.8×10^4	3.3×10^4	NA	9.4×10^3
Range	$5.1 \times 10^3 – 2.9 \times 10^5$	$8.6 \times 10^2 – 1.2 \times 10^5$	NA	$7.6 \times 10^3 – 1.1 \times 10^4$

NOTE : NA, not applicable.
[a] Mice studied included Tg and control non-Tg offspring derived from founder mouse F27367, with some also containing a knockout (KO) of the CD8 receptor alpha chain.
[b] Two mice did not survive until day 352 (assessment days 136 and 193).
[c] Four mice did not survive until day 352 (assessment days 172 to 302).
[d] All mice survived until euthanasia.

Table 3. Cell surface marker analysis of peripheral blood and cervical lymph node lymphocytes from CD4C/HIV^{MutG} CD8 KO, CD4C/HIV^{MutG}, CD8 KO, and non-Tg mice inoculated orally with *Candida albicans*

Mice[a]	No. inoculated	Age at assessment, mean days (range)	Cell population, mean % ± SD			
			Peripheral blood		Cervical lymph nodes	
			CD4+	CD8+	CD4+	CD8+
CD4C/HIV^{MutG} CD8 KO	6	227 (136 - 352)	1.5 ± 1.3[b]	0	11.2 ± 8.3[b]	0
CD4C/HIV^{MutG}	6	257 (172 - 352)	1.2 ± 0.1[b]	5.6 ± 2.3[c]	10.1 ± 5.3[b]	35.3 ± 8.8[c]
CD8 KO	6	337 (321 - 352)	15.1 ± 3.9	0	62.1 ± 3.8[d]	0
Non-Tg	6	337 (321 - 352)	16.2 ± 2.0	13.6 ± 4.6	43.0 ± 5.1	25.6 ± 2.7

[a] Mice studied included Tg and control non-Tg offspring derived from founder mouse F27367, with some also containing a knockout (KO) of the CD8 receptor alpha chain.
[b] Significantly different from CD8 KO and non-Tg mice ($p < 0.001$, one-way analysis of variance).
[c] Significantly different from non-Tg mice ($p < 0.05$, Student's t test).
[d] Significantly different from non-Tg mice ($p < 0.001$, one-way analysis of variance).

Figure legends

Fig. 1 Quantitation of peripheral blood leukocytes in CD4C/HIV^{MutA} Tg and non-Tg mice infected or uninfected with *C. albicans*. Blood samples were drawn from Tg and non-Tg mice at 7 (A), 45 (B) or 70 days (C) after oral infection with *C. albicans*, or from uninfected control animals. There were 4 to 6 mice per determination. Statistically significant differences between groups: *, $p < 0.05$ compared to infected or uninfected non-Tg mice; **, $p < 0.05$ compared to uninfected non-Tg mice; ***, $p < 0.01$ compared to uninfected non-Tg mice; ****, $p < 0.05$ compared to uninfected Tg or non-Tg mice; *****, $p < 0.001$ compared to infected non-Tg mice.

Fig. 2 Oxidative burst of PMNs from CD4C/HIV^{MutA} Tg and non-Tg mice infected or uninfected with *C. albicans*. Blood samples were drawn from Tg and non-Tg mice at 7 or 70 days after oral infection with *C. albicans*, or from uninfected control animals. Data represent the mean ± SD of four independent observations, each obtained by pooling the blood of two mice. *, significantly different from uninfected non-Tg mice ($p < 0.05$).

Fig. 3 Phagocytosis and killing of *C. albicans* blastoconidia by PMNs from CD4C/HIV^{MutA} Tg and non-Tg mice infected or uninfected with *C. albicans*. Blood samples were obtained from Tg or non-Tg mice at 7 or 70 days after oral infection with *C. albicans*, or from uninfected control animals. Analysis was performed at the indicated PMN: blastoconidia ratios. Data represent the mean ± SD of 3 to 9 independent observations, each obtained by pooling the blood of 4-10 mice. *, $p < 0.05$ compared to non-Tg mice.

Fig. 4 Oral burdens of *C. albicans* and absolute circulating PMNs in CD4C/HIV^{MutA} Tg

mice treated with RB6-8C5 monoclonal antibody (red lines) or isotype control activity (blue lines). Control non-Tg mice (green lines) were treated with RB6-8C5 monoclonal activity. Beginning on days 20 (A), 45 (B) or 63 (C) after oral infection with *C. albicans*, each group of mice received its respective treatment every two days until euthanasia 7 days later. Data represent the mean ± SD of 6 to 7 mice in each group.

Fig. 5 CD4C/HIV^{MutG} CD8 knockout (KO) Tg mice (red line), CD8 KO mice (green line), CD4C/HIV^{MutG} Tg (blue line) and non-Tg littermates (orange line) were infected intra-orally with 10^8 CFU of *C. albicans*, and burdens were assessed longitudinally by sampling the oral cavity. Data represent the mean ± SD of 6, 6, 10 and 14 mice in each of these four groups, respectively.

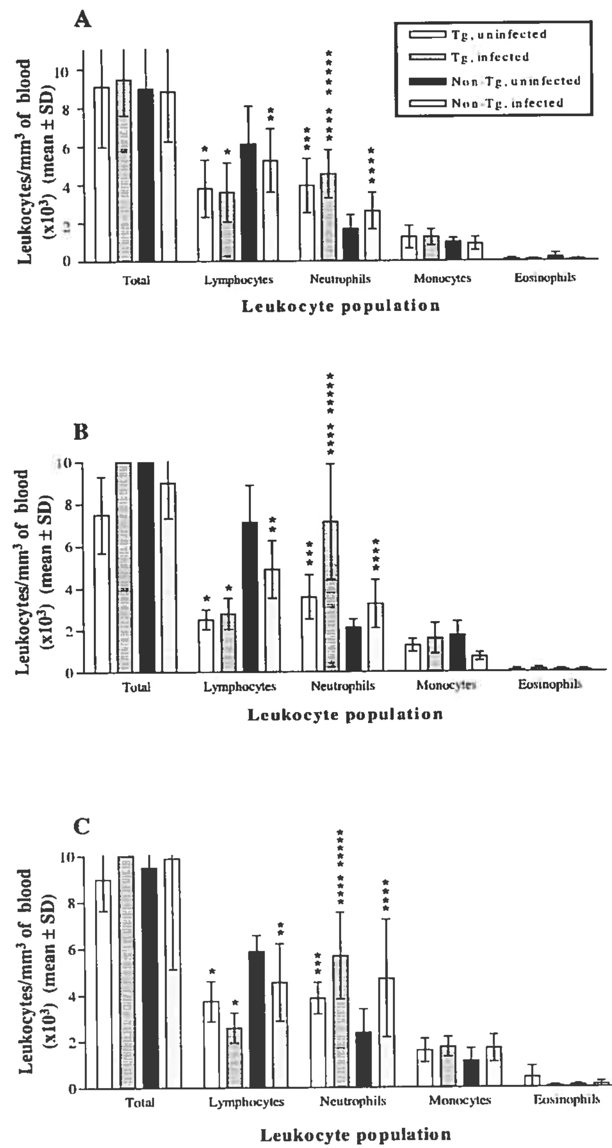

Figure 1

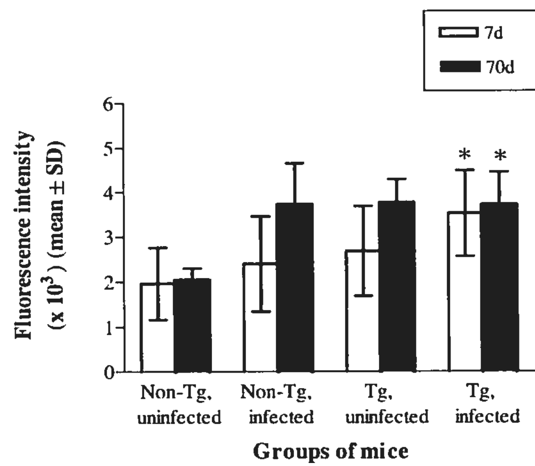

Figure 2

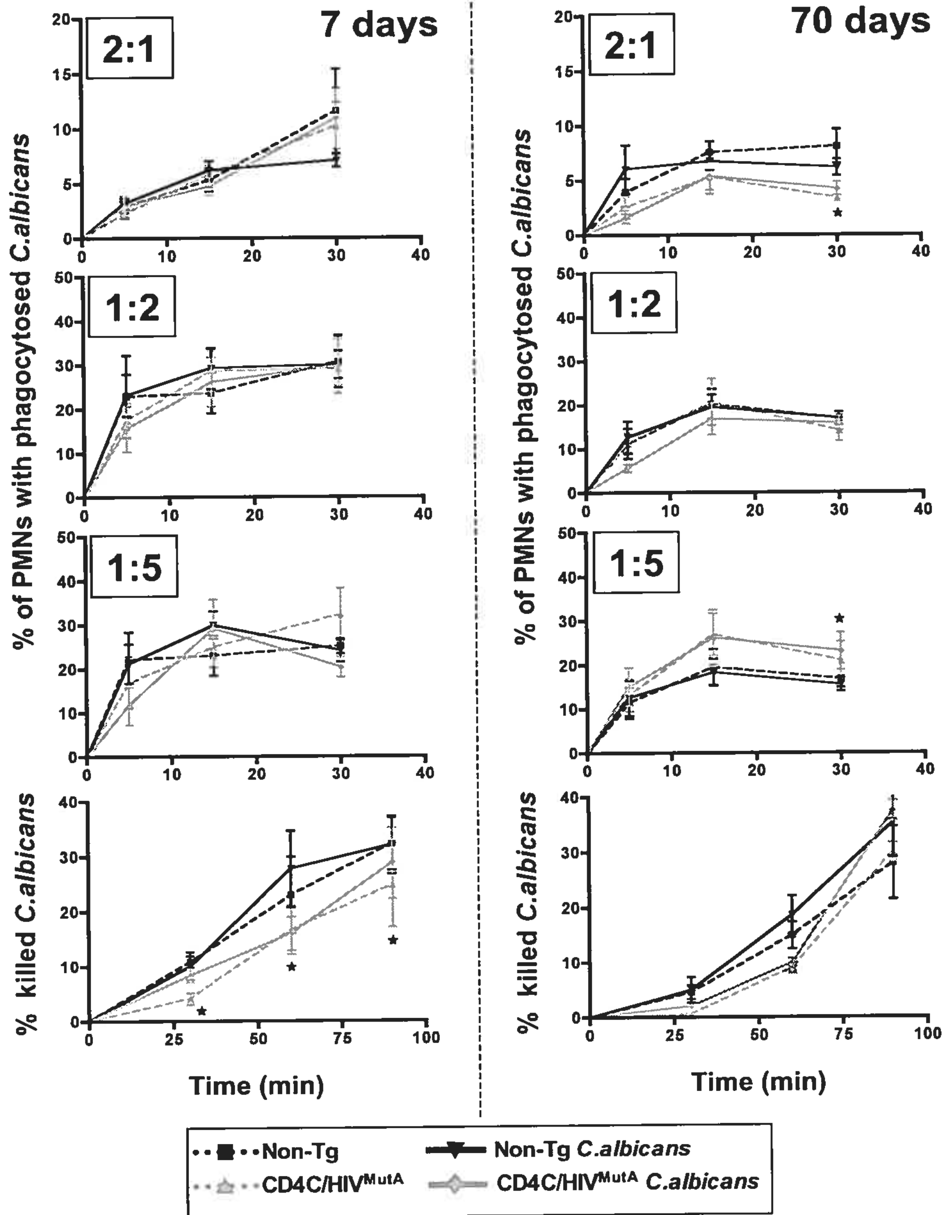

Figure 3

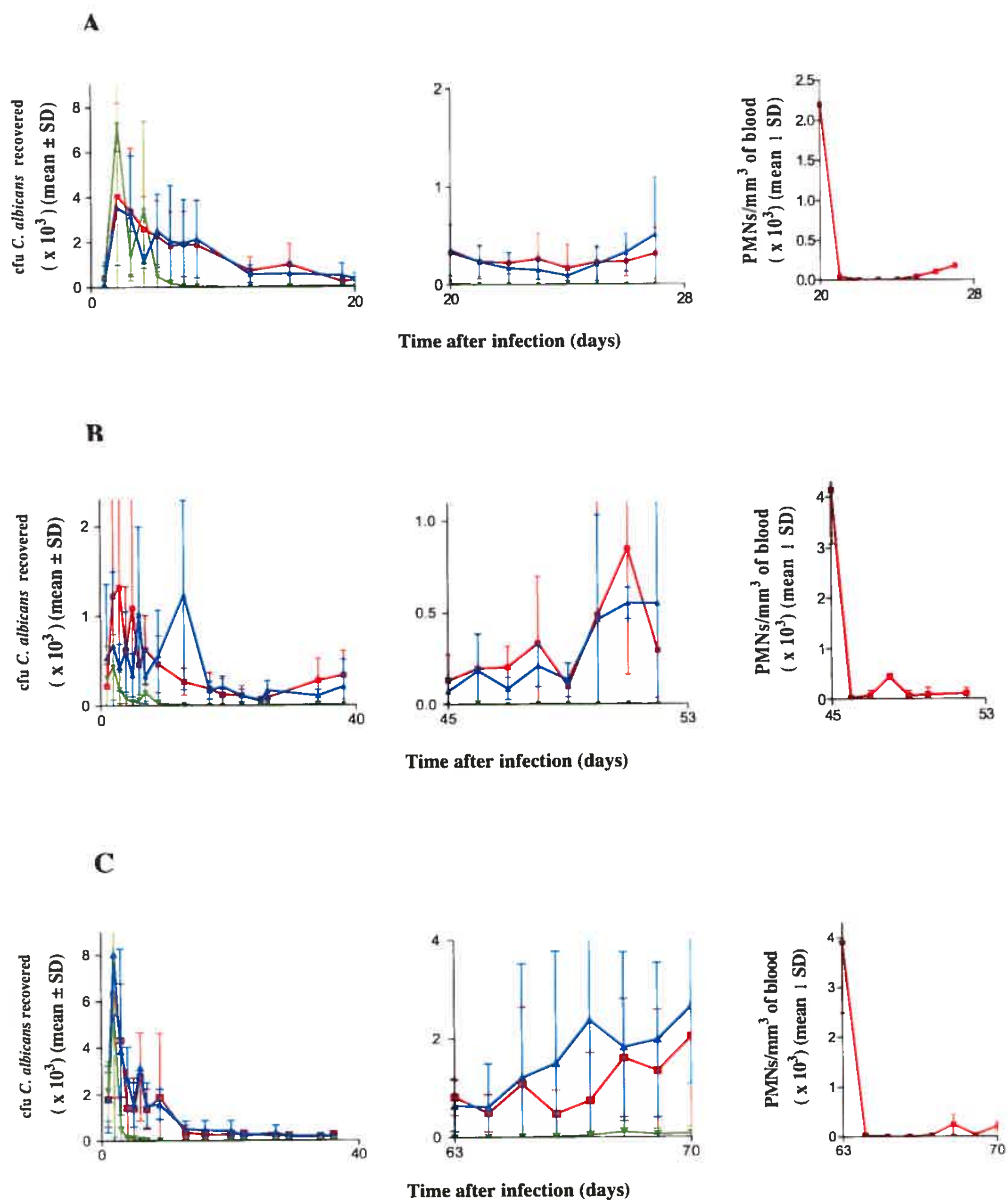

Figure 4

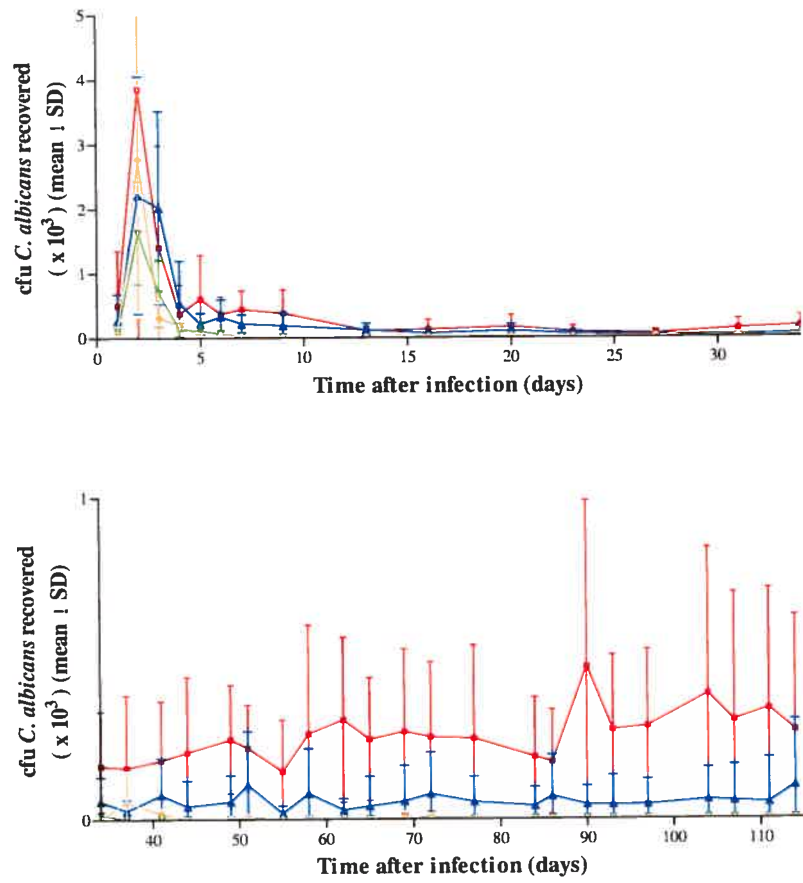

Figure 5

Article 3

Immunopathogenesis of Oropharyngeal Candidiasis in Human Immunodeficiency Virus Infection

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Louis de Repentigny: Introduction, Chapitre 1, 5, Conclusion et correction des chapitres 2, 3 et 4

Daniel Lewandowski: Chapitres 2, 3, 4 et adaptation des figures

Paul Jolicoeur: Chapitre 5

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Immunopathogenesis of Oropharyngeal Candidiasis in Human Immunodeficiency Virus Infection

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INTRODUCTION

Oropharyngeal candidiasis (OPC) is the most frequent opportunistic fungal infection among human immunodeficiency virus (HIV)-infected patients, and it has been estimated that more than 90% of HIV-infected patients develop this often debilitating infection at some time during progression of their disease (374, 375). Although the incidence of OPC in HIV infection has been significantly reduced since the introduction of highly active antiretroviral therapy (HAART) (280), it remains a common opportunistic infection in HIV-infected patients. Clinically, OPC in HIV infection has been classified as exhibiting pseudomembranous and erythematous variants, or angular cheilitis (2). The pseudomembranous form of HIV-associated OPC is characterized by the presence of multifocal

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smooth white papular lesions that can usually be rubbed away, leaving a red surface, and surface pseudohyphae can be readily detected. The erythematous form of OPC typically presents as diffuse and multiple foci of macular erythema involving the palate, oropharynx, buccal mucosa, and dorsal tongue, but hyphae are frequently absent. OPC is frequently complicated by esophageal candidiasis, which may limit food consumption and lead to weight loss, threatening the general health and well-being of HIV-infected patients (428). Furthermore, clinical and in vitro resistance to antifungal azoles frequently occurs in OPC when $CD4^+$ cell counts fall to <200 cells/mm^3 of blood, either by selection or acquisition of resistant strains of *Candida albicans* or by infection with inherently resistant species of *Candida* other than *C. albicans* (273, 332, 343, 344, 354, 380, 445). Of added concern, the full potential impact of antiretroviral therapy on the ability to reconstitute immunity (10, 21, 22, 55, 111, 292, 323, 324, 395) and therefore to reduce the incidence of OPC and esophageal candidiasis (67, 68, 470) has been hampered (362, 429) by suboptimal adherence (235, 441, 443), toxicity (125), and resistance (316, 337) to antiretrovirals, as well as the limited availability of these treatments in developing countries where most HIV-infected patients reside (104).

The leading cause of candidiasis, *C. albicans*, is an imperfect diploid dimorphic fungus that resides as a commensal of the mucosae and the gastrointestinal tract. Intraoral *C. albicans* is found in ~40% of healthy humans (16). However, colonization often leads to opportunistic mucosal or life-threatening deep-organ infection in immunocompromised hosts. Invasion of the human gastrointestinal mucosa by *C. albicans* and its passage across the bowel wall into the bloodstream is an important portal of entry for this opportunistic pathogen into the neutropenic host, leading to systemic or disseminated candidiasis (114, 451). Hematogenous candidiasis is a frequent complication in the treatment of patients with acute leukemia (2, 278). In contrast, *Candida* fungemia is infrequent in HIV-infected patients and is confined mainly to the late stage of HIV infection (247, 333, 438).

The predisposition for OPC and esophageal candidiasis among HIV-infected patients, initially attributed to T-cell impairment, is enigmatic (72, 74, 240, 446). Colonization of oral mucosal surfaces and symptomatic disease are closely correlated with the development and progression of the cellular immunodeficiency of HIV infection (230, 311, 414). However, because *Candida* colonization of the keratinocyte surface occurs without invasion of the submucosa, the occurrence of this superficial fungal disease in a T-cell-poor environment has not been adequately explained. The onset of lesions depends on imbalances between *Candida* virulence attributes and progressively impaired host mucosal defenses in the sequential development of HIV infection, but the exact pathways leading to this imbalance are still unclear. The enhanced risk of OPC and esophageal candidiasis in HIV infection stands in striking contrast to the unenhanced incidence of vaginal candidiasis in HIV-infected women (255, 389), indicating that mucosal immune defense mechanisms and/or their perturbations which favor candidiasis in HIV infection are anatomically compartmentalized (158, 160, 250).

A large body of work conducted with experimentally infected intact or congenitally immunodeficient mice has provided a foundation for understanding the critical roles of Th1 $CD4^+$ T cells, $CD8^+$ T cells, $\gamma\delta$ T cells, macrophages, and polymorphonuclear leukocytes (PMNs) in host defense against mucosal and systemic candidiasis (19, 29, 149, 150, 213, 446). The results of these investigations indicated that protection against mucosal candidiasis involves the recruitment and interactive collaboration of several cell populations which, together, can prevent invasion of mucosal surfaces by *C. albicans* in the normal host. It is thus evident that multiple, rather than single, defects in host defense mechanisms potentially underlie mucosal candidiasis in HIV-infection.

In this review, the salient clinical features of OPC and esophageal candidiasis are correlated with mucosal immune defense mechanisms against *C. albicans* and their perturbations in HIV infection. We also describe how a novel experimental model of oroesophageal candidiasis in transgenic (Tg) mice expressing HIV and developing an AIDS-like disease (116, 363) can be used as a new and powerful tool to investigate critical issues regarding innate and acquired immunity at the level of the oral and esophageal mucosa.

OROPHARYNGEAL AND ESOPHAGEAL CANDIDIASES IN THE SETTING OF HIV INFECTION

Clinical Features and Pathology

The pseudomembranous and erythematous variants of OPC represent the most common clinical presentations of mucosal candidiasis associated with HIV-infection (12). Further clinical variants include angular cheilitis (12), exfoliative cheilitis (360), and *Candida*-associated palatal papillary hyperplasia (359). Recognition of these specific forms of oral candidiasis in HIV-infected patients is facilitated by utilizing established clinical diagnostic criteria (12, 178). Symptoms may include burning pain, altered taste sensation, and difficulty swallowing liquids and solids (155). The pseudomembranous form can be easily diagnosed by demonstrating the presence of candidal yeast and pseudohyphae on wet mounts or stained smears of material obtained by swabbing the lesions and is confirmed by isolation of *Candida* species on culture. In the erythematous form, however, the sparse presence of *Candida* at the mucosal surface frequently requires a biopsy and periodic acid-Schiff staining to establish a formal diagnosis.

At least 75% of HIV-infected patients with OPC have concurrent AIDS-associated (70a) esophageal candidiasis (263) confirmed by histopathology of biopsy material obtained at endoscopic examination (355). While 30 to 43% of these patients may not have symptoms of esophageal involvement, a majority have symptoms including dysphagia and odynophagia (80, 428). For this reason, the combination of OPC and these symptoms of esophagitis is highly predictive of esophageal involvement, and these patients can receive empirical antifungal therapy without confirmation of the diagnosis by endoscopy (15, 349, 428). However, patients who fail to respond to antifungal treatment require esophageal biopsy to assess the possibility of azole-resistant *Candida*, other opportunistic pathogens including herpes simplex virus and cytomegalovirus, and lymphoma or Kaposi's sarcoma.

Because procurement of oral tissue samples is restricted for ethical reasons (358), only a limited number of studies have been conducted to determine the histopathologic and ultrastructural features of OPC in HIV infection (147, 357, 358, 367). In erythematous candidiasis, *Candida* hyphae are few while blastoconidia may be found on an atrophic epithelial surface. In contrast, hyphae are numerous and extend into the spinous cell layer in pseudomembranous candidiasis, accompanied by parakeratosis, acanthosis, and spongiosis of the infected superficial epithelium (357). Of interest, hyphae have been observed to penetrate through intercellular spaces, suggesting that *Candida* can engage in thigmotropism (contact guidance), a phenomenon commonly seen in plant fungi and also recognized in *C. albicans* in vitro (398). In some cases, hyphae are seen to traverse spinous cells and display appressoria-like appendages at their extremities, another common feature in plant fungi which enhances the strength of attachment of the exploring fungal tip (357). Intercellular penetration of hyphae is also facilitated by the detachment of epithelial cell desmosomes, presumably by *C. albicans* secretory aspartyl proteinases (SAPs) and/or phospholipase (357). This particular feature is also observed in non-HIV-infected patients with OPC (294). In addition to the marked contrast in penetration of the epithelium by *C. albicans* in pseudomembranous and erythematous candidiasis, these two forms of OPC are distinguished by the nature and intensity of the mucosal inflammatory cell response (147, 357, 358, 367). The erythematous form in both HIV-infected and uninfected patients is characterized by abundant neutrophilic microabcesses in the parakeratin layer of the epithelium, while microabcesses are rarely found in pseudomembranous candidiasis, even underneath foci of extensive hyphal colonization of the parakeratin layer (147, 358, 367). Indeed, some HIV-infected patients with pseudomembranous candidiasis have almost no epithelial inflammatory response (147, 357). In both clinical forms, however, an abundant mononuclear cell response is observed in the submucosa with no significant difference between HIV-infected and -uninfected patients with the exception of an enhanced infiltration in HIV-infected compared to HIV-uninfected patients with pseudomembranous candidiasis. Immunohistochemical analysis has demonstrated that the inflammatory response in both forms of OPC consists predominantly of $CD8^+$ T cells and $CD1a^+$ Langerhans cells (367). The mechanisms which govern the more intense inflammatory response in erythematous compared to pseudomembranous candidiasis remain unknown but are probably independent of HIV infection and its progression since these differences are also observed in patients who are not infected with HIV (147).

Epidemiology

The development of molecular biology-based methods for discriminating *Candida* strains (121) has provided a vital tool to determine the relationships between progression of HIV infection; acquisition, maintenance, and clonality of oral candidal populations; and selection of resistant *C. albicans* or non-*albicans* species of *Candida* following sustained treatment with antifungal azoles. Using these methods, a number of longitudinal studies have been conducted with HIV-infected patients to prospectively examine the molecular epidemiology of recurrent OPC (32, 246, 344, 354, 380, 445). The majority of patients (77 to 100%) with OPC are infected with *C. albicans*, while the remaining patients are infected with one or more non- *albicans* species of *Candida*, either alone or in combination with *C. albicans* (32, 246, 344, 354, 380, 445). A diversity of non-*albicans* species of *Candida* are found, including *Candida tropicalis*, *Candida parapsilosis*, *Candida guillermondii*, *Candida glabrata*, and *Candida dubliniensis*. However, among these species only *C. dubliniensis* has been specifically associated with and recognized as the sole cause of OPC in HIV infection (88, 246, 290, 386, 422, 445). Analysis of serial isolates has revealed that throughout each episode of OPC, the majority of patients are infected with a unique strain of *C. albicans*, originally present as a commensal of the oral cavity (32, 246, 269, 445). In a minority of patients, other patterns of recurrence are found, including strain replacement with a new genotype of *C. albicans* and species replacement with non-*albicans* species of *Candida* (344, 354, 380, 445). Fluconazole resistance may occur through acquisition of a new resistant genotype of *C. albicans* or by development of resistance in a previously susceptible strain (380). Surprisingly, *C. albicans* strains colonizing HIV-infected patients prior to the first episode of OPC and antifungal therapy exhibit an increased frequency of phenotypic switching which increases the proportion of phenotypes in the colonizing population which are resistant to fluconazole (444). After the first OPC episode, risk factors for the emergence of recurrent OPC caused by fluconazole-resistant *C. albicans* include lower $CD4^+$ cell counts, a greater number of treated episodes of OPC, and a greater duration of prior fluconazole treatment (156, 273). Although colonization with azole-resistant *C. glabrata* increases after treatment with fluconazole (402) it is rarely if ever isolated as the sole cause of recurrent OPC (159, 273, 380, 445). In vitro resistance to fluconazole is strongly correlated with clinical failure of fluconazole treatment of OPC in HIV-infected patients (273, 354, 380) and failure to respond to fluconazole therapy in experimental OPC and esophageal candidiasis (450). The molecular mechanisms of resistance to azole antifungals in *C. albicans* strains isolated from HIV-infected patients are multifactorial, with a predominance of overexpression of genes (*MDR1* and *CDR*) encoding efflux pumps, detected in 85% of all resistant isolates (332). Alterations in the gene encoding the target lanosterol 14-α-demethylase enzyme, including functional amino acid substitutions and overexpression of the gene that encodes the enzyme (*ERG11*), are detected in 65 and 35% of the resistant isolates, respectively (332). Overall, multiple mechanisms of resistance are combined in 75% of the isolates displaying high-level fluconazole resistance (332). Although azole-resistant *C. albicans* strains usually remain confined to a single patient with HIV infection and OPC, the potential for transmission of resistant isogenic strains of *C. albicans* among couples (33, 380) and family members including children (301) has been clearly established.

Although HIV-infected women may develop both OPC and vaginal candidiasis, the risk of OPC alone is enhanced by HIV-infection (255, 389). Molecular typing of *C. albicans* colonizing HIV-infected women revealed that concurrent oral and vaginal isolates were in all cases dissimilar, suggesting that the dominant strains of *C. albicans* colonizing these different mucosal sites are distinct (102). These differences may indicate

an ability of specific genotypes of *C. albicans* to colonize different ecological niches or may result from interhuman transmission of different genotypes to separate mucosal sites.

Correlation with Progression of HIV Infection

OPC and esophageal candidiasis can occur at any time during the course of HIV infection, including primary HIV infection (82, 330), the chronic asymptomatic phase and overt AIDS (1, 80, 152, 177, 237, 277, 285, 329, 366, 389). During the chronic asymptomatic phase, both erythematous and pseudomembranous candidiases are predictive of progressive immunodeficiency and onset of AIDS, independently of the CD4$^+$ cell count (129, 224, 230, 311). Oral burdens of *C. albicans* are augmented in HIV-infected patients even prior to the first episode of OPC (439, 445, 467), and the intensity of carriage increases significantly in the progression from asymptomatic *Candida* carrier to an episode of OPC (445). These observations indicate that normal defenses against *Candida* are perturbed early in the progression of HIV infection before any marked depletion of CD4$^+$ cells has occurred. However, the prevalence of the pseudomembranous form of OPC (152, 237, 277, 285, 329, 445) and esophageal candidiasis (1) increases dramatically in advanced HIV disease associated with CD4$^+$ cell counts of $<200/mm^3$, while erythematous candidiasis and angular cheilitis are less strongly associated with late disease (152, 329, 366). The association of lower CD4$^+$ cell counts and OPC has also been established in HIV-infected women (177, 389) and children (80). Higher HIV RNA burdens are also associated with an enhanced risk of OPC and esophageal candidiasis (1, 53, 277, 329) and inversely correlated with CD4$^+$ cell counts, especially in the absence of treatment with HAART. Overall, these findings suggest that while depletion of CD4$^+$ cells below a critical threshold of 200 cells/mm^3 most often triggers the onset of OPC and esophageal candidiasis, other as yet unidentified perturbations of mucosal immunity against *Candida* appear early during the progression of HIV infection.

Impact of Antiretroviral Therapy

The introduction in 1996 of HAART including protease inhibitors dramatically reduced the prevalence of OPC (17, 68, 127, 280) and esophageal candidiasis (120, 207, 222) in HIV-infected patients. Over a period of 12 months after starting antiretroviral treatment including a protease inhibitor, significant decreases were found in the prevalence of OPC (from 30–56% to 1–9%) (17, 68, 127, 280), the number of relapses of OPC (127), the rate of asymptomatic oral carriage of *C. albicans* (280), and oral candidal burdens (17). An equally striking diminution in the incidence of *Candida* esophagitis ranging from 29 to 42% occurred during the period from 1996 to 1998 compared with the first half of the decade (pre-HAART) (120, 207, 222). A comparable decline in the incidence of esophageal candidiasis has been observed in HIV-infected children since the introduction of HAART (222).

The mechanisms underlying the dramatic impact of HAART on the incidence of OPC and esophageal candidiasis have received close attention (10, 17, 21, 39, 67, 68, 127, 292) and provide valuable insights into understanding the perturbations of mucosal defense mechanisms against *C. albicans* in HIV-infection. Several observations indicate that increases in CD4$^+$ cell counts in response to HAART confer immunologic reconstitution and a decreased incidence of opportunistic infections. Episodes of OPC and esophageal candidiasis that continue to occur despite HAART have done so at low CD4$^+$ cell counts, and patients whose CD4$^+$ cell counts have increased in response to HAART are at lower risk (17, 127, 222, 280), establishing a correlation between CD4$^+$ cell recovery and a decreased incidence of mucosal candidiasis. A three-phase T-cell reconstitution has been demonstrated after HAART, with an early rise in the number of memory CD4$^+$ cells, an improved CD4$^+$ cell reactivity to recall antigens, and a late rise in the number of naive CD4$^+$ cells (21, 22, 292). In addition, proliferative responses to the mitogen phytohemagglutinin develop in the majority of patients in whom responses were absent at baseline (10, 292), and there is increasing interleukin-2 (IL-2), IL-12, and IL-10 production (10). It could therefore be hypothesized that HAART reduces the incidence of mucosal candidiasis by reconstituting delayed-type hypersensitivity to *C. albicans* antigens and a protective mucosal Th1 response to *C. albicans* (42, 70, 211) and rectifying the shift to a nonprotective Th2 response resulting from HIV infection (83). However, in contrast to the frequent recovery of a proliferative response to phytohemagglutinin, treatment with HAART results only in late and inconsistent recovery of anti-candidal cellular immunity, as assessed either by skin test reactivity for delayed-type hypersensitivity or by a proliferative response to *C. albicans* antigens (17, 67, 68, 292). These findings, associated with the resolution of refractory OPC in some HAART-treated patients well before the recovery of CD4$^+$ cell counts and response to *Candida* antigens (67, 68), indicate that the decreased incidence of OPC in patients receiving HAART cannot be fully accounted for by reconstitution of *Candida* cell-mediated immunity (67, 68). Indeed, decrease of the viral load after HAART therapy (10) may also ameliorate mucosal candidiasis by correcting a dysfunction of neutrophils induced by HIV envelope glycoprotein gp41 (143, 168, 454) or by increasing the neutrophil count in HIV-infected patients with neutropenia (127, 471). Evidence has also been presented that HAART has an early, immune reconstitution-independent inhibitory effect on *C. albicans* Saps in the oral cavities of HIV-infected patients (67), and that HIV protease inhibitors attenuate adherence of *C. albicans* to epithelial cells in vitro (39). It has been shown that *C. albicans* strains from HIV-infected patients with OPC have increased expression of Saps (107, 321), possibly enhanced by HIV envelope gp160 and gp41 binding to *C. albicans* (180). Therefore, inhibition of *C. albicans* Saps by HIV protease inhibitors may also contribute to the amelioration of OPC and esophageal candidiasis in HIV-infected patients treated with HAART.

HISTOLOGY OF THE ORAL MUCOSA

The oral mucosa has histological features in common with the vaginal and esophageal mucosas, including a superficial stratified squamous epithelium and an underlying lamina propria of dense collagenous connective tissue, separated by a basal membrane. However, the mucosa of the oral cavity varies in cellular layer composition, depending on the position and function (145, 369, 408). The lining mucosa, including that

found on the cheeks, floor of the mouth, underside of the tongue, and soft palate, represents 60% of the surface area of the oral mucosa. The stratified squamous epithelium in these areas contains a germinating layer (stratum basale) overlying the basal membrane, a spinous layer (stratum spinosum), and a superficial granular layer (stratum granulosum) and is generally nonkeratinized and therefore similar to the esophageal epithelium. The cells undergo structural differentiation as they migrate from the stratum basale to the epithelial surface (408). The masticatory mucosa, found on the gingiva and hard palate, represents 25% of the surface area of the oral mucosa and has an additional keratinized or parakeratinized surface layer resembling that of the skin but lacking a stratum lucidum (145, 369). The specialized stratified squamous epithelium of the dorsum of the tongue (15% of the surface area of the oral mucosa) contains abundant lingual papillae differentiated into four different types: filiform, fungiform, circumvallate, and foliate. The outer surface of the papillae is covered by keratinized epithelium and thus resembles the hard palate, while the interpapillary region is covered by nonkeratinized epithelium similar to that of the lining mucosa (388). The oral epithelium thus varies in the degree of keratinization, cornification, and orthokeratinous and parakeratinous layer thickness found in areas (gingiva, hard palate, and dorsal surface of the tongue) where frictional forces created by biting, chewing, or movement of food occur. Although the thickness of the human oral stratified squamous epithelium shows regional variation ranging from 190 ± 40 μm (floor of the mouth) to 580 ± 90 μm (cheeks) (388), the width of the epithelium is three to five times less in the mouse oral mucosa at each site (197).

Keratinocyte proliferation is stimulated by epidermal growth factor, transforming growth factor α, platelet-derived growth factor, and IL-1 (408). The switch between proliferation and differentiation is modulated by extracellular calcium, phorbol esters, retinoic acid, and vitamin D_3 (408). To ensure a 14- to 20-day median turnover time of oral epithelial cells (408), the keratinocytes attached to the basal membrane lose integrin expression, leading to progressive morphologic changes during migration to the mucosal surface (408). Interestingly, the turnover times of mouse palate and cheek epithelia are slightly shorter than that of tongue epithelium, and the times for all of these tissues are threefold that for epidermis (197). Keratinocytes are linked by desmosomes, which increase in number from the basal to the superficial layer of the epithelium, and by nexus-like (gap) junctions (388, 392). Polygonal and more flattened, upwardly migrating cells discharge the contents of membrane-coating granules by an exocrine process into the intercellular space, forming broad lipid lamellae containing ceramides and acylceramides which serve as a permeability barrier in the keratinized stratified squamous epithelium (399, 408, 455). In nonkeratinized epithelium, intercellular lipid is nonlamellar, contains mainly cholesterol and glycosphingolipids but no acylceramides and only small amounts of ceramide, and provides a less efficient permeability barrier (399, 408, 455). Continuous desquamation of surface keratinocytes of the oral epithelium plays a pivotal role in maintaining a healthy oral mucosa and in limiting candidal colonization and infection (378).

In several regions of the oral cavity, there are nodules of lymphoid tissue consisting of crypts formed by invagination of the epithelium into the lamina propria. These areas are extensively infiltrated by lymphocytes, which play an important role in host defense against oral infections.

ALTERATIONS IN MECHANISMS OF ORAL INNATE RESISTANCE TO *C. ALBICANS* IN HIV INFECTION

The skin and mucosal tissues represent the primary portal of entry for opportunistic pathogens, leading to life-threatening systemic dissemination in the immunocompromised host. In the normal host, however, several redundant immunological and nonimmunological defense mechanisms directly limit the proliferation of pathogenic microorganisms, thus reducing the burden of organisms available for binding to potential attachment sites. In the oral cavity, the flow and composition of saliva establish a dynamic equilibrium between *C. albicans* (361) and other members of the commensal microbiota, preventing the establishment of oral candidiasis in the normal host (37). Salivary flow protects the oral cavity by dislodging yeasts and bacteria, which are then removed by swallowing, and studies have provided evidence that this process may be facilitated by binding of *C. albicans* to salivary mucins (139, 140) or to a nonmucin proteoglycan (198, 199). In patients with Sjögren's syndrome, however, decreased salivary flow and buffering capacity are associated with an increased frequency of carriage of *C. albicans* (361) and of oral candidiasis (7). The prevalence of oral candidiasis in these patients has been estimated at about 35% (7). A similar effect is observed in patients with advanced HIV infection, in whom the salivary flow rate is reduced by 40% and is also correlated with enhanced recovery of *Candida* from saliva (258). The incidence of oral candidiasis is also enhanced in patients with acidic saliva (373), and a low pH increases the adherence of *C. albicans* to epithelial surfaces in vitro (377). Glucose supplementation of saliva augments the growth rate of *C. albicans* in vitro, and the resulting acidic pH provides the required environment for activity of *Candida* Saps, which enhance virulence by degrading a wide variety of host substrates including the mucins, which play an important role in lubrication of epithelial surfaces and host defense (89, 115, 253, 307, 363, 376, 384). The pH of the mucosa also regulates the expression of the *C. albicans* virulence genes *PHR1* and *PHR2* (108) and is thus a significant environmental signal in determining the virulence capacity of *Candida* and/or modulation of the host defenses (372). Finally, biofilm formation by *C. albicans* has been implicated in the ability of the fungus to cause persistent infection on bioprosthetic materials, including denture acrylic, as well as mucosal surfaces (75, 76). However, there were no significant quantitative differences in biofilm formation between *C. albicans* oral isolates from HIV-infected and noninfected patients, indicating that the biofilm-forming ability of *C. albicans* is unlikely to contribute to high levels of oral yeast carriage in cases of HIV infection (212).

Several salivary anticandidal proteins, including lysozyme, lactoferrin, the histatins, calprotectin, and antileukoprotease, inhibit the growth of *C. albicans* and its attachment to the oral epithelium. Because saliva from HIV-infected patients shows decreased anticandidal activity (258), several investigations have focused on identifying putative defects in salivary antimicrobial proteins which may favor oral candidiasis in HIV infection. Lysozyme and lactoferrin are two major nonimmu-

nological antimicrobial proteins in saliva which possess concentration-, time-, and strain-dependent fungicidal activity against *C. albicans* in vitro (379, 466). Lysozyme is found at a concentration range of 1.5 to 57 μg/ml of saliva (350, 419), and its antifungal properties are thought to be mediated by the enzymatic hydrolysis of N-glycosidic linkages in the microbial cell wall and injury to the cytoplasmic membrane following direct cationic-protein binding (279). Interestingly, concentrations of salivary lysozyme are increased in HIV-infected patients with or without oral candidiasis (20, 199, 274, 467), and a trend toward progressive in vitro resistance to lysozyme has been observed in genetically similar, sequential oral *C. albicans* isolates from patients infected with HIV (379). Because the concentration of lysozyme is increased in HIV-infected patients while the anticandidal activity of saliva is decreased, the contribution of salivary lysozyme to limiting the proliferation of *C. albicans* in the oral cavity of these patients appears doubtful.

Lactoferrin is a member of the transferrin family of nonheme iron-binding glycoproteins and is found at the mucosal surface, where it functions as a prominent component of the first line of host defense against infection (452). The concentration of lactoferrin in unstimulated saliva is about 7 to 20 μg/ml (126, 371), and its fungicidal activity against *C. albicans* (404) has been attributed not only to sequestration of ferrous ions (284) but also to structural damage to the fungal cell wall (313) and activation of intracellular autolytic enzymes (243). Salivary concentrations of lactoferrin in patients with HIV infection have been variously reported to be increased (20, 258), unchanged (276), or decreased (266, 300). These variable results have been at least partly ascribed to the source of the saliva, because increased concentrations of lactoferrin in HIV infection have been found in submandibular but not parotid saliva (20, 258, 274). The predisposition to oral candidiasis in HIV-infected patients is thus not convincingly associated with defective production of lactoferrin. In contrast to lysozyme, serial genotypically identical oral isolates of *C. albicans* from HIV-infected patients did not develop progressive in vitro resistance to lactoferrin (379). The therapeutic potential of lactoferrin for the treatment of OPC has recently led to the development of mucoadhesive lactoferrin tablets with fungicidal activity against *C. albicans* and *C. glabrata* (239). This novel approach to the treatment of mucosal candidiasis will require further validation in clinical trials.

The family of salivary histatins consists of at least 12 low-molecular-weight, structurally related, histidine-rich, cationic proteins, which also contribute to nonimmunological host defense of the oral mucosa (138, 437). The histatins have broad fungicidal activity against pathogenic fungi, including *C. albicans*, *Cryptococcus neoformans*, and *Aspergillus fumigatus* (194), and are present at 50 to 425 μg/ml (244) in the saliva of healthy adults. Histatin 5, which exerts potent candidacidal activity (138), is internalized by *C. albicans*, inhibits the respiration of mitochondria, and induces the formation of reactive oxygen species leading to mitochondrial and cytoplasmic membrane damage, efflux of ATP and other nucleotides, and cell death (183, 194). The mechanism of action of the histatins is thus distinct from that of other cationic peptides such as the defensins, which can directly insert into and disrupt cell membranes because of the strongly amphipathic nature of their α-helical structures (138). The concentration of histatins in the saliva of HIV-infected patients has been determined to be increased (20) unchanged (258), or decreased (244, 274), and these seemingly discordant results may have been caused by the different stages of HIV infection among the patients under study as well as by the analytical methods employed. However, decreased concentrations of histatins appeared to correlate with an increased tendency to oral candidiasis in a subgroup of HIV-infected patients (274), suggesting that decreased histatin concentrations and/or an inability of these proteins in saliva to interact with *C. albicans* may contribute to the defective salivary anticandidal activity seen in HIV-infected patients (244). Interestingly, transfer of the gene encoding histatin 3 in the salivary glands of rats by using recombinant adenovirus vectors resulted in its expression at up to 1,045 μg/ml of saliva, suggesting that a gene transfer approach to overexpression of naturally occurring antifungal proteins may be potentially useful in the management of mucosal candidiasis (317).

The heterodimeric calcium- and zinc-binding protein calprotectin is produced by PMNs, monocytes, macrophages and mucosal keratinocytes (54, 73, 403). In vitro, calprotectin quantitatively inhibits the growth of *C. albicans* by depriving the fungus of zinc, which is essential for microbial growth (138). Salivary calprotectin concentrations and oral keratinocyte expression of calprotectin are augmented in response to oral candidiasis, in both HIV-infected and -uninfected patients (146, 231, 424). However, the results of two independent studies demonstrated that salivary concentrations of calprotectin are deficient in HIV-infected patients with oral candidiasis or high salivary *Candida* counts compared to those in HIV-infected patients without oral candidiasis or with low salivary *Candida* counts (298, 424). These results suggested that a diminution of this antimicrobial factor may predispose to oral candidiasis in HIV infection. On examination of the oral mucosa of HIV-infected patients with OPC, however, *Candida* hyphae were found to penetrate through the epithelial parakeratin layer yet did not extend beyond the zone of spinous-layer keratinocyte calprotectin expression (147). Further studies are required to determine whether reduced salivary calprotectin is not simply associated with but directly contributes to the predisposition to oral candidiasis in HIV-infected patients.

Antileukoprotease (436), also known as secretory leukocyte protease inhibitor (141), is produced by keratinocytes (457) and constitutes the last member of the family of antimicrobial proteins involved in nonimmunological defense against *C. albicans* at mucosal sites. Like other cationic antimicrobial polypeptides, the antimicrobial activity of antileukoprotease is limited to conditions of low ionic strength. In addition to its inhibition of leukocyte-derived proteinases, antileukoprotease has fungicidal activity by an unknown mode of action against *C. albicans* which is localized primarily in the NH_2-terminal domain (436) and it may thus play an important role in the innate mucosal defense. Interestingly, antileukoprotease exhibits anti-HIV-1 activity in vitro and may contribute to the antiviral activity of saliva associated with the infrequent oral transmission of HIV-1 (287).

ORAL MUCOSAL IMMUNE SYSTEM AND HOST DEFENSES AGAINST *C. ALBICANS*

Cells with Immune Potential in the Oral Mucosa

The oral mucosa is continuously challenged by the resident microbial flora and occasionally by microbial pathogens; it is therefore armed with several cell populations which individually, or in association, can produce a protective innate or acquired immune response. Mucosal cell populations with immune potential include Langerhans' cells, macrophages, αβ- and γδ-T cells, keratinocytes, and PMNs. We now review the specific properties of these cell populations and their role in host defense.

Lymphoid cells. The oral mucosal immune system possesses features in common with both the skin immune system and the mucosal immune system (442). The normal oral mucosa shares with normal skin an absence of B lymphocytes, which are present in the mucosal immune system (50, 442). In contrast to the skin, however, T lymphocytes in the normal human oral mucosa are not organized in rows around postcapillary venules of the superficial and deep vascular networks (50) but are distributed singly or in small clusters on both sides of the basal membrane (442). In addition, T lymphocytes are only rarely found in the more superficial layer of the epithelium. The oral mucosal epithelium contains about 37 times as many T lymphocytes as the epidermis of normal skin (442). The vast majority of T lymphocytes in the oral mucosa express the memory $CD45R0^{+}$ phenotype (86, 340). T lymphocytes within the oral epithelium are not activated ($CD25^{-}$), in contrast to $CD25^{+}$ T cells in the underlying stroma (86). The conversion of T cells from the naive $CD45RA^{+}$ to the memory $CD45R0^{+}$ phenotype requires repeated antigenic stimulation, suggesting that apoptotic $CD25^{-}$, $CD45RA^{+}$ intraepithelial T cells die after unsuccessful antigen presentation by Langerhans' cells (86).

The CD4/CD8 ratio of 1:2 in the human oral epithelium and 1:4 in the skin indicates the preferential presence of the $CD8^{+}$ subset in both sites, but $CD4^{+}$ cells are proportionately more frequent in the oral mucosa than in the skin (442). However, a CD4/CD8 ratio of 1 within the epithelium of the normal human gingiva indicates regional variation in the oral cavity (86). In contrast to normal humans, $CD4^{+}$ cells are twice as numerous as $CD8^{+}$ cells in the normal murine oral mucosa (47). $CD4^{+}$ T cells are required for a Th1-type protective response against oral candidiasis in mice (149) and therefore play a central role in host defense against OPC.

Of direct relevance to host defense against OPC in HIV infection, the buccal epithelium is an inductive site for the generation of cytotoxic T-lymphocyte responses mediated by major histocompatibility complex (MHC) class I-restricted $CD8^{+}$ T cells, independent of $CD4^{+}$ cell help (119). It has been suggested that $CD8^{+}$ T lymphocytes are attracted to the epithelium by IL-8 produced by keratinocytes (267, 442). Moreover, IL-2 (but not gamma interferon [IFN-γ])-activated $CD8^{+}$ cells exert direct growth inhibition against the hyphal form of *C. albicans* (40). However, $CD8^{+}$ cells may not be in proximity to *C. albicans* hyphae, which are usually confined to the upper layers of the epithelium (72, 147, 357). Alternatively, $CD8^{+}$ cells may produce cytokines which enhance the antimicrobial activity of macrophages and neutrophils against *C. albicans*. In addition, MHC class I molecules expressed constitutively on keratinocytes may represent a target for $CD8^{+}$ cytotoxic T lymphocytes after internalization by keratinocytes of microbial pathogens (448).

γδ T cells represent at most 2% of the T-cell population in the human oral epithelium (331). Oral mucosal γδ T cells display ultrastructural features typical of large granular lymphocytes, also found in cytotoxic $CD8^{+}$ and NK cells (267), and probably represent a first immunologic line of defense. γδ T cells are located within the epithelium in both normal and inflamed gingiva, often in close proximity to $CD1a^{+}$ and/or $CD1c^{+}$ Langerhans' cells and keratinocytes (268). In inflamed mucosa the γδ T cells show the phenotype of activated cells ($CD45RO^{+}$, $CD8^{+}$, or $CD4^{+}$), whereas in normal mucosa the cells are $CD4^{-}$ $CD8^{-}$ and express CD45RA (268). In the connective tissue, under the basal membrane, $V\delta2^{+}$ γδ T cells are predominant, whereas the epithelium contains mostly $V\delta1^{+}$ γδ T cells (206, 331). γδ T cells participate in the immune response to microbial pathogens including *C. albicans* by producing cytokines such as IFN-γ (213, 267) or by direct cell-to-cell contact leading to cytotoxicity (267, 303). Increases in the numbers of γδ T cells have been found in the oral mucosa soon after mice are colonized and infected with *C. albicans* (71), coinciding with resolution of infection.

Finally, natural killer (NK) cells are large granular lymphocytes which represent 6 to 39% of human gingival (268) and 3% of lower lip (283) mononuclear cells. NK cells are cytotoxic in vitro to certain tumor cell lines and to virally infected cells (72) and have direct antimicrobial activity against *Cryptococcus neoformans* (256) but little or no effect against *C. albicans* hyphae in vitro (40).

Langerhans' cells. Langerhans' cells develop from bone marrow stem cells as one of three distinct subsets of dendritic cells (DCs) which home in to selected tissues (30, 99, 448). The bone marrow stem cells appear to be common precursors of both macrophages and DCs (30). Serving as an essential link between innate and acquired immunity, dendritic cells function as antigen-presenting cells (APCs) that patrol all tissues of the body, capturing pathogens for processing and presentation to T cells in the secondary lymphoid organs. Two subsets of human DCs, Langerhans' cells and interstitial (or dermal) DCs, belong to the myeloid lineage, while the third subset is composed of lymphoid DCs (99). Culture of human $CD34^{+}$ hematopoietic progenitors in the presence of granulocyte-macrophage colony-stimulating factor (GM-CSF) and tumor necrosis factor alpha (TNF-α) gives rise to $CD1a^{+}$ DCs related to Langerhans' cells and $CD14^{+}$ DCs closely related to interstitial DCs, which can differentiate into macrophages in the presence of M-CSF (69). DCs are thus phenotypically and functionally heterogeneous depending on their specific differentiation pathways (11, 468, 469). Serving as sentinels for pathogen entry at the epithelium of the skin and mucosa, Langerhans' cells, identified by expression of CD1a, Lag, and langerin, are localized on the basal and suprabasal layers and represent 2 to 4% of the cells in the epithelium (6, 34, 49, 59, 96, 99, 103, 216, 245, 262, 351, 356, 365, 390, 391, 442). These cells express MHC class II molecules and are also $CD11b^{+}$ and $CD11c^{+}$. Langerhans' cells have a pronounced dendritic shape and contain rod-shaped organelles called Birbeck granules. Immature epithelial Langerhans' cells are equipped to capture antigens by

phagocytosis, macropinocytosis, and receptor-mediated absorptive endocytosis, including the macrophage mannose receptor, DEC-205, as well as Fcγ and Fcε receptors (30). The loose interaction of DC-specific, ICAM-3 grabbing, nonintegrin (DC-SIGN) with ICAM-3 establishes the initial contact of the Langerhans' cell with a resting T cell in the apparent absence of foreign antigen (416). To successfully present antigens for T-cell activation, Langerhans' cells must undergo a maturation process (334) triggered by whole bacteria, bacterial lipopolysaccharide, cytokines such as TNF-α and IL-1β, or the T-cell CD40 ligand (CD40L) (30, 99, 216, 458). Mature Langerhans' cells lose the ability to take up antigens but express surface molecules required for communication with T cells at the immunologic synapse (416). During maturation, Langerhans' cells express high levels of surface MHC class I and II and the costimulatory molecules CD54, CD58, and CD86 that interact with receptors on T cells to enhance adhesion (30, 99, 416). In addition, high CD40 expression on mature Langerhans' cells favors binding to CD40L on T cells, which in turn up-regulates the expression of CD80 and CD86, secretion of IL-12, and release of chemokines such as IL-8 and macrophage inflammatory proteins 1α and 1β (MIP-1α and MIP-1β) (30). Antigen presentation via MHC class II molecules in the presence of IL-12 and collaborating IL-18 induces $CD4^+$ cells to differentiate into IFN-γ-producing Th1 cells, leading to activation of the antimicrobial properties of macrophages, and is therefore critical to the induction of a protective acquired cell-mediated immune response (30, 99, 308). Furthermore, Langerhans' cells receiving T-cell help mediated by CD40-CD40L interactions (385) can also present antigen on MHC class I molecules to cytotoxic $CD8^+$ cells, which can be loaded through both endogenous and exogenous pathways (30, 99). More recent work, however, indicates that Langerhans' cells do not need to receive a signal from T cells to become fully mature DCs capable of stimulating $CD4^+$ T cells and cytotoxic $CD8^+$ T cells (110). Interestingly, the murine oral mucosa is an inductive site for priming class I-restricted $CD8^+$ cytotoxic T cells in vivo (119). The expression of MIP-3α by TNF-α-stimulated keratinocytes in the spinous layer (77, 435) and the production of defensins (464), which both recognize the CCR6 chemokine receptor in immature DCs, may explain the positioning of Langerhans' cells in the epidermis and their ready access to microbial pathogens (99). The mobilization of Langerhans' cells and their migration via afferent lymphatics to draining lymph nodes for antigen presentation (208) is governed by the upregulation of the chemokine receptor CCR7 and the production of MIP-3β (405). In addition, IL-18 produced by Langerhans' cells and keratinocytes also contributes to the regulation of Langerhans' cell migration by a TNF-α and IL-1β-dependent mechanism (98).

In normal humans, the density of Langerhans' cells in nonkeratinized oral mucosa is apparently the same as in the skin, but keratinized oral mucosa has fewer Langerhans' cells (96, 103, 442). Although murine palate implants are repopulated by Langerhans' cells within 2 weeks (365), the numerical densities of Langerhans' cells in old mice is reduced compared with that in young mice (364). At the ultrastructural level, murine and human Langerhans' cells in the oral mucosa exhibit no significant differences (59). In the normal human oral mucosa, however, Langerhans' cells present a highly variable morphology according to their epithelial location (391). In contrast to the upper epithelium, where $CD1a^+$ Langerhans' cells have long dendrites forming a network, Langerhans' cells in the basal layer are more rounded and have very few short dendrites. Functionally, the well-developed dendritic morphology of Langerhans' cells in the upper epithelium could reflect optimal immune surveillance (391). Conventionalization of germfree mice with a bacterial flora results in enhanced migration of Langerhans' cells to the oral epithelium (49), and the densities of oral epithelial Langerhans' cells are increased in patients with chronic periodontitis compared to healthy controls (216), demonstrating that Langerhans' cells are recruited to the oral epithelium in response to a bacterial challenge. Purified human (35) or rat (192) oral mucosal Langerhans' cells can serve as APCs in vitro and are more efficient than skin Langerhans' cells in providing costimulatory signals to T cells (193). *C. albicans*-specific T-cell activation by human epidermal Langerhans' cells (85, 223) requires not only the ligation of the T-cell receptor to the antigen-MHC complex but also costimulation by the combination of adhesion molecules CD54 and CD58 with CD11a and CD2 on T cells, respectively (433). As described in further detail below, productive infection of oral mucosal Langerhans' cells by HIV-1 may contribute to their selective depletion (81) and perturb their ability to generate a primary immune response (44), which may impair protective mucosal immunity against colonization and infection by opportunistic microbial pathogens. In addition, Langerhans' cells serve as the portal of entry for HIV-1 at mucosal sites and are critical to the initiation and subsequent spread of infection to draining lymphoid tissue (340).

Keratinocytes. Keratinocytes are of primary importance in the pathogenesis of OPC since they constitute a physical barrier after adhesion of *C. albicans* to the epithelial surface. In addition to their role as a mechanical barrier, epithelial keratinocytes function as fixed or immobile immunocytes and are capable of producing a number of soluble factors and expressing receptors that are involved in up-regulating and down-regulating immune responses (179, 415, 440). The major growth factors produced include basic fibroblast growth factor, platelet-derived growth factors, transforming growth factors α and β, and TNF-α. Keratinocytes also produce several cytokines including IL-1, IL-3, IL-6, IL-7, IL-8, IL-10, IL-12, IL-15, IL-18, and IL-20, and a number of CSFs such as GM-CSF, G-CSF, and M-CSF (14, 45, 179, 440, 448). Under normal conditions, most of these mediators are not constitutively produced (162), but their gene expression and release is up-regulated during inflammation by a variety of external stimuli derived from leukocytes, Langerhans' cells, and keratinocytes themselves, including IFN-γ, TNF-α and IL-17 (14, 241, 257, 282, 432, 448). Interestingly, mRNA expression of IL-1α, IL-1β, IL-8, GM-CSF, and TNF-α is up-regulated in experimental cutaneous *C. albicans* infection with reconstituted human epidermis, demonstrating that the fungus induces a brisk cytokine response by host keratinocytes (383). *C. albicans* also triggers the production of IL-1α and TNF-α (410), as well as GM-CSF (131), by primary oral epithelial cells and oral epithelial cell lines in vitro. In addition, proteolytic activation of the IL-1β precursor by *C. albicans* Sap (38) suggests that candidal proteinases may contribute to the activation and maintenance of the inflammatory response at the epithelial surface. IL-1, IL-8,

and IL-12 possess attractant effects on PMNs, macrophages, and lymphocytes (448). In addition, some of the cytokines produced by keratinocytes (IL-1 and TNF-α) promote the maturation of DCs and therefore could differentially modify the ability of Langerhans' cells to respond to antigens (448). Keratinocyte-derived IL-7 and IL-15 are involved in epidermal T-cell trafficking (179, 448). In addition to the production of soluble factors, keratinocytes express the adhesion molecules CD54 and CD58, and CD54 expression is increased by IFN-γ (136, 448). MHC class I molecules are expressed constitutively and may be a target for $CD8^+$ T cells (448). MHC class II molecules are not expressed constitutively but can be induced by IFN-γ produced by infiltrating T lymphocytes (448). Keratinocytes may function as accessory cells in antigen presentation and interact with lymphocytes to produce a Th2 cytokine response (448).

Of potential relevance to the fragile equilibrium between epithelial colonization and infection, IFN-γ promotes expression of the glycoprotein desquamin, a cell adhesion molecule in the stratum corneum of the human epidermis which possesses lectin-like properties for amino sugars (58), as well as trypsin-like serine proteinase (57) and RNase (393) activity. Desquamin may thus play a crucial role in desquamation and shedding of *Candida* from the superficial portion of the epithelium.

In addition to these indirect mechanisms, keratinocytes possess several potential antimicrobial mechanisms which may directly contribute to host defense against *Candida*. (i) Keratinocytes have been shown to express inducible nitric oxide synthase activity (43), and NO has been associated with candidacidal activity and resistance to mucosal candidiasis (213). (ii) Human oral keratinocytes produce numerous antimicrobial peptides, including β-defensins 1 to 3 (134, 135, 191, 261, 281, 387), cathelicidins (132, 166, 167, 182, 465), adrenomedullin (220, 221), calprotectin (54, 73, 146, 147, 231, 298, 370, 403, 424), and bactericidal/permeability-increasing protein (BPI) (61), which, as natural antibiotics, contribute to the innate immunity of the epithelium (170, 453). β-Defensins exhibit potent antimicrobial activity against *Candida* (387), and their expression by keratinocytes at the mRNA and protein level is enhanced by TNF-α, IL-1β, whole bacteria, and bacterial lipopolysaccharide (191, 261, 281, 387). Although epithelial injury or inflammatory disorders augment the expression and release of the human cathelicidin LL-37 from keratinocytes (132, 166), its antimicrobial activity in vitro has so far been demonstrated only against bacteria and the MBCs against *Candida* species are >100 μg/ml (182). In addition to their direct antimicrobial properties, human β-defensins and the cathelicidin LL-37 are chemotactic for immature DCs and neutrophils and for monocytes and T cells, respectively (465). Secretion of the vasoactive peptide adrenomedullin from oral keratinocytes is stimulated by IL-1α, IL-1β, TNF-α, LPS, and live bacteria but not by *C. albicans* (220, 221). Although adrenomedullin possesses antimicrobial properties, it is not yet known whether it contributes to host defense against oral candidiasis. As outlined previously in this review, oral keratinocytes also express calprotectin, a heterodimer of MRP8 and MRP14 with antimicrobial activity against *C. albicans*. The up-regulated expression of calprotectin by oral keratinocytes in response to infection has been investigated in vitro and appears to be independent of IL-1β (370). Finally, keratinocytes express on their cell membranes BPI, which is also an abundant constituent of PMNs (61, 170). BPI on keratinocytes contributes to the killing of gram-negative bacteria that become closely adherent to epithelial cells (61, 170). The role, if any, of BPI in limiting *C. albicans* colonization or infection of the oral mucosa remains to be determined. (iii) Human oral keratinocytes directly inhibit the growth of blastoconidia and/or hyphae of *Candida* species in vitro, with a strict requirement for cell contact (411). Growth inhibition appears to involve a carbohydrate moiety on the surface of the keratinocytes but is not mediated by phagocytosis, nitric oxide, superoxide-hydrogen peroxide pathways, or defensin and calprotectin antimicrobial peptides (412). Direct growth inhibition of *Candida* by oral keratinocytes appears to occur through a novel and distinct mechanism, complementary to other components of the antimicrobial armamentarium of oral keratinocytes. Oral epithelial keratinocytes are thus equipped with numerous redundant defense mechanisms, acting either directly or indirectly against the continuous microbial challenge at the oral mucosal surface. The role of keratinocytes in host protection against *Candida* at mucosal surfaces appears likely, since *C. albicans* hyphae are restricted to the upper layers of the oral epithelium in OPC and are some distance away from lymphocytes and Langerhans' cells located in deeper layers.

Macrophages and PMNs. Macrophages and PMNs originate from monoblasts and myeloblasts, distinct populations of myeloid stem cells which differentiate into monocytes and neutrophils in the bloodstream. In the normal uninfected host, circulating monocytes differentiate into resident tissue macrophages, in contrast to PMNs, which are retained almost exclusively within the circulation. Because of their key role in the innate immune response (289), these two cell populations are critical effectors in the first line of defense against oral microbial pathogens. In the normal human oral mucosa, macrophages are located mainly in the lamina propria (86) while PMNs appear in the lamina propria and epithelium only in response to inflammation (268). Macrophages are not a homogeneous cell population but can be separated into biologically active subpopulations which appear at early, intermediate, or late stages of inflammation (185).

Oral mucosal resident macrophages express MHC class II molecules and CD11b, as well as Fc receptors that bind IgG (FcγR) (31). Like Langerhans' cells, macrophages present antigenic peptides to $CD4^+$ T cells after induction of CD86 costimulatory molecules (112, 113). Th1 $CD4^+$ T cells secrete IFN-γ and IL-2, which activate both macrophages (97) and $CD8^+$ cytotoxic T cells to kill intracellular pathogens (113). After activation, macrophages produce TNF-α, which activates PMNs, further amplifying the innate immune response (19). For this reason, macrophages play a critical dual role at the crossroads of innate and acquired cell-mediated immunity. Indeed, activation of a specific T-cell response to *C. albicans* antigens in vitro has been found to require macrophages expressing MHC class II molecules (314).

To date, Langerhans' cells, monocytes, macrophages, and PMNs are the only cells that have been reported to be candidacidal (132a, 213, 310). Macrophages and PMNs have the ability to kill both *C. albicans* blastoconidia and hyphae by both oxygen-dependent and -independent mechanisms (446). Oxy-

gen-dependent killing by PMNs is mediated by superoxide anion and the myeloperoxidase-hydrogen peroxide-halide system, with the added participation of reactive nitrogen intermediates including NO and peroxynitrite in the candidacidal activity of macrophages which lack myeloperoxidase (446). Production of IFN-γ by γδ T cells augments NO production by macrophages and enhances resistance to orogastric candidiasis, indicating that γδ T cells indirectly contribute to macrophage killing of *C. albicans* (213). Macrophages and PMNs are also equipped with oxygen-independent mechanisms including the cationic protein defensins (446) and calprotectin (54, 73, 403), demonstrating the use of an extensive array of oxidative and nonoxidative mechanisms to kill *C. albicans* blastoconidia and hyphae (446).

In experimental OPC in the mouse model, the early inflammatory response 24 to 48 h postinfection is composed of large numbers of PMNs migrating from the lamina propria to accumulate in the superficial epithelial layers (242). During recovery from primary infection, at 5 to 6 days postinfection, the initial influx of PMNs is replaced by a massive recruitment of macrophages in the lamina propria (71). The presence of both macrophages and PMNs in experimental candidiasis concurs with similar histologic findings in HIV-infected patients with OPC, suggesting a major role for these professional phagocytes in mucosal containment of *C. albicans*.

Mechanisms of Protective Cellular Immunity to *C. albicans* in the Oral Mucosa

A foundation for understanding the complex mechanisms critical to host defense against *C. albicans* at mucosal sites was provided by a large body of work conducted with experimentally infected, congenitally immunodeficient mice (26, 27, 63, 64, 209, 210, 309, 446), as well as in intact (94) or knockout (28) mice depleted of specific factors ($CD4^+$ cells or IFN-γ). These studies demonstrated that functional T cells play a role in resistance to *C. albicans* colonizing or infecting mucosal surfaces and that an added defect of phagocytes is required to produce dissemination of *C. albicans* from the gastrointestinal tract (63, 209). Further investigation showed that, although Th1 and Th2 $CD4^+$ cells are involved in recovery from primary gastrointestinal candidiasis in immunocompetent mice, activation of a Th1 response occurs in animals that show delayed-type hypersensitivity to *Candida* and protection after a second gastrointestinal inoculation (70). Studies of B-cell knockout mice demonstrated that antibodies do not play a role in protection against mucosal candidiasis or dissemination from the gastrointestinal tract (449). However, a protective role of antimannan antibodies has been demonstrated in experimental vaginal candidiasis (106, 184). Overall, these investigations have produced the current paradigm of a central role for a Th1 $CD4^+$ response in host defense against mucosal candidiasis (42, 70, 211, 406).

In contrast to gastrointestinal and vaginal candidiasis, relatively few hypothesis-driven, cause-and-effect investigations have been conducted to specifically elucidate the mechanisms of host defense against *C. albicans* in the oral mucosa (157). The accumulated evidence indicates that normal host defense against OPC is the sum of individual redundant mechanisms which include several salivary anticandidal proteins, growth inhibition of *C. albicans* by oral keratinocytes, and the presence of T-cell-mediated delayed-type hypersensitivity to *C. albicans*. The evidence implicating anticandidal proteins and oral keratinocytes, described in previous sections of this review, has so far been derived solely from observations of in vitro activity against *C. albicans*. Although their role in host defense appears likely, no direct demonstration has been presented using compelling approaches such as their depletion, augmentation, or transfer in an experimental model of OPC. Consequently, mechanistic investigations of host defense in experimental OPC have been focused almost entirely on dissecting the precise role of an acquired cell-mediated immune response to *C. albicans*.

Although this has not yet been directly studied in experimental OPC, oral mucosal Langerhans' cells are most probably involved in the initiation of an acquired cell-mediated immune response to *C. albicans*. Both human (310) and murine (132a) DCs recognize *C. albicans* by the mannose-fucose receptor, can phagocytose and degrade *Candida*, and can subsequently present *Candida* antigens to T cells. Interestingly, the yeast and hyphal forms of *Candida* are ingested by different mechanisms and receptors. Phagocytosis of the yeast cells by DCs occurs by coiling phagocytosis, characterized by the presence of overlapping bilateral pseudopods, whereas ingestion of hyphae occurs through a more conventional zipper-type phagocytosis (132a). Human DCs kill *Candida* as efficiently as human monocyte-derived macrophages do, and killing appears to be mainly oxygen independent, possibly via lysosomal hydrolases (310). In contrast, killing of *C. albicans* yeast cells or hyphae by murine DCs is correlated with the production of NO (132a). The T-cell proliferation observed with a mixture of human DCs, *Candida*, and T cells most probably represents a secondary immune response, since *C. albicans* is a commensal in humans (310). However, analogous experiments conducted using murine DCs required the presence of IL-2 to elicit a priming response since *C. albicans* is not a commensal organism in mice (132a). In vitro, ingestion of the yeast form of *C. albicans* activated DCs for IL-12 production and priming of Th1 cells whereas ingestion of hyphae inhibited IL-12 and Th1 priming and induced IL-4 production (132a). The pivotal role of DCs in initiating the immune response to *C. albicans* was elegantly demonstrated by the generation of protective immunity against intravenous infection after injection of DCs ex vivo pulsed with *C. albicans* yeasts but not hyphae (132a). Yeast-pulsed DCs from IL-12 knockout mice primed lymphocytes for IL-4 production in vitro and were unable to confer resistance to candidiasis (132a), consistent with the lack of Th1 response development (272). Finally, work from the same group showed that murine DCs pulsed with yeast but not hyphal RNA induce protective immunity to *C. albicans* in allogeneic bone marrow-transplanted mice (24).

In addition to Langerhans' cells, macrophages and keratinocytes could be potentially involved in the processing and presentation of *Candida* antigens to $CD4^+$ cells and could therefore also participate in the induction of an adaptive immune response to *C. albicans* in the oral cavity. Keratinocytes of the reproductive tract express MHC class II molecules and can function as APCs (459). In addition, expression of MHC class II molecules by epithelial keratinocytes is enhanced in patients with angular cheilitis (320) or OPC (214), possibly in response

to IFN-γ produced by infiltrating T lymphocytes (18, 448). However, the ability of oral keratinocytes to engage in presentation of *Candida* antigens is uncertain, since these cells do not appear to have the capacity to engulf *C. albicans* (412), and the epithelial CD4$^+$ cells are located above the basement membrane and therefore not in proximity to the superficial keratinocyte layer where *C. albicans* is localized. Although not formally demonstrated in OPC, the participation of macrophages in *Candida* antigen presentation is more likely, since these cells are a prominent component of the innate immune response to *C. albicans* and fulfill all the requirements for engulfment, killing, and presentation of *C. albicans* antigens to CD4$^+$ cells (18, 173, 314). Of direct relevance to this process, the ability of human monocytes to phagocytose the yeast but not the hyphal form of *C. albicans* is correlated with enhanced induction of IL-12, again indicating that *C. albicans* yeasts are specifically involved in promoting Th1 protective immunity (79).

The initiation of the mucosal immune response to *C. albicans* in OPC requires the maturation and mobilization of the Langerhans' cells (30) resulting either directly from exposure to *C. albicans* or from cytokines produced by the T-cell response to infection (30). Maturation of DCs, including mucosal Langerhans' cells, is characterized by a strong up-regulation of MHC class II expression, secretion of IL-12, and expression of the adhesion molecules CD54, CD58 and CD86 (30). Enhancement of MHC class II expression on APCs by coculture with *C. albicans* or exposure to *C. albicans* antigens has been demonstrated in vitro (18), in accordance with the ability of microbial pathogens and their products to directly induce the maturation of DCs (30, 458). Cytokines such as IL-1, GM-CSF, and TNF-α, as well as the T-cell ligand CD40L, which binds CD40 on dendritic cells, may also contribute to maturation of Langerhans' cells in mucosal candidiasis (30). MHC class II alloantigens (173, 314, 433) and the adhesion molecules CD54 and CD58 (433) are all directly involved in *Candida*-specific T-cell activation by APCs and therefore most probably participate in this critical step in the afferent limb of the specific immune response in OPC.

Several lines of evidence in support of the protective role of acquired cell-mediated immunity in OPC have been presented by Ashman and colleagues, using experimentally infected mice (142, 149, 150). The differences between the colonization patterns of *C. albicans* in infection-resistant BALB/c mice and infection-prone DBA/2 mice following oral inoculation correlated with both *Candida* antigen-specific T-cell proliferation and early expression of IL-4, IFN-γ, and IL-12 in cervical lymph nodes, supporting the concept of a balanced Th1 and Th2 response in clearing OPC (142). A constitutive mixed Th1/Th2 cytokine expression (Th0) was also found in whole saliva of healthy HIV-negative individuals with (251) or without (252) *Candida*-associated denture stomatitis. Although associated with a Th2 response, IL-4-enhanced resistance to OPC may be mediated by the promotion of a protective Th1 response (291) and by enhanced killing of *Candida* by both PMNs (46) and macrophages (153). Although systemic depletion of CD4$^+$ cells alone did not increase the severity of oral infection in immunocompetent BALB/c and CBA/CaH mice (150), reconstitution of immunodeficient BALB/c and CBA/CaH *nu/nu* mice with naive CD4$^+$ but not CD8$^+$ T cells significantly decreased oral colonization compared to that in controls and was correlated with expression of IL-12 and IFN-γ in cervical lymph nodes (149), demonstrating the direct requirement for T lymphocytes in recovery from OPC. The depletion of PMNs and the inactivation of monocytes/macrophages increased the severity of infection in immunocompetent BALB/c and CBA/CaH mice, clearly demonstrating the critical role of these professional phagocytes in the efferent limb of the immune response (150). It thus appears that the clearance of OPC is dependent on CD4$^+$ T-cell augmentation of monocyte/macrophage and PMN functions exerted by Th1-type cytokines such as IL-12 and IFN-γ (150). A significant expansion of γ/δ T cells in the cervical lymph nodes was demonstrated after oral inoculation of *C. albicans* (142), and these cells may also augment the function of phagocytes by their production of IFN-γ and indirectly contribute to clearing OPC, as previously demonstrated in experimental gastrointestinal candidiasis (213).

PATHOGENESIS OF OROPHARYNGEAL AND ESOPHAGEAL CANDIDIASES IN HIV/AIDS

Evidence Implicating *C. albicans* Virulence Factors

The ability of *C. albicans* to colonize, penetrate, and damage host tissues depends on imbalances between *Candida* virulence attributes and specific defects in host immune defenses. *C. albicans* possesses a multiplicity of properties, including adhesins, dimorphism, phenotypic switching, molecular mimicry of mammalian integrins, and secretion of hydrolytic enzymes, each with a low propensity for enhancing fungal infection and none necessarily dominant, and all, even in combination, unable to fully overcome intact host defenses (319). Hydrolytic enzymes are probable virulence factors in pathogenic *Candida* species (reviewed in references 87, 100, 109, 202, 203 and 456). Among these, *C. albicans* Saps, under the control of a multigene family (*SAP1* to *SAP10*) expressing distinct isoenzymes that are regulated differentially at the mRNA level in vitro (204, 295, 456), are implicated in the breakdown of several host substrates (202). Evidence has been presented that phospholipase B, expressed by at least two genes (*PLB1* and *PLB2*) (249, 421), also contributes to the pathogenesis of candidiasis by the degradation of host tissues (172, 205).

Several lines of evidence implicate Saps in the pathogenesis of OPC in the setting of HIV infection. Sap antigens have been detected on the surface of blastoconidia and hyphae adhering to human oral mucosa (48), and *C. albicans* isolates from HIV-infected patients with OPC not only exhibited enhanced adherence to buccal epithelial cells (423) but also produced higher Sap levels in vitro than did strains isolated from an HIV-negative control group (107, 321, 462). In addition, in vivo expression of individual members of the *C. albicans SAP* gene family was found to be differentially regulated in a murine model of esophageal candidiasis (409), during oral infection in intact and HIV-1 Tg mice (363), and in HIV-positive and -negative patients with OPC (307). Specifically, assessment of the expression of the *SAP1* to *SAP6* genes by in vivo expression technology revealed that the *SAP5* and *SAP6* genes were strongly activated at a single time point examined during infection of the esophageal mucosa in experimentally infected, immunocompromised mice whereas only low-level expression

of *SAP1* to *SAP4* occurred (409). A controlled sequential reverse transcription-PCR analysis of the temporal expression of individual members of the *SAP* gene family was conducted in a model of OPC in intact and transgenic mice that expressed HIV-1 and developed an AIDS-like disease (363). In contrast to the sustained expression of other *SAP* genes, *SAP7* and *SAP8* were conspicuously distinguished by their transient expression in both intact and transgenic mice. *SAP5* and *SAP9* were most strongly expressed throughout the course of infection in the transgenic mice (363). In accordance with these findings, reverse transcription-PCR analysis of the in vivo expression of the *SAP1* to *SAP7* genes on single samples of saliva, collected from a limited number of HIV-positive and -negative patients with OPC, revealed that *SAP2* and *SAP4* to *SAP6* were uniformly expressed and that all seven *SAP* genes were simultaneously expressed in some patients (307). Expression of specific *SAP* genes was comparable in HIV-positive and -negative patients with OPC (307) and in immunocompetent C3H and DBA/2 mice, non-Tg controls, and HIV-1 transgenic mice (363), indicating that the HIV status does not in itself alter *SAP* expression. Finally, a temporal progression of *SAP* expression in the order *SAP1* and *SAP3*, *SAP6*, and *SAP2* and *SAP8* was observed in an in vitro model of OPC that made use of reconstituted human epithelium (384). Taken together, these results suggest that some *SAP* gene products may be involved in specific steps in the onset, progression, and maintenance of OPC in HIV infection by their ability to degrade particular host substrates. This possibility is supported by the observation that although recombinant Sap1p, Sap2p, Sap3p, and Sap6p cleave peptide bonds between larger hydrophobic amino acids, substrate specificities differ among the four Sap proteins (236). Evidence implicating *C. albicans* Saps in the pathogenesis of OPC in HIV infection was further strengthened by studies demonstrating that the decreased prevalence of OPC in patients treated with HIV-1 protease inhibitors results not only from immune reconstitution but also from inhibition of Sap activity (65, 67, 128, 238, 304). The requirement for specific *SAP* genes in the pathogenesis of mucosal candidiasis may differ according to the microenvironment at individual anatomic sites, as exemplified by the loss of virulence of null *sap1* to *sap3* but not *sap4* to *sap6* mutants in an estrogen-dependent rat vaginitis model (105). In this regard, a direct role for individual *C. albicans SAPs* in the pathogenesis of OPC will require assessment of the virulence of targeted null mutants at this specific mucosal site.

In contrast to the substantial evidence implicating *SAP* genes in the pathogenesis of OPC in HIV infection, the potential role of *C. albicans* phospholipase at this specific mucosal site has received less attention. *PLB1* mRNA transcripts were detected during the entire course of OPC, with the exception of primary infection, in Tg mice expressing HIV-1 (363). This was the first report of *PLB1* expression at the mRNA level in vivo and corroborated the finding of caPlb1p secretion in the stomach (172) and kidneys (249) of experimentally infected mice. In vitro, blastoconidia, pseudohyphae, and hyphae of *C. albicans* expressed higher levels of *PLB1* mRNA than did germ tube-forming cells, suggesting that expression of ca*PLB1* is regulated as a function of morphogenic transition (200). The presence of both blastoconidia and hyphae in the oral cavities of the Tg mice was therefore consistent with detection of expression of *PLB1*. In addition, the optimal activity of caPlb1 at pH 6.0 (172) makes it likely that the enzyme is functional in oral candidiasis. The attenuated virulence of *plb1* null mutants in a hematogenous-dissemination murine model (249) and an oral-intragastric infant mouse model (172), combined with the involvement of *PLB1* in the penetration and damage of host tissues (172, 249), indicates that *C. albicans* phospholipases also contribute to fungal virulence. However, determination of the precise role of *PLB* gene products in the pathogenesis of OPC in HIV-infected patients will require further analysis, including but not limited to the demonstration of attenuated virulence of *plb1* null mutants in a clinically relevant model of OPC and an assessment of *C. albicans* phospholipase gene expression in HIV-infected patients with OPC.

Perturbed Mucosal Immune Defense Mechanisms against *C. albicans* in HIV-Infected Patients

Humoral immune response. Secretory immunoglobulin A (sIgA) constitutes the predominant immunoglobulin isotype in secretions, including saliva, and is considered to be the first line of defense of the host against pathogens which colonize or invade surfaces bathed by external secretions (275). Salivary sIgA binds to a group of polydispersed heat shock mannoproteins expressed on *C. albicans* yeast cells and germ tubes grown at 37°C, and the highest reactivity is observed with yeast grown at pH values between 5.9 and 7.5, a range similar to that found in normal saliva (41). The possibility that specific anti-*Candida* sIgA antibodies may be protective was suggested by their higher concentrations in patients with oral candidiasis than in controls (144), as well as their ability to quantitatively inhibit the adherence of *C. albicans* to buccal epithelial cells in vitro (144, 447). However, a primary role for sIgA in protection against mucosal candidiasis remains inconsistent with the rarity of oral candidiasis in patients with selective IgA deficiency (8). In HIV infection, salivary concentrations and secretion rates of total sIgA and its subclasses have been found to be unchanged (274), increased (20, 258), or decreased (299, 425), with a lower avidity of sIgA antibodies (73, 91). In contrast, the concentration of *Candida*-specific salivary sIgA has been repeatedly found to be increased in HIV-infected patients despite the decreased salivary flow rate (92, 133). Furthermore, *Candida*-specific salivary IgA production significantly correlated with the salivary *Candida* load (92), suggesting that an adequate mucosal humoral immune response to *C. albicans* is maintained in HIV infection. It was further suggested that increased salivary *C. albicans*-specific sIgA antibody concentrations may be a consequence of infection instead of playing a protective role (92). In support of this interpretation, increased salivary sIgA concentrations were correlated with decreased salivary anticandidal activity in HIV-infected patients (258). More recently, a comprehensive analysis of *Candida*-specific antibodies in the saliva of HIV-positive patients revealed that despite changes in total immunoglobulin levels, when levels of *Candida*-specific antibodies were normalized to total protein or total immunoglobulin levels of the corresponding isotype, no distinct differences in IgA or sIgA were seen, regardless of the OPC status or $CD4^+$ cell count (461). Therefore, there is no evidence of appreciable changes in levels of *Candida*-specific

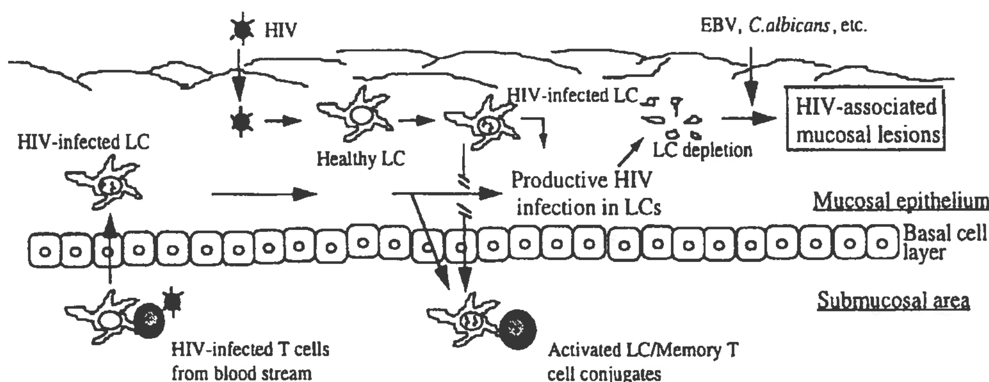

FIG. 1. Hypothetical model of the role of mucosal Langerhans' cells (LC) in HIV infection. Oral mucosal Langerhans' cells serve as an initial target for HIV infection. The cytopathic changes of Langerhans' cells with a productive HIV infection contribute to selective depletion of Langerhans' cells, which may impair mucosal immunologic protection against colonization by microorganisms causing HIV-associated oral mucosal lesions. Reprinted from reference 81 with permission of the publisher.

IgA in saliva that would account for the prevalence of OPC among patients infected with HIV.

Cellular immune response. The devastating impact of HIV infection on mucosal cell populations is most probably central to the pathogenesis of mucosal candidiasis in HIV-infected patients. In several investigations, possible defects of cells with immune potential against *Candida* were specifically examined in the HIV setting of infection.

Oral epithelial keratinocytes play a critical role in the pathogenesis of OPC because of their close interaction with *C. albicans* in the superficial epithelium. Because oral epithelial keratinocytes from HIV-infected patients contain integrated HIV proviral DNA and HIV Tat/Rev RNA (347, 348), possibly acquired through contact with submucosal HIV-positive lymphocytes and/or Langerhans' cells, the anticandidal properties of these cells could be potentially impaired. However, although HIV-positive patients with OPC had a significant decrease in oral epithelial cell-mediated growth inhibition of *C. albicans* in vitro compared to those without OPC, there was no difference in epithelial cell activity between HIV-noninfected and -infected persons without OPC (411). Expression of IL-1 and IL-8 by keratinocytes was equivalent in HIV-positive and -negative patients with OPC, and no constitutive expression of either cytokine was found in control patients without OPC (147). As mentioned above, expression of calprotectin by keratinocytes was preserved in HIV-infected patients with OPC and appeared to serve as a keratinocyte barrier to hyphal penetration (147). Consequently, further investigation is required to firmly establish whether defects in the anticandidal mechanisms of keratinocytes contribute to the predisposition to OPC in HIV infection.

Mucosal Langerhans' cells are the initial target cells after primary mucosal contact with the virus, facilitating the transfer of HIV to $CD4^+$ cells (81, 340, 341). Tonsils and adenoids from HIV-infected patients contain multinucleated syncytia expressing high levels of intracellular HIV Gag protein in the DC- and T-cell-rich crypt lymphoepithelium (164, 165). DCs within these areas express the viral coreceptors CD4 and CCR5 required for cell entry (217) and are selectively infected by R5 (macrophage-tropic) strains, promoting vigorous replication of HIV. DCs play two critical roles in the pathogenesis of HIV infection, first by spreading infection to and then by inducing virus-specific immune responses in draining lymph nodes (217, 340, 341). Despite being infected by HIV, oral mucosal Langerhans' cells are challenged to perform a vital task: the uptake of *Candida* antigens in the mucosa and their presentation to $CD4^+$ T cells in draining lymph nodes. Several lines of evidence have now clearly demonstrated multiple defects of oral Langerhans' cells, which probably contribute to a progressive loss of protective acquired cell-mediated immune responses to *C. albicans* antigens in HIV infection. Numbers of both oral (81) and esophageal (78) Langerhans' cells are depleted in HIV infection, congruent with decreased numbers of cervical (368), splenic (286), and blood (36, 130, 175, 271, 322) DCs (Fig. 1). It remains unclear whether decreased DC populations result from cytopathic changes caused by productive HIV infection, cytotoxic T-cell responses resulting in lysis of targeted DCs, migration to lymph nodes where active viral replication occurs, or down-regulation of DC surface markers (36, 81). In addition, impairment of terminal differentiation of Langerhans' cells was demonstrated by decreased expression of MHC class II antigens (338, 367), as well as the presence of blunt dendrites, very limited development of organelles, and lack of Birbeck granules (367). In contrast, however, increased expression of CD40 and CD86 costimulatory molecules was observed on blood DCs from HIV-infected patients (36). HIV and its transcriptional transactivator (Tat) block the expression of MHC class II genes in HIV infection by competing with the class II transactivator (218). Expression of MHC class I antigens in APCs is also down-regulated, but to a lesser degree than expression of MHC class II molecules (218), and results from a combination of their transcriptional blockade by Tat (56, 201) and intracytoplasmic sequestration by the HIV Nef protein (176, 248). Several of the defects identified in Langerhans' cells are also present in monocytes and macrophages, including reduced expression of MHC class II and formation of

MHC class II-antigen complexes (339) and altered capacity to stimulate and present antigen to CD4+ T cells (265). In addition to these defects, expression of HIV gp120 in APCs and impaired CD40L induction on CD4+ cells activated by antigens contribute to impaired IL-12 and IFN-γ production by APCs, thus preventing a protective CD4+ Th1 response as well as differentiation of CD8+ cells into cytotoxic lymphocytes (335, 420).

Progressive depletion of CD8+ cells in HIV infection results from apoptosis mediated by macrophages through interaction of HIV gp120 with the chemokine receptor CXCR4 (195). Despite a progressive diminution in absolute numbers, remaining CD8+ T cells nevertheless successfully accumulate in the basal epithelial layer of the oral mucosa in HIV-infected patients with OPC, demonstrating that these cells can be actively recruited to the mucosa in response to candidiasis (305, 367). However, the precise role of CD8+ cells in mucosal containment of *C. albicans* in HIV infection, either by direct growth inhibition of *Candida* or, more likely, by an indirect mechanism, remains to be determined (157, 305).

Numbers of CD4+ T cells are strikingly reduced in the oral mucosae of HIV-infected patients with or without OPC (318, 367, 413). In addition, *C. albicans*-specific peripheral CD4+ cells become depleted with HIV disease progression in patients with concurrent OPC (240), but these findings have not yet been demonstrated for CD4+ cells isolated from cervical lymph nodes draining the mucosal surface. HIV-infected patients have a Th2 cytokine profile in saliva (252) consistent with the well-documented switch from Th1 to Th2 in HIV infection (83), which correlates with a loss of lymphocyte proliferation in response to *Candida* antigens in patients with advanced HIV infection (252, 346). However, deficiencies in *Candida*-specific systemic cell-mediated immunity do not solely account for susceptibility to OPC in HIV-infected patients (250). The overall evidence nevertheless suggests that the depletion and immaturity of oral Langerhans' cells may interfere with normal processing and presentation of *C. albicans* antigens to CD4+ cells, which are themselves depleted in HIV infection, and that perturbation of these protective mechanisms probably plays a preponderant role in the pathogenesis of OPC in HIV infection.

Potential defects of phagocytes could also predispose to OPC in patients HIV with infection. HIV-infected patients with chronically inflamed gingivae have increased numbers of mucosal macrophages and PMNs, demonstrating that HIV infection does not prevent a normal innate mucosal immune response by these cells (306). Although recruitment of phagocytes to the oral epithelium does not appear to be perturbed by HIV infection, their anticandidal properties could be impaired either directly by HIV infection or by altered stimulation by cytokines. In several investigations yielding conflicting results, phagocytosis of *C. albicans* by blood monocyte-derived macrophages from HIV-infected patients has been found to be either normal (312, 315) or reduced (95), possibly by HIV Nef (345). Likewise, although HIV gp41 suppresses the reduction of nitroblue tetrazolium by PMNs in vitro (168), growth inhibition of *C. albicans* and IL-1 and IL-6 production by PMNs were found to be preserved in HIV infection (66), while in other studies phagocytosis (143) and killing (454) of *C. albicans* by PMNs were impaired but without altered production of reactive oxygen intermediates (454). Interestingly, the candidacidal activity of PMNs from healthy subjects and HIV-infected patients is impaired by the Th2 cytokines IL-4 and IL-10, suggesting a role for these cytokines in mediating increased susceptibility to OPC in HIV infection (427).

In conclusion, HIV infection severely perturbs APCs and CD4+ cells, and may also reduce the function of phagocytes against *C. albicans* in the oral mucosa, leading to the onset of OPC. However, these critical defects may be partly compensated by preserved host defense mechanisms (calprotectin, keratinocytes, CD8+ T cells, and some activity of phagocytes), which individually or together may limit *C. albicans* proliferation to the superficial mucosa and prevent systemic dissemination in HIV infection.

AIDS-Like Disease in Transgenic Mice Expressing HIV-1

The above description of the numerous manifestations of HIV infection, including multiple perturbations of immune cells which express HIV as well as of the immune response to *C. albicans*, highlights the difficulties in studying and understanding the pathogenesis of candidiasis in the context of HIV infection. Undoubtedly, the availability of an adequate animal model would facilitate this task enormously. Unfortunately, there is no animal species in which HIV-1 can replicate and induce an AIDS-like disease. The most widely used model for AIDS remains the rhesus macaque infected with simian immunodeficiency virus (SIV) (117, 118). These primates are not widely available to researchers, the model requires the use of a virus different from HIV-1, and the tools to probe the macaque immune system, especially at the genetic level, are very limited. For studies of the immune system, the mouse has been the species of choice. Decades of research have provided a panoply of biological and molecular reagents to study virtually any cell population of the immune system. More recent advances in transgenesis and in embryonic stem cell technology, coupled with the development of efficient homologous recombination, have allowed the generation of Tg and gene-deficient mice. These technologies have been used largely for the investigation of the immune system.

Early attempts to express HIV-1 gene products in Tg mice were not totally successful in initiating an AIDS-like disease (52, 122–124, 254, 259, 381, 401, 434; for reviews, see references 51 and 232). Novel phenotypes not usually seen in HIV-1-infected individuals were observed in some of these Tg mice, while others showed either minimal or no permanent perturbation of immune cell populations, or severe immune defects, distinct from those usually associated with HIV-1 infection in human individuals. However, more recent attempts to generate a mouse model of AIDS were more successful, and the CD4C/HIV Tg mice were found to develop an AIDS-like disease very similar in its manifestations to human AIDS (186, 187).

Structure and expression of HIV-1 in CD4C/HIV Tg mice. A key feature of the CD4C/HIV transgene is the nature of its regulatory elements (CD4C) allowing the expression of HIV-1 in a selected subset of immune cells: thymic immature CD4+ CD8+ and mature CD4+ CD8− T cells, peripheral mature CD4+ T cells, and cells of the myeloid lineage such as macrophages, DCs, and Kupffer cells. These cells represent the ma-

jority of the natural cell populations found to be infected with HIV-1 in humans (325). The CD4C regulatory elements were indeed chosen to target as faithfully as possible the expression of HIV-1 in the same cell populations as those infected in humans.

It was initially thought that the best way to achieve this selected cell tropism was to use the regulatory elements of the CD4 gene itself, which codes for the receptor of HIV-1. The human CD4 (hCD4) gene was chosen, since expression of the mouse CD4 gene is absent in most myeloid cell populations, including macrophages, while several subsets of human myeloid cells, including macrophages and DCs, express CD4 at their surface (228, 460). Using a reporter gene, the cell surface hCD4 itself and the hCD4 regulatory elements were dissected (first by generating the CD4A, CD4B, and CD4C constructs) in order to identify which sequences were required to drive expression in $CD4^+$ T cells and macrophages and at the same time to silence the expression in $CD8^+$ T cells, B cells, and other nonhematopoietic cells (189). An in vivo (Tg) approach was used, since initial experiments with established human cell lines in vitro appeared unreliable. Among the DNA constructs tested, the CD4C regulatory elements were found to drive the expression of the reporter gene most faithfully (Fig. 2A). These CD4C elements represent a human/mouse chimeric construct (14.4 kbp) containing the murine T-cell-specific enhancer (1.9 kbp) (382) fused to human genomic elements (12.5 kbp) representing 4.5 kbp of sequences upstream of the first noncoding exon (exon 1), intron 1 (9.9 kbp), exon 2, and part of exon 3 (189). Further work has now confirmed that intron 1 contains a silencer (repressing expression in $CD8^+$ T cells, B cells, and nonlymphoid cells [426]) and DNA elements necessary for macrophage expression (188).

The CD4C regulatory elements have been used in Tg mice to express the genome of wild-type HIV-1 (186), of point and deletion mutants of HIV-1 (187, 190) (Fig. 2A), of natural HIV-1 variants, of an experimental variant of HIV-1 encoding green fluorescent protein (Z. Hanna, C. Simard, and P. Jolicoeur, unpublished data), and of the wild-type SIV genome (400). They have also been used to express other nonviral genes, such as Notch1 (IC) (X. Zang, Z. Hanna, J. Poudrier, D. G. Kay, and P. Jolicoeur, unpublished data). In each case, the expression of the transgene, assessed with a very large number of Tg founder lines, has been remarkably reproducible and faithful, the major differences between lines being the levels of expression, most probably reflecting the distinct sites of transgene integration within the host genome. A variety of techniques were used to identify the cells expressing the transgene: immunohistochemistry with cell-specific markers coupled with in situ hybridization (ISH) with transgene-specific probes, cell sorting of specific populations followed by ISH or Western blot analysis, and fluorescence-activated cell sorter (FACS) analysis on cells expressing a cell surface reporter (CD4C/hCD4 Tg mice) or expressing a fluorescent reporter ($CD4C/HIV^{GFP}$ Tg mice). The consensus emerging from these studies is that the transgene is expressed in the same murine cell populations which have been found to express the cell surface CD4 molecules in human cells, i.e., thymic immature $CD4^+$ $CD8^+$ T cells and mature $CD4^+$ $CD8^-$ T cells, peripheral $CD4^+$ T cells, and cells of the myeloid lineage (peripheral macrophages, Kupffer cells, DCs, and microglial cells). Expression was not detectable in many cell populations which are not thought to express hCD4, notably hepatocytes, kidney epithelial cells, lung epithelial cells, cardiomyocytes, endothelial cells, intestinal epithelial cells, muscle cells, neurons, oligodendrocytes, and astrocytes.

It therefore seems that HIV-1 gene products are expressed in CD4C/HIV Tg mice in the same cell populations which are thought to be infected with HIV-1 in human individuals. This specific targeted expression of HIV-1 genes is thought to be critical for the development of the AIDS-like disease observed in these Tg mice (186, 187). However, the latency of disease was found to correlate very closely with the levels of transgene expression (187). Indeed, Tg mice from very-high-expressor lines died within weeks after birth, and these lines could not be maintained. Tg mice from medium-expressor lines survived 4 to 6 months, while Tg mice expressing low levels of HIV-1 Nef survived as long as 1 year but still developed essentially the same multiorgan phenotypes as Tg mice dying early (Fig. 2B). This feature of fast and slow disease progression in Tg mice is reminiscent of the human clinical situation.

To determine whether all or only a subset of the HIV-1 gene products were required to induce this AIDS-like disease in mice, various mutated forms of the HIV-1 genome were constructed and assessed in Tg mice (Fig. 2). It was found that only Tg mice expressing Nef developed disease, while Tg mice expressing all other HIV-1 genes or a subset of them did not develop apparent phenotypes and apparently led a disease-free life (187). This mutational analysis established that Nef was the major determinant of this AIDS-like disease in Tg mice.

Clinical and pathological features of AIDS-like disease in CD4C/HIV Tg mice. The CD4C/HIV Tg mice, even those expressing only Nef, develop a severe AIDS-like disease whose latency correlated with the levels of Tg expression (186, 187). Clinically, these Tg mice show wasting, failure to thrive and/or weight loss, premature and sudden death, and sometimes edema and diarrhea. Wasting is a known consequence of human AIDS (90, 181, 270). The early death and edema are most probably caused by a severe renal disease (see below). The sudden deaths are probably due to a cardiac disease (see below). The Tg mice are fragile: often minor manipulations such as preparation for echocardiography will precipitate an early death. They also appear to survive better in a specific-pathogen-free environment than in non-specific-pathogen-free animal rooms. Tg females have lower fertility than non-Tg female littermates and typically give birth to fewer pups per litter. Tg males also show lower fertility, although this is not as apparent as with females. This lower fertility may reflect a general disease state or more specific problems of the autonomic nervous system.

A more detailed macroscopic and histological examination of these CD4C/HIV Tg mice showed multiorgan AIDS-like disease.

(i) Kidneys. Smaller, atrophic, and pale kidneys with an irregular surface are the most macroscopically visible manifestation of the disease. This phenotype also has the highest penetrance (in virtually 100% of Tg mice), at least for Tg mice bred on the C3H/HeN background. This kidney disease represents a tubulointerstitial nephritis associated with tubular atrophy, interstitial mononuclear cell infiltration, and fibrosis, as well as with lumen dilatation forming cysts. In addition,

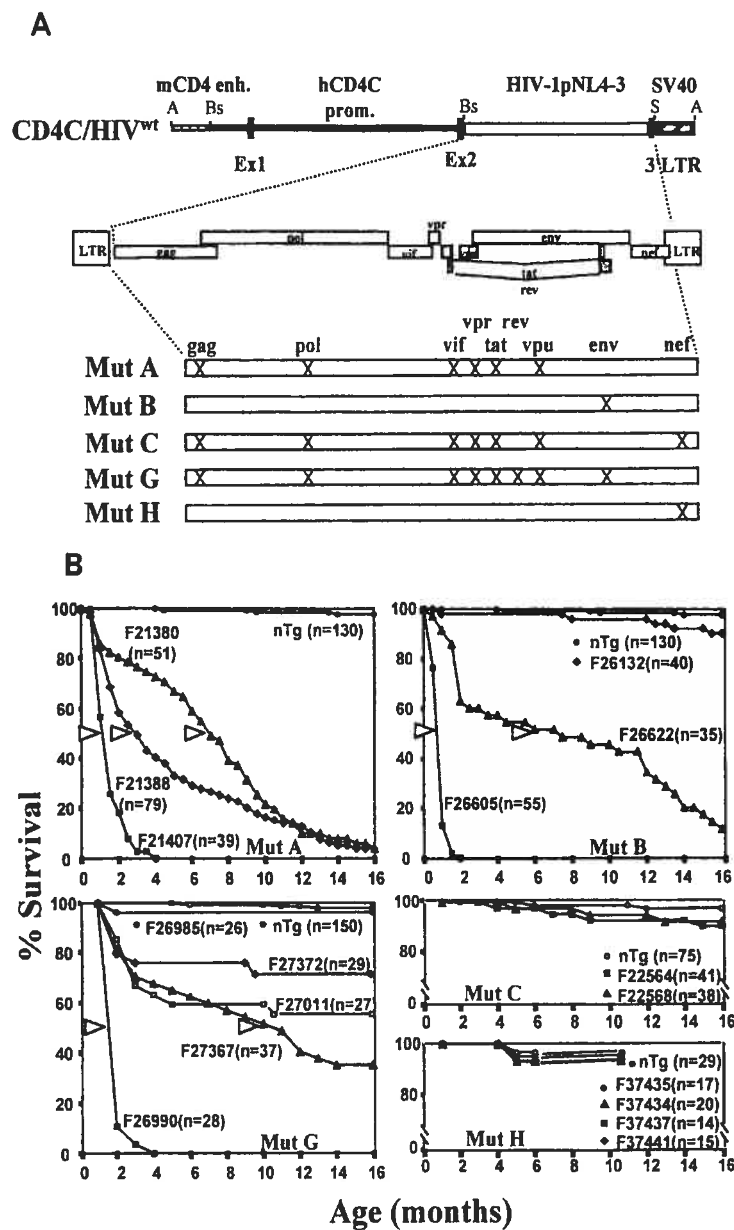

FIG. 2. Incidence of death in CD4C/HIV^{Mut} Tg mice. (A) Structure of the CD4C/HIV^{Mut} transgenes. The mouse CD4 enhancer (mCD4enh.), the human CD4 promoter (hCD4C prom.), each of the HIV-1^{NL4-3} mutant genomes, and the polyadenylation sequences from simian virus 40 (SV40) were ligated. Ex1 and Ex2 are the first two untranslated exons of the hCD4 gene; 3′ LTR is part of the 3′ long terminal repeat of the HIV-1 genome. The symbol × means that the open reading frame of the indicated HIV gene was interrupted. Restriction sites: A, AatII; B_s, BssHII; S, SstI. (B) Cumulative incidence of death in non-Tg and CD4C/HIV^{Mut} Tg mice. The arrows show the time of death of 50% (TD_{50}) of the Tg mice from different founders (F) for each mutant. Each point represents the death of a mouse. Symbols: n, number of animals observed. Reprinted from reference 187 with permission of the publisher.

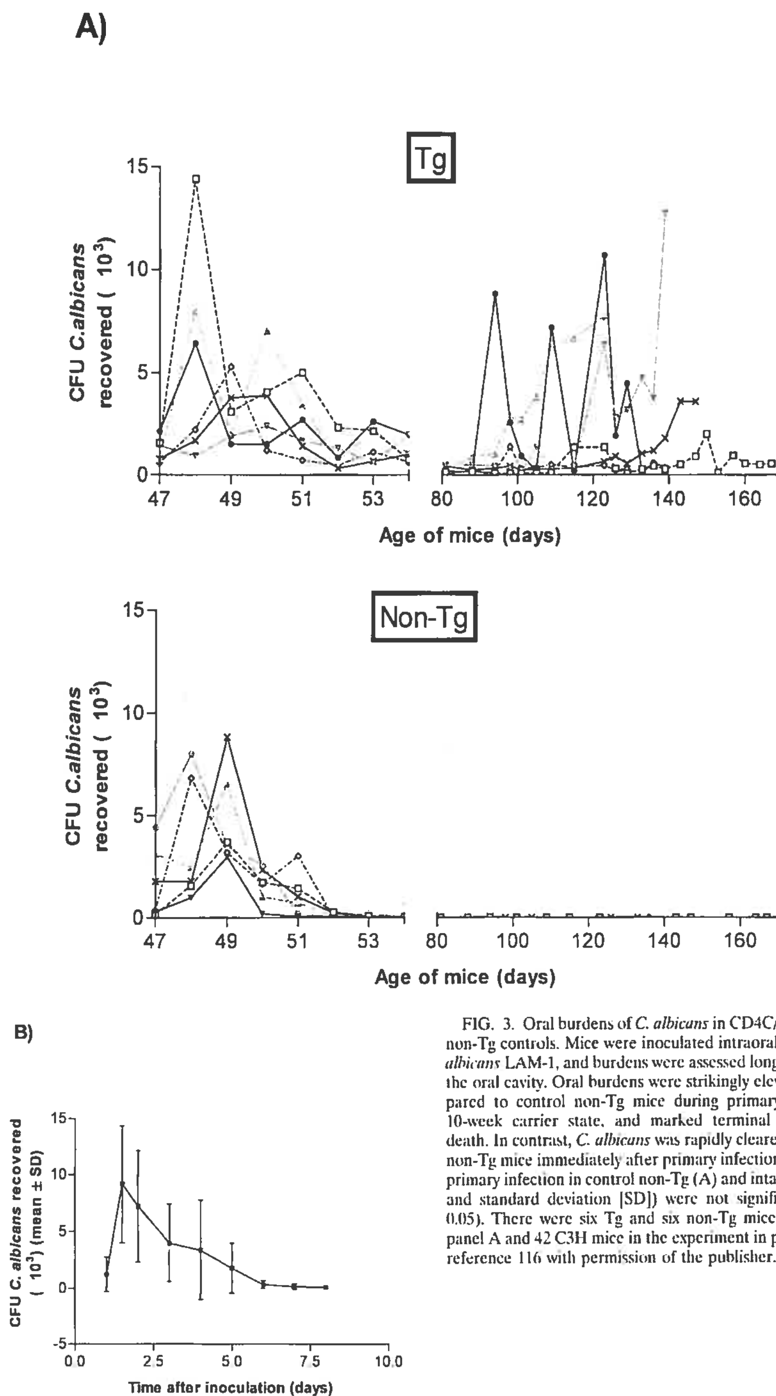

FIG. 3. Oral burdens of *C. albicans* in CD4C/HIV^{MutA} Tg mice and non-Tg controls. Mice were inoculated intraorally with 10^8 CFU of *C. albicans* LAM-1, and burdens were assessed longitudinally by sampling the oral cavity. Oral burdens were strikingly elevated in Tg mice compared to control non-Tg mice during primary infection, the 6- to 10-week carrier state, and marked terminal outgrowth preceding death. In contrast, *C. albicans* was rapidly cleared from oral cavities of non-Tg mice immediately after primary infection. Oral burdens during primary infection in control non-Tg (A) and intact C3H mice (B; mean and standard deviation [SD]) were not significantly different ($P > 0.05$). There were six Tg and six non-Tg mice in the experiment in panel A and 42 C3H mice in the experiment in panel B. Adapted from reference 116 with permission of the publisher.

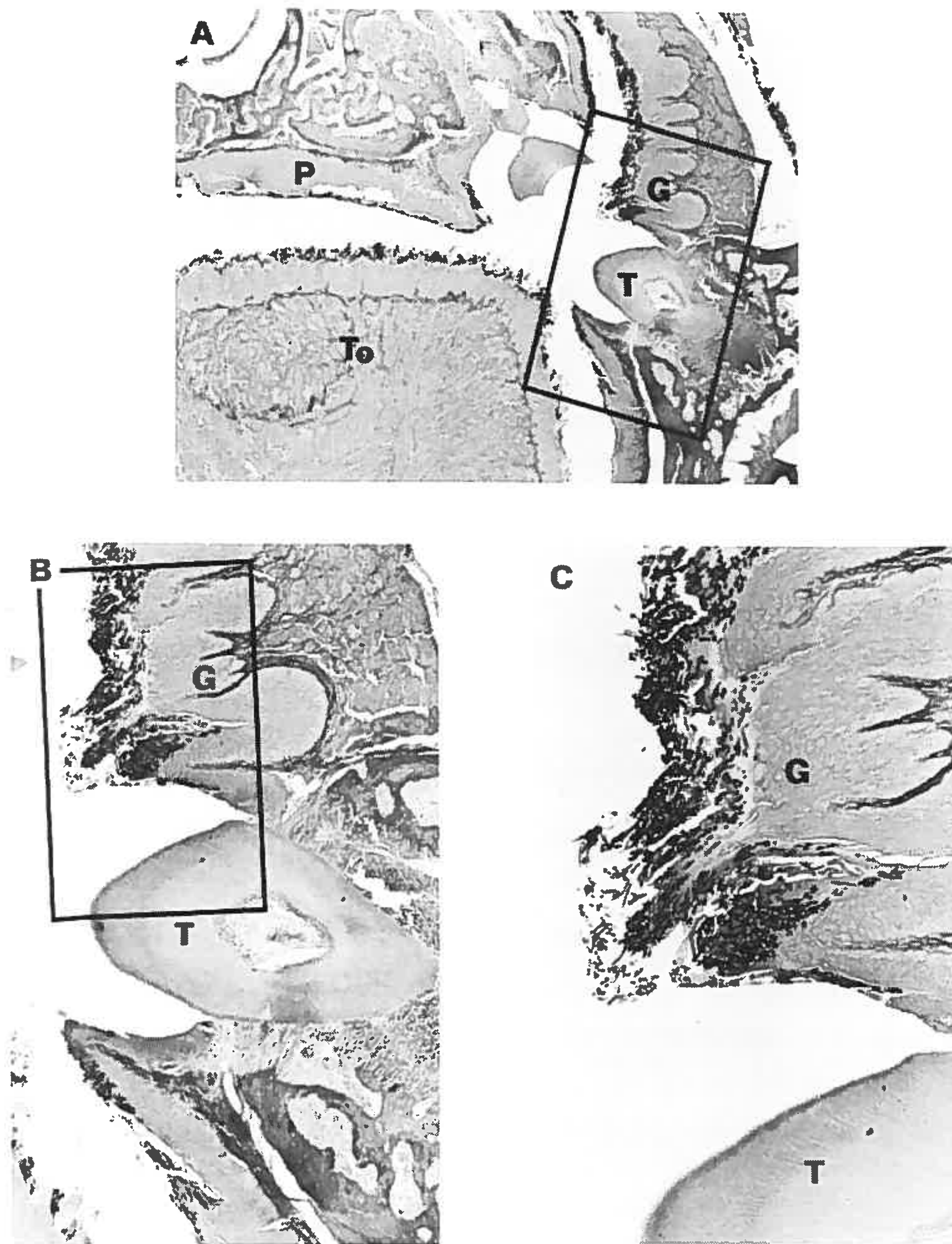

FIG. 4. Histopathology of oral candidiasis in CD4C/HIV[MutA] Tg mice. *Candida* hyphae penetrate the superficial keratin layer of the stratified squamous epithelium of the tongue (To), palate (P), and gingiva (G) surrounding the tooth (T). Stain, Gomori-Grocott methenamine silver. Magnifications: A, ×100; B, ×250; C, ×500. Adapted from reference 116 with permission of the publisher.

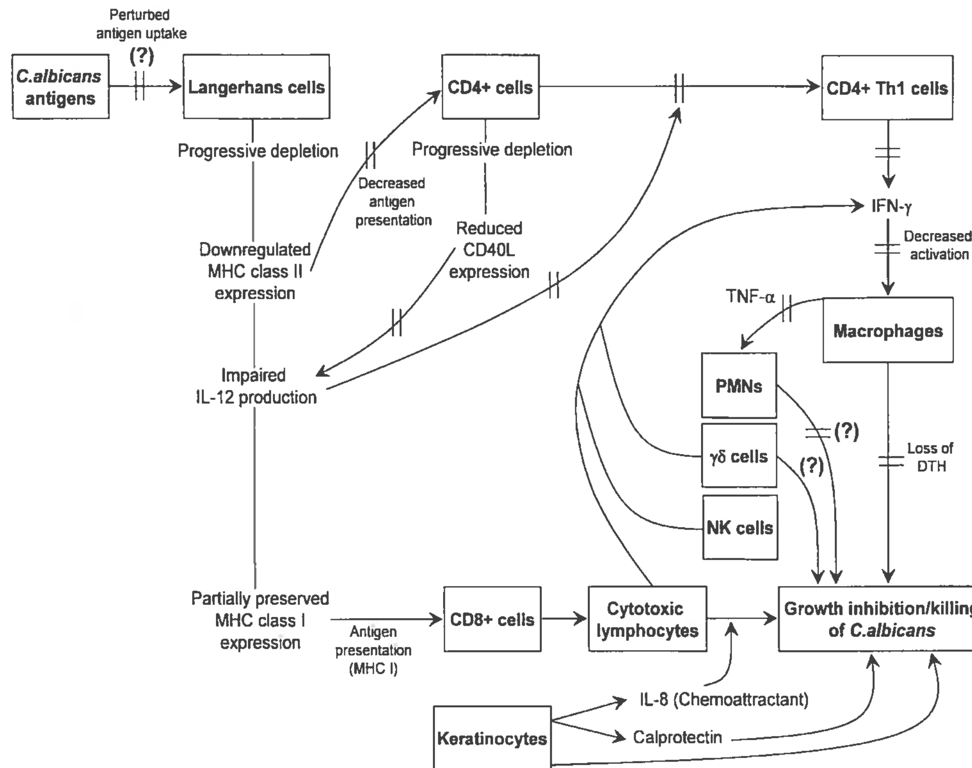

FIG. 5. Hypothetical defects in host defenses against oroesophageal candidiasis in HIV infection. Progressive depletion and functional dysregulation of mucosal Langerhans' cells alters normal processing and presentation of *C. albicans* antigens to $CD4^+$ cells, which are themselves depleted in HIV infection, resulting in diminished numbers of $CD4^+$ Th1 cells and perturbed adaptive immunity to *C. albicans*. However, substantially preserved innate defense mechanisms (calprotectin, PMNs, and γδ T cells) and cytotoxic T lymphocytes prevent systemic dissemination.

glomerular changes are observed and often represent segmental glomerulosclerosis. Kidney disease such as tubulointerstitial nephritis and focal and segmental glomerulosclerosis are relatively frequent in SIV-infected macaques (417) and in AIDS patients, especially in children (352, 394, 418). The early expression of the Tg along with the specific C3H/HeN background may explain the high penetrance of this phenotype in the CD4C/HIV Tg mice.

(ii) Lungs. A lymphocytic interstitial pneumonitis (LIP) (thickening of alveolar walls caused by infiltrating mononuclear cells) was observed in a high proportion of Tg mice bred on a mixed C3H-C57BL/6 background. The incidence of LIP decreased somewhat after inbreeding within the C3H background. LIP also develops in SIV-infected macaques (23) and is most often observed in human pediatric AIDS and constitutes an AIDS-defining condition (4, 5, 60, 215, 288). Since the CD4C/HIV transgene is expressed early in life, it is therefore not surprising to observe this phenotype in these Tg mice.

(iii) Heart. The sudden deaths observed in these Tg mice prompted a detailed analysis of the heart. Noninvasive echocardiography revealed signs of depressed cardiac functions (increased systolic left ventricular internal dimension and decreased fractional shortening, ejection fraction, stroke volume, and cardiac output) compared to those of their non-Tg littermates (227). Cardiac disease was also observed very frequently at autopsy, especially when the animals were bred on a C3H-C57BL/6 mixed background. The hearts often appeared enlarged and dilated. After exsanguination, they become smaller, suggesting dilated cardiomyopathy. Histologically, multifocal lesions representing focal areas of myocytolysis, sometimes associated with infiltrating mononuclear cells and fibrosis, were observed. In addition, a more diffuse interstitial fibrosis compared to that in non-Tg mouse hearts was revealed by Sirius red staining. This was not always associated with focal lesions. More recently, evidence of disturbances of the cardiac rhythm was found (D. G. Kay and P. Jolicoeur, unpublished data). Analysis by ISH with HIV-1-specific probes revealed no detectable expression in cardiomyocytes of Tg mice (227), suggesting that the pathogenesis of this cardiomyopathy is indirect and reflects abnormal functions of immune cells, some of them most probably expressing the transgene. Interestingly, cardiac disease is a relatively frequent manifestation of simian (396,

397) and human (3, 9, 84, 154, 196, 225, 260, 293) AIDS, and the cardiac lesions of the CD4C/HIV Tg mice are similar to those found in individuals with AIDS.

(iv) Bones. Often, the bones of CD4C/HIV Tg mice have a white appearance and are more fragile than those of non-Tg mice. Again the severity and penetrance of this phenotype was more apparent when these Tg mice were bred on a C3H-C57BL/6 background. These two phenotypes suggest the presence of a myelodysplasia and of osteoporosis. These phenotypes have not been extensively investigated, but preliminary FACS and X-ray analyses conducted on bone marrow cells and bones, respectively, are consistent with such anomalies (J. Caceres-Cortes, C. Simard, and P. Jolicoeur, unpublished data). Hematological abnormalities have been described in AIDS (93, 169, 297, 353).

Immune defects in CD4C/HIV Tg mice. In addition to the various organ diseases described above, the CD4C/HIV Tg mice developed several phenotypes of the immune system involving many cell populations and structures, which are very similar to those found in human AIDS.

(i) Thymus. The most striking macroscopic feature of the immune system is a severe thymic atrophy (186, 187). Histologically, loss of the normal thymus architecture is noted, with the cortical and medullary regions being poorly defined in contrast to the well-defined structures observed in non-Tg mice. In addition, the thymuses of the Tg mice are hypocellular. Thymocyte cell counts are close to normal at birth and during the first few weeks of life, but thereafter they progressively decrease to reach very small numbers, with loss of more than 90% of thymocytes. FACS analysis data show that the relative proportion of thymocytes is often almost normal, except for a lower proportion of mature $CD4^+$ $CD8^-$ cells (P. Chrobak, M. C. Simard, T. Ndolo, Z. Hanna, and P. Jolicoeur, Abstr. 10th Conf. Retroviruses Opportunistic Infect., 2003). This appears to reflect a block in differentiation at the transition stage of double-positive (DP) $CD4^+$ $CD8^+$ to single-positive $CD4^+$ $CD8^-$ T cells (Chrobak et al., Abstr. 10th Conf. Retroviruses Opportunistic Infect, abstr. 210, 2003). This thymic depletion appears to be cell autonomous and independent of stromal epithelial cells. It can indeed be reproduced in lethally irradiated normal non-Tg mice transplanted with bone marrow or fetal liver cells from Tg mice. Depletion of thymic cell populations is a feature of human AIDS, in both children and adults (171, 215, 229).

Phenotypically, these Tg DP $CD4^+$ $CD8^+$ T cells are abnormal and show a significant downregulation of the cell surface CD4 molecule (186, 187), also a characteristic of Nef-expressing human cells. In addition, DP cells from Tg mice show dysregulation of development marker expression (Chrobak et al., Abstr. 10th Conf. Retroviruses Opportunistic Infect., 2003), suggesting an impaired differentiation. Moreover, biochemical studies revealed constitutively enhanced levels of phosphotyrosine-containing proteins and further enhancement of tyrosine phosphorylation of several protein species, notably lysophospholipid:acyl-CoA acyltransferase and mitogen-activated protein kinases (ERK 1/2), on in vitro stimulation with anti-CD3 (187) compared to the situation for DP cells from non-Tg mice.

(ii) Peripheral lymphoid organs. Macroscopic and microscopic examination of peripheral lymphoid organs (lymph nodes [LN] and spleen) from CD4C/HIV Tg mice showed that they were atrophic, in contrast to organs from control non-Tg mice (186, 187). They had lost their normal architecture and were hypocellular and often fibrotic. On stimulation with an antigen (ovalbumin), the CD4C/HIV Tg mice produced a significantly smaller number of germinal centers (GC) in their LN (342). In spleens of Tg mice, the T-cell zone was markedly decreased and the marginal zone (MZ) appeared larger and contained numerous Mac-1^+ and $CD11c^+$ cells, likely to represent macrophages and DCs (342). In addition, the follicular DC network was found to be markedly reduced in Tg mice compared to non-Tg mice (342). An atrophic follicular DC network and smaller numbers of GC were observed early during the course of the AIDS pandemic and were considered one of the pathological hallmarks of the disease (326, 336, 430, 431). The CD4C/HIV Tg mice show this phenotype. Such a phenotype, reminiscent of that found in CD40- or CD40L-deficient mice (226, 463), is likely to reflect a lack of $CD4^+$ T-cell help (342).

(iii) $CD4^+$ T cells. Peripheral $CD4^+$ T cells are present in smaller numbers in Tg mice than in non-Tg mice (186, 187, 342). At later stages of the disease, they often almost totally disappear from peripheral organs. They also show a downregulation of the cell surface CD4 molecule. Therefore, the loss of this population is best assessed by quantitating the $TcR\alpha\beta^+$ $CD8^-$ T-cell population, which essentially represents the entire $CD4^+$ T-cell population, including the subpopulation which expresses no or very low levels of CD4. FACS analysis of $CD4^+$ T cells from Tg mice revealed a lower expression of CD40L than that in cells from non-Tg mice (342). Also, an increased population of $CD4^+$ T cells in Tg mice, compared to that in non-Tg mice, exhibit an activated/memory phenotype ($CD69^{Hi}$, $CD44^{Hi}$, $CD25^{Hi}$, $CD45RB^{Low}$, $CD62L^{Low}$) (342; X. Weng, E. Priceputu, J. Poudrier, D. G. Kay, Z. Hanna, T. W. Mak, and P. Jolicoeur, unpublished data). Assessment of the division capacity of these cells carried out by in vivo bromodeoxyuridine labeling showed that a higher proportion of these $CD4^+$ T cells from Tg mice than from non-Tg mice divided in vivo. However, in vitro, the purified $CD4^+$ T cells appear to be restricted in their ability to divide. They also appear to die by apoptosis at a higher rate. Finally, in a mixed leukocyte reaction, they performed poorly compared to cells from non-Tg mice (Weng et al., unpublished). It therefore appears that the peripheral $CD4^+$ T cells are functionally impaired, especially in their helper function, despite being in a state of activation.

(iv) $CD8^+$ T cells. The numbers of peripheral $CD8^+$ T cells of CD4C/HIV Tg mice are proportionally increased, as in human AIDS (186, 187). At later stages of the disease, these cells are lost, along with the $CD4^+$ T cells, as occurs in human AIDS (151, 327). Coupled with the smaller $CD4^+$ T-cell numbers, this leads to a lower CD4/CD8 ratio, also a characteristic of human AIDS.

(v) B cells. The proportion of B cells in lymphoid organs of CD4C/HIV Tg mice is increased compared to that in non-Tg mice (186, 187, 342). The presence of hyperglobulinemia is associated with this peripheral B-cell expansion in Tg mice. Most of the immunoglobulins are of the IgM isotype because of a failure to class switch (342). After ovalbumin immuniza-

tion, most of the ova-specific antibodies (Ab) were of the IgM isotype (342). Interestingly, these Tg mice also appear to exhibit signs of autoimmunity, producing autoantibodies, notably anti-DNA Ab (342) and anti-heart Ab (227).

(vi) Macrophages. The macrophages from the Tg mice seem to have a constitutively activated phenotype. Preliminary experiments also indicate that they can produce higher levels of NO and TNF-α on stimulation than those from non-Tg control littermates (D. Jovanovic, P. Vincent, and P. Jolicoeur, unpublished data). In addition, they were found to be more numerous in the spleen MZ (342).

(vii) Dendritic cells. DCs express the transgene and are abnormal in CD4C/HIV Tg mice (342a). In LN, the total number of DC were found to progressively decrease and to express lower levels of MHC class II, CD40, and CD86 cell surface markers, suggesting an immature phenotype. Interestingly, a subpopulation of $CD11b^{Hi}$ DC was found to accumulate in these lymphoid organs. LN DC also show a reduced capacity to deliver costimulatory signals to syngeneic $CD4^+$ T cells in the presence of anti-CD3 and to stimulate a mixed leukocyte reaction in coculture with allogeneic $CD4^+$ T cells from non-Tg mice. In addition, LN DC exhibit an impaired capacity to present antigen, both as proteins (pigeon cytochrome *c* [Pcc]) and as Pcc peptides. In contrast, in the spleen, DC were present in large numbers, especially in the MZ. In the thymus of Tg mice, recovery of $CD8\alpha^+$ DCs tended to be low compared to that in non-Tg control mice. Consistent with these findings, maturation of bone marrow-derived DCs from Tg mice was impaired and expression of MHC class II, CD86, and MHC class I cell surface proteins was lower than on DCs from non-Tg mice. Therefore, it appears that DCs from Tg mice fail to undergo full maturation. This may well affect their capacity to fully activate T cells. Also, the abnormal DC phenotypes and functions may contribute to the expanded B-cell compartment described above and the Ab production found in these Tg mice.

The number of DCs in HIV-infected individuals has been reported by some investigators to be small in the blood (130, 148, 175, 271, 322, 328), skin (302), lymphoid tissues (264), and oral mucosa (407). Also, DCs with an immature phenotype have been observed in some HIV-infected patients (137, 286, 338). In addition, reduced DC function has been detected in AIDS patients (reviewed in references 233 and 234). Although the benefit for the virus to elicit such a phenotype is unclear, it may favor viral replication. Indeed, it has been reported that HIV-1 replicates preferentially in immature DCs (25, 62, 163, 174).

This brief summary highlights the main features of the AIDS-like disease which develops in this CD4C/HIV Tg mouse model. All of the immune and organ disease phenotypes studied to date appear to be similar to human AIDS, not only pathologically but also at the cellular and molecular levels. In addition, no major phenotypes which are absent in human AIDS arise in the Tg mice. Conversely, almost all of the phenotypes found in human AIDS develop in these Tg mice. This strong similarity suggests that this murine AIDS model is relevant to the human disease and may be instrumental in understanding several manifestations of AIDS, in particular, opportunistic infections with pathogens such as *C. albicans*.

Oroesophageal Candidiasis in CD4C/HIV Transgenic Mice: a New Tool To Study Pathogenesis

Controlled studies of the pathogenesis of mucosal candidiasis in HIV infection have been hampered by the lack of a relevant animal model. The availability of CD4C/HIV Tg mice expressing HIV-1 in immune cells and developing an AIDS-like disease has recently provided a unique opportunity to devise a novel model of mucosal candidiasis that closely mimics the clinical and pathological features of candidal infection in human HIV infection (116). The following features of mucosal candidiasis in the Tg mice were found to be identical to those in patients with HIV infection: a sustained enhancement of oral burdens of *C. albicans*, becoming more manifest in the later stage of HIV disease (161, 439, 467) (Fig. 3); penetration by *Candida* hyphae of the stratified squamous epithelium of the oral cavity and esophagus, limited to the superficial epithelial layer (147, 357, 367) (Fig. 4); a low incidence of systemic dissemination of *C. albicans* (219, 296, 438); and a mononuclear inflammatory cell infiltrate of the mucosa (147, 367). In addition to closely mimicking the features of mucosal candidiasis in patients infected with HIV, the model obviates the problematic procurement of tissue samples from human patients; avoids the potentially confounding effects of antiretroviral therapy and of mixed infection by different strains of *C. albicans* or different *Candida* species, often found in HIV-infected patients (344); and allows for longitudinal observations at fixed times during the progression of HIV infection by direct comparison with non-Tg littermates. The Tg mice thus provide a novel opportunity to study the pathogenesis of mucosal candidiasis in HIV infection under controlled conditions in a small laboratory animal.

In accordance with the findings in humans with advanced HIV infection, the frequencies of cells expressing MHC class II antigen and $CD4^+$ cells were markedly reduced in the oral mucosa of the Tg compared to the non-Tg mice at the time of marked terminal outgrowth of *C. albicans* preceding death (116). However, in the Tg mice, infection with *C. albicans* also resulted in a substantial increase in the frequencies of mucosal cells expressing MHC class II and a more modest enhancement in the frequencies of $CD4^+$ cells, demonstrating the ability of the Tg mice to partially respond to mucosal infection despite the reduction in these cell populations (116).

Continuing investigation using this novel model in Tg mice is under way and is allowing, for the first time, a precise cause-and-effect analysis of the immunopathogenesis of mucosal candidiasis in HIV infection.

FUTURE DIRECTIONS AND CONCLUSION

The accumulated evidence presented in this review suggests a number of hypothetical defects which may underlie the susceptibility to mucosal candidiasis in HIV-infection (Fig. 5). Depletion of Langerhans' cells and their reduced expression of MHC class II molecules and IL-12 probably perturb the development of *Candida*-specific $CD4^+$ Th1 cells which are instrumental in orchestrating a protective adaptive cell-mediated immune response to *C. albicans* in the oral and esophageal mucosa. The depletion of $CD4^+$ cells and a shift in expression from Th1 to Th2 cytokines may reduce the anticandidal activity

of macrophages and PMNs and thus trigger the onset of OPC. Partly preserved expression of MHC class I antigens on Langerhans' cells may allow the recruitment of a compensatory protective $CD8^+$ T-cell response to *C. albicans* in the mucosa despite HIV infection: this, combined with the anticandidal activity of preserved innate defense mechanisms (calprotectin and keratinocytes), may limit the proliferation of *C. albicans* to the mucosa and prevent systemic dissemination to deep organs.

Oral biopsy material from HIV-infected patients will continue to provide clinically relevant, descriptive information about critical alterations in mucosal host defense mechanisms which may predispose to mucosal candidiasis in HIV-infection and may also suggest a protective role for cell populations which appear in the mucosa in response to candidiasis. The specificity of these identified alterations will be strengthened by examining tissue samples from normal and infected areas of the oral cavity in HIV-infected patients with OPC in comparison to HIV- and non-HIV-infected patients without OPC. Complementary to these investigations, the novel model of mucosal candidiasis in CD4C/HIV Tg mice will for the first time allow a longitudinal assessment of the alterations in oral mucosal and cervical lymph node cell populations and their production of cytokines at the single-cell level under controlled conditions. New Tg mouse constructs and replenishment with specific cell populations and cytokines should provide the opportunity to achieve a precise cause-and-effect analysis of the immunopathogenesis of mucosal candidiasis in HIV infection. The new knowledge gained is a prerequisite to designing novel approaches to immune reconstitution.

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Chapitre 3

Discussion

Discussion générale

La candidose oropharyngée (OPC) est la plus fréquente des infections fongiques opportunistes chez les individus immunodéficients. Elle est causée par le *Candida albicans*, une levure dimorphe, diploïde et imparfaite, qui réside de manière commensale dans les muqueuses et le tractus gastro-intestinal chez l'homme. Il a été estimé que plus de 90% des individus infectés par le virus de l'immunodéficience humaine (VIH) développeraient au moins un cas d'OPC durant la progression de la maladie (551). La colonisation des muqueuses buccales est étroitement corrélée avec le développement et la progression de la déficience cellulaire induite par l'infection au VIH. Cependant, les mécanismes de défense des muqueuses qui protègent l´hôte à l´égard de *C. albicans* demeurent inconnus et le dysfonctionnement précis qui favorise la croissance de *C. albicans* à la surface des muqueuses buccales dans le développement séquentiel de l´infection au VIH n´a pas été clairement défini.

La prédisposition à l'OPC chez les patients infectés au VIH semble préférentiellement impliquer une atteinte de l'immunité à médiation cellulaire locale plutôt que systémique, rendant compte d'un déséquilibre entre la virulence de *C. albicans* et la dysrégulation des défenses immunitaires de la muqueuse buccale. Les lymphocytes T CD4+ Th1, T CD8+, T γ/δ, les macrophages et les polymorphonucléaires (PMNs) semblent interagir et collaborer au maintien et à l'efficacité des mécanismes de défense de l'hôte à l'égard de l' OPC. De plus, les nombreux travaux menés sur des souris normales, infectées, ou présentant une immunodéficience congénitale ont montré que les lymphocytes T CD4+ fonctionnels jouaient un rôle dans la résistance à la colonisation et à l'infection des muqueuses buccales par *le C. albicans* (21, 35). Des études menées au laboratoire sur des souris MAIDS suggèrent que, bien que les lymphocytes T CD4+ Th1 jouent un rôle central dans la résistance à l'OPC, les lymphocytes T CD8+ et/ou T γ/δ ainsi que les *natural killer* (NK) peuvent être en partie impliquées dans un mécanisme de compensation de l'immunité (144). Bien que l'incidence de l'OPC augmente avec la déplétion des

lymphocytes T CD4+ (<200/mm^3), les perturbations de l'immunité des muqueuses à l'égard de *C. albicans* apparaissent précocement durant la progression de l'infection au VIH. Par conséquent, les études ont convergé vers le rôle précis de la réponse immunitaire acquise à médiation cellulaire à l'égard de *C. albicans.* À ce titre, et afin de promouvoir la réponse Th1, la présence de cellules présentatrices compétentes et fonctionnelles au sein des muqueuses buccales semble être un pré-requis dans l'initiation de la réponse cellulaire et dans l'instauration de l'hypersensibilité de type retardée à l'égard de *C. albicans.* L'établissement récent de lignées de souris transgéniques (CD4C/HIV) exprimant le VIH-1 dans les cellules immunitaires (cellules thymiques CD4+CD8+ et CD4+CD8-, lymphocytes T périphériques CD4+CD8-, cellules de Langerhans (LCs) et macrophages) est apparu comme une nouvelle opportunité d´examiner la pathogenèse de l'OPC à l'aide d'un modèle animal qui reproduit les principales manifestations cliniques et biologiques de l'infection au VIH (248, 249). Ces manifestations incluent de la difficulté à avaler, une atrophie sévère et une fibrose des organes lymphoïdes, une perte des lymphocytes T CD4+, une pneumonie interstitielle, et une néphrite tubolo-interstitielle (248, 249).

Nous avons émis l´hypothèse que l´expression du transgène dans les LCs des muqueuses buccales de souris CD4C/HIV^{MutA}, exprimant les gènes *REV*, *ENV* et *NEF*, altère leur développement normal et la présentation des Ag de *C. albicans* aux lymphocytes T CD4+, eux-même déplétés chez ce modèle murin. Nous voulions déterminer si ces perturbations résultaient en une incapacité à développer une réponse locale CD4+ Th1 spécifique ainsi qu'une immunité acquise à l'égard de *C. albicans.* Le développement d'un mécanisme de compensation de l'immunité par les lymphocytes T CD8+, les monocytes/ macrophages, les PMNs ou l'association de ces populations cellulaires pourrait réduire la charge buccale de *C. albicans* et prévenir de la dissémination systémique de *C. albicans* chez la souris Tg.

Au cours de mes études de doctorat, j'ai démontré que l'expression du transgène du VIH-1 dans les DCs et les lymphocytes T CD4+ est requis et suffisant pour la persistance de *C. albicans* dans les muqueuses buccales des souris Tg. Cette persistance est associée à

une altération du nombre et de la fonction des DCs et des lymphocytes T CD4+, des muqueuses buccales et des ganglions lymphatiques cervicaux des souris Tg CD4C/HIV^{MutA}. Ces altérations ont résulté en une incapacité d'initier une réponse immunitaire adaptée à l'égard de *C. albicans* in vivo et in vitro. Par ailleurs, j'ai démontré que le transfert adoptif de lymphocytes T CD4+ de souris non-Tg dans les souris Tg CD4C/HIV^{MutA} restaure l'immunité protectrice à l'égard de *C. albicans* et que les lymphocytes T CD8+, bien que diminués en nombre chez les souris Tg, augmentent néanmoins dans les muqueuses buccales en réponse à la persistance de la candidose. Nous en avons conclu que l'altération du phénotype et des fonctions des DCs et des lymphocytes T CD4+ détermine la susceptibilité à la candidose buccale chez les souris transgéniques exprimant le VIH-1.

Associé aux travaux effectués par Miriam Marquis, nous avons démontré que la candidose buccale chronique des souris Tg CD4C/HIV^{MutA} ne peut pas être attribuée à une altération du nombre ou des fonctions des PMNs à l'égard de *C. albicans*. Néanmoins, l'utilisation de souris Tg CD4C/HIV^{MutG} homozygotes KO pour l'Ag CD8, exprimant uniquement le gène *NEF*, nous a permis de démontrer que les lymphocytes T CD8+ participent in vivo aux défenses de l'hôte à l'égard de la candidose buccale. Nous en avons conclu que les lymphocytes T CD8+ et non les PMNs sont requis pour limiter la charge buccale chronique de *C. albicans* chez la souris transgénique exprimant le VIH-1. L'ensemble de ces travaux n'auraient pas pu être réalisé sans la collaboration du laboratoire du Dr Paul Jolicoeur qui a développé et caractérisé toutes les lignées de souris Tg exprimant le VIH-1 que nous avons utilisées.

Au cours de ce chapitre, je discuterai et critiquerai les résultats obtenus afin de dégager de futures perspectives de recherche et de compréhension des mécanismes qui protègent l'hôte à l'égard de l'OPC.

le phénotype de candidose buccale chez les souris transgéniques exprimant le VIH-1

L'utilisation de souris Tg **CD4C/HIV^{MutG}** (expression du gène *NEF* dans les

lymphocytes T CD4+ matures et immatures, dans les macrophages et les DCs), **CD4E/HIV^{MutG}** (expression du gène *NEF* dans les lymphocytes T CD4+ matures et immatures et dans les DCs) et **mCD4/HIV^{MutG}** (expression du gène *NEF* dans les lymphocytes T CD4+ matures et immatures) (249, 251) a permis d'investiguer plus précisément le rôle de ces populations cellulaires dans l'apparition de la candidose buccale chez ces souris Tg. L'infection buccale à *C. albicans* de souris Tg **CD4C/HIV^{MutG}** a entraîné l'apparition d'une candidose similaire à celle observée chez la souris Tg **CD4C/HIV^{MutA}** (exprimant les gènes *NEF*, *REV* et *ENV* dans les lymphocytes T CD4+ matures et immatures, dans les macrophages et les DCs) (141), démontrant que l'expression du gène *NEF* est nécessaire et suffisante à l'apparition du phénotype de candidose. Les souris Tg **CD4E/HIV^{MutG}** infectées à *C. albicans* sont également atteintes de candidose buccale suggérant que l'expression du gène *NEF* dans les macrophages serait dispensable pour son établissement. Néanmoins, les perturbations affectant les lymphocytes T CD4+ et les DCs pourraient être suffisantes pour altérer les fonctions des macrophages, sans nécessiter l'expression du transgène. L'absence de phénotype de porteur chronique de *C. albicans* observée chez la souris Tg **mCD4/HIV^{MutG}** a suggéré que l'expression du gène *NEF* uniquement dans les lymphocytes T CD4+ serait insuffisante à l'établissement d'une candidose buccale chez cette souris Tg. Les DCs saines de souris Tg **mCD4/HIV^{MutG}** pourraient néanmoins induire une immunité protectrice à l'égard de *C. albicans*. Cette interprétation est renforcée par l'observation en coculture d'une activation préservée des lymphocytes T CD4+ provenant de souris Tg **CD4C/HIV^{MutA}** infectées à *C. albicans* en présence de DCs provenant de souris Non-Tg ayant phagocyté le *C. albicans* et inversement d'une altération de l'activation de ces mêmes lymphocytes en présence de DCs provenant de souris Tg. Ces résultats suggèrent que les DCs saines des souris Tg **mCD4/HIV^{MutG}** seraient capables d'induire une immunité protectrice à l'égard de *C. albicans* en activant néanmoins les lymphocytes T CD4+ exprimant le gène *NEF*. Cependant, au cours de la primo-infection à *C. albicans*, les DCs saines de souris Tg **mCD4/HIV^{MutG}** pourraient contribuer à l'élimination du champignon des muqueuses buccales en compensant à l'altération de ces lymphocytes T CD4+ par

l'activation d'un mécanisme moléculaire ou cellulaire indépendant tel que l'activation des lymphocytes T CD8+. En effet, les CTL peuvent générer une forte réponse primaire indépendante des lymphocytes T CD4+ lors d'une première infection par des micro-organismes (82, 507). Les CTL provenant de souris KO pour le CMH II ou pour l'Ag CD4 infectées par le virus influenza présentent une augmentation de leur activité cytotoxique in vitro à l'égard de ce micro-organisme (82, 507, 688). L'activation des APCs par des micro-organismes via les TLRs pourrait expliquer l'activation directe des CTL sans nécessiter l'aide des lymphocytes T CD4+ (53). Nous avons observé chez les souris Tg **CD4C/HIV^{MutA}** exprimant le transgène dans les DCs, une augmentation significative du nombre de lymphocytes T CD8+ dans les muqueuses buccales au cours de la primo-infection à *C. albicans* et pendant toute la phase de porteur chronique. Les souris non-Tg infectées à *C. albicans* ont présenté une augmentation des lymphocytes T CD8+ uniquement au cours de la primo-infection et la diminution de cette population cellulaire a coïncidé avec l'élimination du champignon des muqueuses buccales chez ces souris non-Tg. Ces observations renforcent l'idée que la perturbation des DCs chez la souris Tg pourrait contribuer à elle-seule à l'établissement d'une candidose buccale.

Néanmoins, chez la souris Tg **mCD4/HIV^{MutG}**, la sous-population de DCs CD8- (251), habituellement présente dans la rate (661) et dans les ganglions lymphatiques (550), pourrait exprimer le transgène. Les DCS CD8- expriment fortement l'Ag CD11b (491) et pourraient être apparentées aux sous-populations cellulaires CD11b^{hi} CD11c+ observées dans les muqueuses buccales des souris Tg, n'excluant pas la possibilité que les DCs buccales des souris Tg **mCD4/HIV^{MutG}** expriment le gène *NEF*. De plus, les DCs CD8+ des ganglions lymphatiques diminuent au profit des DCs myéloïdes chez la souris Tg (491). Les souris Tg **CD4F/HIV^{MutG}** présentent une faible expression du transgène dans les DCs myéloïdes (1-9%) et une expression variable dans les DCs lymphoïdes (11-40% dépendant du taux d'expression du transgène dans les différentes lignées générées) (251). L'étude longitudinale de l'infection buccale chez les souris Tg **CD4F/HIV^{MutG}** permettrait de valider l'hypothèse que seule l'altération des DCs induite par l'expression du gène *NEF* est

responsable de l'apparition du phénotype de candidose buccale observée chez la souris Tg.

<u>Altération du phénotype et du nombre des DCs exprimant le transgène</u>

L'expression du transgène induit une importante diminution des DCs CD11b^{low} I-A^{k}+ CD11c+ matures dans les muqueuses buccales des souris Tg **CD4C/HIV^{MutA}**, indépendamment de l'infection à *C. albicans*. Cette diminution a également été observée chez les souris Tg **CD4C/HIV^{MutG}** et **CD4E/HIV^{MutG}** suggérant que l'expression du gène *NEF* dans les DCs matures des muqueuses buccales pourrait être responsable de leur déplétion. Cette interprétation a été confirmée par l'absence de déplétion de cette sous-population observée chez les souris Tg **mCD4/HIV^{MutG}**. L'absence de perturbation des DCs CD11b^{low} I-A^{k}+ CD11c+ matures mais également des CD11b^{high} I-A^{k}+ CD11c+ immatures des muqueuses buccales des souris Tg **mCD4/HIV^{MutG}** pourrait suggérer que ces sous-populations cellulaires n'expriment pas le transgène bien qu'il puisse être exprimé dans les DCs CD8- de cette souris Tg. Les DCs des muqueuses buccales exprimant le transgène présentent également une diminution de l'expression membranaire du CMH II, en accord avec la diminution observée à la surface des DCs des ganglions lymphatiques des souris Tg **CD4C/HIV^{MutA}** (491). Le nombre de DCs des ganglions lymphatiques des souris Tg **CD4C/HIV^{MutA}** est diminué et cette population cellulaire présente un défaut de maturation (491), suggérant que les DCs des muqueuses buccales de cette souris Tg pourrait également présenter ce défaut. L'altération de la maturation des DCs pourrait résulter des effets directs de la protéine Nef ou indirectement d'un environnement perturbé par la diminution des lymphocytes T CD4+ (491). Chez les patients infectés au VIH, la protéine Nef a été observée tantôt diminuant l'expression du CMH II (564, 613) en séquestrant dans les compartiments intracellulaires les complexes CMH II-peptide (612), tantôt induisant la différentiation des DCs immatures en activant l'expression membranaire du CMH II et de ses co-récepteurs CD40, CD80 et CD86 (501). Néanmoins, les LCs des patients infectés au

VIH présentent une altération de leur différentiation terminale (486, 523) et sont diminuées en nombre dans les muqueuses buccales (118, 592) et dans l'oesophage (111). La déplétion des DCs chez l'homme pourrait résulter des changements cytopathiques induit par l'infection au VIH, d'une réponse médiée par les CTL induisant la lyse des DCs cibles, ou de la diminution de ses marqueurs de surface (39, 118).

Cependant, la diminution progressive du nombre total de DCs des ganglions lymphatiques de souris Tg **CD4C/HIV^{MutA}** est concomitante à une accumulation significative des DCs CD11b^{hi} immatures (491). Néanmoins, bien que les DCs CD11b^{low} I-A^{k}+ CD11c+ matures soient déplétées dans les muqueuses buccales de souris **CD4C/ HIV^{MutA}**, aucune accumulation de DCs CD11b^{high} I-A^{k}+ CD11c+ immatures a été observée chez ces souris Tg. De plus, la proportion de DCs CD11b^{low} I-A^{k}+ CD11c+ matures des muqueuses buccales des souris Tg **CD4C/HIV^{MutA}** est 3 à 4 fois inférieure à celle des souris non-Tg et reste constante au cours de la progression de la maladie apparentée au SIDA, indépendamment de l'infection à *C. albicans*. Le même rapport entre le nombre de DCs CD8+ du thymus de souris Tg **CD4C/HIV^{MutA}** et celui de souris non-Tg a également été observé (491). La diminution du nombre de DCs CD8+ dans le thymus des souris Tg pourrait résulter d'une altération de la distribution des différentes sous-populations de DCs dans les tissus probablement induite par l'expression du transgène (491). On ne peut donc pas exclure que les DCs CD11b^{high} I-A^{k}+ CD11c+ étiquetées immatures et DCs CD11b^{low} I-A^{k}+ CD11c+ étiquetées matures observées dans les muqueuses des souris Tg **CD4C/HIV^{MutA}** et non-Tg seraient des sous-populations de DCs distinctes d'origine lymphoïde ou myéloïde. La déplétion des DCs CD11b^{low} I-A^{k}+ CD11c+ matures observée chez la souris Tg **CD4C/HIV^{MutA}** pourrait résulter d'une altération de la distribution de cette sous-population de DCs dans les muqueuses buccales induite par l'expression du transgène. Cette sous-population de DCs pourrait être d'origine myéloïde, en accord avec le retard de maturation des DCs dérivées de la moelle osseuse observé in vitro chez la souris Tg (491).

Les DCs dérivées de la moelle osseuse provenant de souris Tg **CD4C/HIV^{MutA}**

conservent la capacité de phagocyter le *C. albicans*, mais présentent une diminution de l'expression d'IL-12 associée à cette phagocytose. L'endocytose de *C. albicans* par les DCs permet d'activer leur production d'IL-12 nécessaire à l'activation des lymphocytes Th1 CD4+ et de l'immunité acquise protectrice à l'égard du champignon (134). La diminution de la proportion de DCs dérivées de moelle osseuse provenant de souris Tg **CD4C/HIV^{MutA}** exprimant l'IL-12 concorde avec le retard de maturation de ces populations cellulaires et la diminution de l'expression membranaire du CMH II induite par l'expression du transgène observés chez ces souris Tg (491). L'expression de Nef par les DCs immatures humaines et du macaque induit la sécrétion de cytokines et de chemokines, et active la protéine de signalisation intracellulaire STAT3, sans augmenter l'expression de HLA-DR et du CD86 associée au profil de maturation des DCs (410, 411). La protéine STAT3 est associée à la production d'IL-10 et à l'expression membranaire de son récepteur IL-10R (4, 158, 667). L'IL-10 joue un rôle important dans le blocage de la production de chemokines, des molécules de stimulation CD80, CD86 et du CMH II (482). L'activation de cette voie de signalisation par Nef pourrait contribuer à la perturbation de la production des cytokines telle que l'IL-12 par les DCs des souris Tg **CD4C/HIV^{MutA}**. La population de DCs CD11b^{low} CD11c+ matures, déplétées chez la souris Tg, produit majoritairement les cytokines Th1 IFN-γ et IL-2 (423), tandis que la population de DCs CD11b^{high} CD11c+, population cellulaire préservée chez la souris Tg, produit de forts taux des cytokines Th2 IL-4 et IL-10, et plus modérément des cytokines Th1 IFN-γ et IL-2 (423). La perturbation des DCs des muqueuses buccales chez ces souris Tg pourrait concourir à l'établissement de la colonisation des muqueuses par *C. albicans*, soit par une absence de présentation de ces Ag aux lymphocytes T CD4+, soit par un défaut de migration des DCs et/ou des lymphocytes T CD4+, entraînant la perte de l'immunité acquise Th1, protectrice à l'égard de *C. albicans*. De plus, l'expression de la gp120 dans les APCs et l'altération de l'activation du CD40L des lymphocytes T CD4 par ces Ag contribuent à la diminution de la production d'IL-12 et d'IFN-γ par les APCs, prévenant ainsi d'une réponse Th1 protectrice (483, 614). Les souris Tg **CD4C/HIV^{MutA}** expriment non seulement le gène *ENV*, mais leurs

lymphocytes T CD4+ présentent également une diminution de l'expression membranaire de l'Ag CD40L (491).

<u>Altération du phénotype et du nombre de lymphocytes T CD4+ exprimant le transgène</u>

L'expression du gène *NEF* induit la perte des lymphocytes T CD4+ chez les souris Tg (491), tant au niveau des muqueuses buccales et des ganglions lymphatiques, que dans le sang périphérique telle qu'observée chez les patients infectés au VIH (257, 401). Nef induit également la diminution de l'expression membranaire du CD4 à la surface des lymphocytes T des souris Tg et leur activation permanente, résultant en l'apoptose de ces lymphocytes T CD4+ (672). Nef est capable de réduire l'expression membranaire du CD4 (17, 96, 112, 224, 279, 358, 360, 389, 609) en activant son endocytose et sa dégradation dans les lysosomes (323, 382, 514). Chez les patients infectés au VIH, Nef contribue à l'apoptose des lymphocytes T CD4+ (5, 533, 641) en activant la voie Fas/FasL (28, 152, 690, 702). Les lymphocytes T CD4+ et T CD8+ des souris Tg **CD4C/HIV$^{\text{MutA}}$** ont une expression augmentée de Fas et FasL (496). Néanmoins, les gènes codant pour ces molécules sont dispensables pour le développement de la maladie apparentée au SIDA chez les souris Tg, tant au niveau du système immunitaire que dans les organes profonds (496). En effet, le croisement de souris Tg **CD4C/HIV$^{\text{MutA}}$** et **CD4C/HIV$^{\text{MutG}}$** avec des souris C3H lpr/lpr (Fas), C3H/gld/gld (FasL), Tg Bcl2/Wehi25 et des souris déficientes en ICE (IL-1β-Converting Enzyme) et en TNF-R1, a permis au laboratoire du Dr Paul Jolicoeur d'étudier l'implication des voies de signalisation apoptotique (Fas, FasL, TNF-R1) ou anti-apoptotique (ICE, Bcl2) dans la maladie apparentée au SIDA et dans la perte des lymphocytes T CD4+ chez la souris Tg (496). Fas, FasL, ICE et TNF-R1 sont non seulement dispensables pour le développement de la maladie, mais la sur-expression de Bcl2 ne protège pas les souris Tg de la perte des lymphocytes T CD4+, suggérant que les voies d'apoptose activées par Nef chez la souris Tg sont distinctes de celles étudiées (496). Bien que Nef soit capable d'activer les lymphocytes T CD4+ de façon indirecte par son

expression dans les macrophages (622), aucune activation de l'apoptose a été observée chez les souris Tg **CD68/HIV** (données non publiées, (496)). Chez ces souris Tg, l'expression de Nef, dirigée par les séquences régulatrices du gène codant pour l'Ag CD68, est présente dans les macrophages, mais absente des lymphocytes T CD4+ (données non publiées, (496)). Néanmoins, le croisement de souris Tg **CD4C/HIV^{MutA}** avec des souris déficientes en CD4 a démontré que les lymphocytes T CD4+ étaient dispensables pour le développement de la maladie (672), suggérant que l'altération de la lignée myéloïde monocyte/macrophage serait suffisante pour générer le phénotype de ces souris Tg.

Le phénotype activé/mémoire des lymphocytes T CD4+ exprimant le transgène

L'analyse de la proportion des sous-populations de lymphocytes T CD4+ des ganglions lymphatiques cervicaux de souris Tg **CD4C/HIV^{MutA}** a révélé une diminution de la population de lymphocytes T naïfs (CD44-/^{lo}, CD62L+) et une augmentation de celle présentant un phénotype activé/mémoire (CD44^{hi}, CD62L-), comparativement aux souris non-Tg. Ces observations indiquent la persistance d'un stade activé des lymphocytes T CD4+ chez la souris Tg. La proportion de lymphocytes T CD4+ présentant un phénotype activé/mémoire provenant des ganglions lymphatiques de souris Tg **CD4C/HIV^{MutG}** est également augmentée (672), suggérant que l'expression du gène *NEF* pourrait induire ce phénotype. Cette interprétation est renforcée par l'observation chez la souris Tg **CD4C/HIV^{MutG}** d'une plus large proportion de lymphocytes T CD4+ exprimant de hauts taux de la protéine Nef présentant un phénotype activé/mémoire (672). Une augmentation de l'expression de l'Ag CD44 et une diminution de l'Ag CD62L à la surface des lymphocytes T CD4+ ont été observées chez d'autres souris Tg exprimant Nef (358), ainsi qu'une augmentation de l'activation des lymphocytes T CD4+ exprimant Nef in vitro (186, 384, 583).

L'infection à *C. albicans* n'altère pas la proportion des lymphocytes T CD4+ présentant un phénotype activé/mémoire chez la souris Tg, en accord avec l'observation

que l'activation des lymphocytes T CD4+ chez ces souris est indépendante de la stimulation du TCR par les Ag (672). Les lymphocytes T CD4+ présentant un phénotype activé/mémoire expriment faiblement l'Ag CD4, fortement le transgène, et sont plus susceptibles à l'apoptose (496), suggérant un lien possible entre l'activation des lymphocytes T CD4+ médiée par Nef et la mort cellulaire. L'activation permanente de l'immunité chez la souris Tg pourrait contribuer à l'épuisement de la réserve de lymphocytes T CD4+ naïfs (672), tel qu'observé dans l'infection au VIH (257, 401). Chez les patients infectés au VIH, l'activation immunitaire chronique des lymphocytes T CD4+ engage une partie de cette population dans un processus d'apoptose, entraînant ainsi une accélération de la déplétion de la réserve de lymphocytes T CD4+ (257).

La proportion et le nombre de lymphocytes T CD4+ présentant un phénotype mémoire au repos ($CD44^{hi}$, CD62L+) sont conservés chez la souris Tg comparativement à ceux de la souris non-Tg. Sept jours après l'infection à *C. albicans*, toutefois, le nombre absolu de lymphocytes T CD4+ présentant un phénotype mémoire au repos est augmenté chez la souris non-Tg. Cette population pourrait être apparentée aux lymphocytes T CD4+ à mémoire centrale chez l'homme (548), suggérant que non seulement les lymphocytes T CD4+ demeurent dans un état d'activation constante, mais sont incapables de générer des cellules mémoires à long terme spécifiques aux Ag de *C. albicans*. Cette hypothèse est renforcée par l'observation d'une anergie des lymphocytes T CD4+ de souris Tg infectées à *C. albicans* en réponse à la restimulation in vitro par ses Ag. De plus, la stimulation du TCR des lymphocytes T CD4+ à mémoire centrale induit leur prolifération et la sécrétion d'IL-2 (547). Les expériences de coculture ont révélé que les lymphocytes T CD4+ provenant de souris Tg infectées à *C. albicans* en présence de DCs dérivées de la moelle osseuse de souris Tg ayant phagocyté le champignon présentaient une diminution de leur prolifération comparativement à celle observée lors de la coculture de ces populations cellulaires provenant de souris non-Tg. Cette diminution de prolifération s'accompagne d'une diminution de la quantité d'IL-2 produite, lors de la coculture.

Le profil Th1/Th2 des lymphocytes T CD4+ exprimant le transgène

Les lymphocytes T CD4+ provenant de souris Tg **CD4C/HIV^{MutA}**, stimulés in vitro à l'aide d'un cocktail anti-CD3/anti-CD28 et PMA/ionomycine, ont présenté une évolution vers un profil Th2 au stade plus tardif de la maladie apparentée au SIDA. Cette interprétation provient de l'observation d'une augmentation de la proportion de lymphocytes T CD4+ de souris Tg à exprimer l'IL-4 et d'une diminution de cette population cellulaire à exprimer l'IFN-γ au stade plus tardif de la maladie apparentée au SIDA, et d'une augmentation de cette population cellulaire à exprimer l'IL-10 tout au long de la maladie. L'augmentation de la production d'IL-4 et d'IL-10 retrouvée chez la souris Tg est étroitement associée à une réponse immunitaire non-protectrice à l'égard de *C. albicans* (524). La charge buccale chronique de *C. albicans* observée chez la souris Tg pourrait provenir d'un déplacement de la réponse Th1 protectrice à l'égard de *C. albicans* vers le développement d'une réponse non-protectrice Th2 par les lymphocytes T CD4+ des ganglions lymphatiques cervicaux. Cette hypothèse est renforcée par l'observation d'une diminution de la proportion de DCs dérivées de la moelle osseuse provenant de souris Tg **CD4C/HIV^{MutA}** à exprimer l'IL-12 comparativement à celles provenant de souris non-Tg. De plus, l'IFN-γ, diminué au stade plus tardif de la maladie apparentée au SIDA chez la souris Tg **CD4C/HIV^{MutA}**, est requis pour la sensibilité à l'IL-12 chez les souris présentant une infection à *C. albicans* (103). L'altération fonctionnelle des lymphocytes T CD4+ de ganglions lymphatiques cervicaux chez la souris Tg **CD4C/HIV^{MutA}** pourrait résulter non seulement d'une réduction de la production d'IL-12 par les DCs de la souris Tg, mais également d'une altération de la sensibilité à l'IL-12 induite par la perte de la production d'IFN-γ. L'IFN-γ est requis pour annuler l'inhibition induite par l'IL-4 de l'expression du récepteur IL-12-Rβ2 (623). L'expression de Nef pourrait moduler l'expression des cytokines par les lymphocytes T CD4+ chez les souris Tg. La protéine Nef est capable d'induire directement la production de TNF-α (346) et d'IL-10 (75), retrouvées augmentées chez la souris Tg **CD4C/HIV^{MutA}**. L'IL-10 est capable d'induire la diminution de l'expression

du CMH II à la surface des DCs (45, 695), que nous avons observée chez la souris Tg **CD4C/HIV^{MutA}** (491). Le *knock out* du gène codant pour l'IL-10 chez la souris augmente la réponse immunitaire Th1 nécessaire à l'élimination de *C. albicans* (657), suggérant que cette cytokine pourrait participer à l'établissement d'une réponse Th2 non-protectrice à l'égard de *C. albicans* chez la souris Tg **CD4C/HIV^{MutA}**. La progression vers un profil Th2 non-protecteur à l'égard de *C. albicans* observée chez cette souris Tg est en accord avec l'observation d'un profil de cytokines Th2 retrouvé dans la salive des patients infectés au VIH (352).

La prolifération des lymphocytes T CD4+ à l'égard de C. albicans

Nous avons observé que les lymphocytes T CD4+ des ganglions lymphatiques cervicaux des souris Tg **CD4C/HIV^{MutA}** infectés à *C. albicans* sont anergiques à la re-stimulation par les Ag de *C. albicans* et ne se différentient pas en lymphocytes T CD4+ effecteurs (CD62L-). Les lymphocytes T CD4+ des ganglions lymphatiques de souris Tg **CD4C/HIV^{MutG}** présentent également une capacité de division limitée en réponse à la stimulation par les Ac anti-CD3 et anti-CD28 ou lors d'un réaction allogénique leucocytaire mixte (672). La majorité des lymphocytes T_{reg} CD4+CD25+ prolifèrent lors de la stimulation par les Ac anti-CD3 et anti-CD28 (670), mais sont anergiques à la re-stimulation par les Ag (670), suggérant que l'augmentation de la proportion et/ou du nombre de lymphocytes T_{reg} pourrait jouer un rôle dans la perturbation de l'hypersensibilité de type retardée à l'égard de *C. albicans.* Cette hypothèse est renforcée par l'observation d'une augmentation de l'expression du CD25 à la surface des lymphocytes T CD4+ des ganglions chez les souris Tg (496, 672).

Les expériences de co-culture de lymphocytes T CD4+ en présence de DCs ayant phagocyté le *C. albicans* ont révélé que l'expression du transgène dans une seule ou les deux populations cellulaires réduit nettement la prolifération des lymphocytes T CD4+ et la production d'IL-2. Ces résultats démontrent que les altérations fonctionnelles de ces

deux populations cellulaires empêchent la réponse immunitaire à l'égard de *C. albicans* chez la souris Tg.

Les DCs dérivées de la moelle osseuse provenant de souris Tg phagocytent normalement le *C. albicans*. Néanmoins, la diminution de l'expression du CMH II, du CD40 et du CD86 observée à la surface des DCs provenant de souris Tg induit une réduction de la capacité à présenter le cytochrome c de pigeon aux lymphocytes TcR AD10 CD4+ (491). La réduction de la capacité à présenter à la fois le cytochrome c de pigeon et les peptides par ces DCs a suggéré que cette réduction ne serait probablement pas due à une altération de leur capacité à apprêter les Ag (491). Chez les patients infectés au VIH, la capacité des APCs à présenter les Ag et à stimuler la prolifération des lymphocytes T CD4+ est diminuée (316, 366) et s'accompagne d'une diminution de l'expression du CMH II et des co-récepteurs CD40 et CD86 (365, 488) impliqués dans l'activation des lymphocytes T CD4+ spécifiques à l'égard de *C. albicans* (226, 456, 636).

La co-culture en présence de DCs dérivées de la moelle osseuse provenant de souris non-Tg ayant phagocyté ou non le *C. albicans* a révélé que les lymphocytes T CD4+ provenant de souris Tg **CD4C/HIV$^{\mathrm{MutA}}$** présentaient une diminution de leur prolifération. Cette diminution a également été observée en présence de la concanavaline A démontrant que l'altération des DCs et des lymphocytes CD4+ réduit la capacité des lymphocytes T CD4+ à proliférer. Néanmoins, l'ajustement de la quantité de lymphocytes T CD4+ provenant de souris Tg **CD4C/HIV$^{\mathrm{MutA}}$** infectées à *C. albicans* dans l'expérience de co-culture en présence de DCs ayant phagocyté le *C. albicans*, a permis d'observer une prolifération plus importante que celle obtenue lors de l'expérience de re-stimulation par ces Ag. Les souris Tg **CD4C/HIV$^{\mathrm{MutA}}$** infectées à *C. albicans* pourraient donc développer une réponse mémoire partielle à l'égard de *C. albicans*. Bien que les DCs dérivées de la moelle osseuse provenant de souris Tg **CD4C/HIV$^{\mathrm{MutA}}$** présentent un retard de maturation (491), la différentiation de ces DCs induite par le *C. albicans* pourrait permettre de générer une meilleure prolifération des lymphocytes T CD4+ que celle induite par les DCs immatures des ganglions lymphatiques.

Nous avons démontré que l'altération de la prolifération des lymphocytes T CD4+ provenant de souris Tg était associée à une diminution de la sécrétion d'IL-2. L'altération de la prolifération et la diminution de la sécrétion d'IL-2 pourraient être induites par l'expression du transgène et plus particulièrement Nef dans les lymphocytes T CD4+ des souris Tg. Cette hypothèse est renforcée par l'observation que Nef est non seulement capable d'interférer avec l'activation de la p56 Lck, perturbant la signalisation via l'IL-2R (236, 237), mais également la progression du cycle cellulaire en diminuant les cyclines D1 et A (237, 438). La prolifération des lymphocytes T CD4+ provenant de souris Tg en réponse à la stimulation par le PMA/inonomycine est conservée. Puisque le PMA active directement la protéine kinase C (640), l'altération du signal de transduction chez la souris Tg pourrait être en aval du TcR et en amont de la protéine kinase C (672). Les lymphocytes T CD4+ des patients infectés au VIH présentent une diminution de leur prolifération en réponse aux mitogènes (12, 270, 414) et aux Ag de *C. albicans* (101, 102, 414, 503), et cette altération de la prolifération est associée à une diminution de la production d'IL-2 (285).

La restauration de l'immunité des souris Tg à l'égard de C. albicans

Bien que la perturbation des lymphocytes T CD4+ joue un rôle important dans la susceptibilité à la candidose buccale chronique chez la souris Tg **CD4C/HIV^{MutA}**, l'utilisation de différentes lignées de souris Tg a démontré qu'une déficience additionnelle des DCs est nécessaire pour observer ce phénotype. Néanmoins, nous avons observé que le transfert adoptif des lymphocytes T CD4+ provenant de souris non-Tg est capable de réduire, de façon transitoire, la charge buccale de *C. albicans* chez les souris **CD4C/HIV^{MutA}**. Les lymphocytes T CD4+ des ganglions lymphatiques cervicaux de souris Tg transférées ont révélé une capacité de prolifération partiellement restaurée à l'égard des Ag de *C. albicans*. La restauration partielle de la prolifération pourrait être attribuée à la capacité de présentation, partiellement préservée, des DCs des souris Tg observée en co-

culture.

Bien que les CTL peuvent générer une forte réponse primaire indépendante des lymphocytes T CD4+ lors d'une première infection par des micro-organismes (82, 507), les APCs activés par les lymphocytes T CD4+ seraient capables de recevoir l'information et de la transmettre aux lymphocytes T CD8+ (53). On ne peut donc pas exclure la possibilité que le transfert de lymphocytes T CD4+ sains ait permis de restaurer transitoirement la prolifération des CTL à l'égard de *C. albicans* chez la souris Tg **CD4C/HIV^{MutA}**. Cette hypothèse est renforcée par l'observation d'une diminution plus modérée du CMH I à la surface des DCs dérivées de la moelle provenant de souris Tg **CD4C/HIV^{MutA}** comparativement à celle du CMH II (491), et d'une prolifération des lymphocytes T CD8+ de ganglions lymphatiques cervicaux partiellement préservée en réponse aux Ag de *C. albicans* chez la souris Tg **CD4C/HIV^{MutA}** ***(Figure 1)***.

Le nombre de lymphocytes T_{reg} diminue moins vite que les autres lymphocytes T CD4+ chez certains patients en phase avancée du SIDA (175), résultant en une augmentation du ratio régulateur:*helper* pouvant contribuer au dysfonctionnement des lymphocytes T CD4+ effecteurs (175). La diminution non-proportionnelle du nombre des sous-populations de lymphocytes T CD4+ (naïfs, activés/effecteurs, régulateurs) pourrait favoriser l'activité de suppression de la prolifération des lymphocytes T CD4+ spécifiques à l'égard de *C. albicans* par les lymphocytes T_{reg} chez la souris Tg **CD4C/HIV^{MutA}**. Ainsi, le transfert adoptif de lymphocytes T CD4+ pourrait ré-équilibrer les proportions des sous-populations des lymphocytes T CD4+ des ganglions lymphatiques cervicaux, levant ainsi la suppression de la prolifération par les lymphocytes T_{reg} et permettant la réduction transitoire de la charge buccale de *C. albicans* chez les souris Tg **CD4C/HIV^{MutA}** transférées.

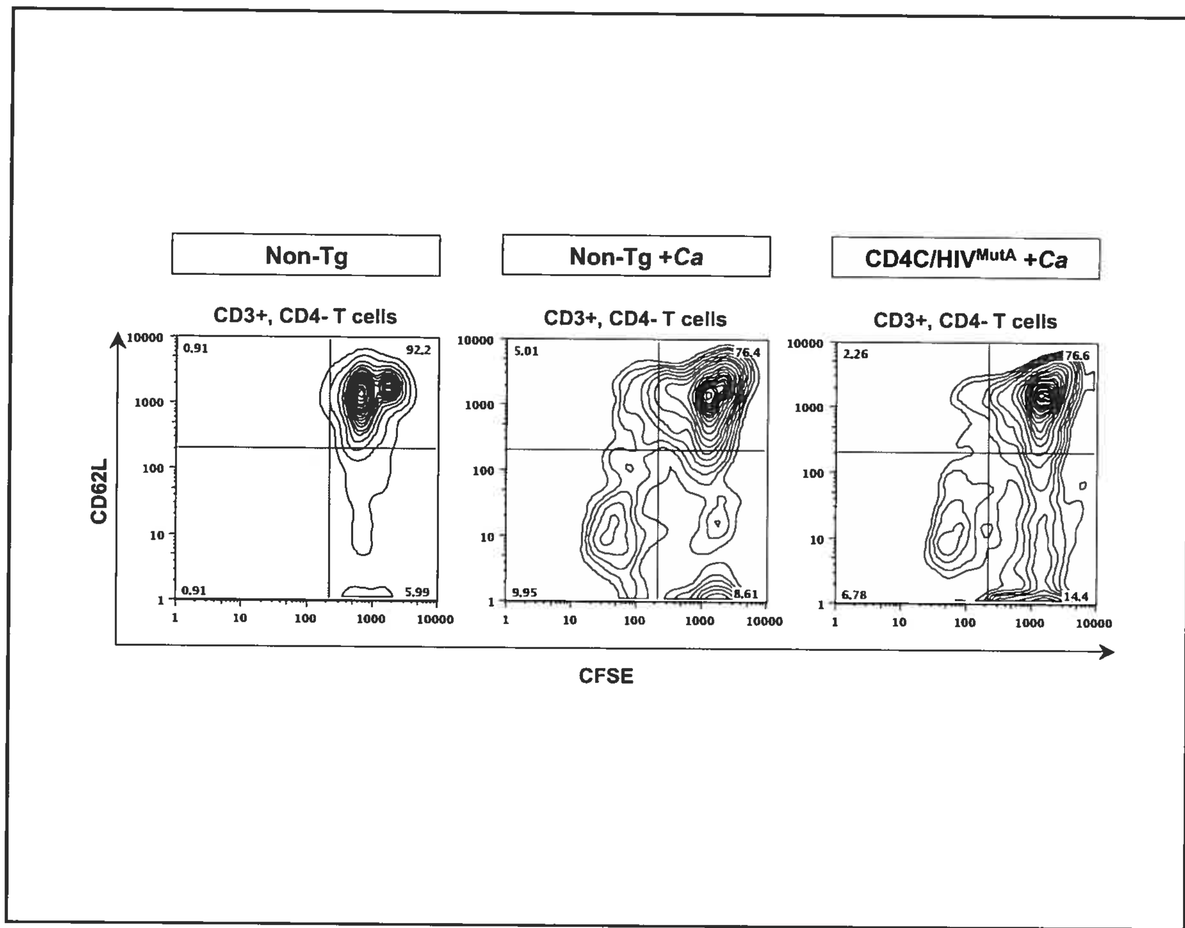

Figure 1: Étude préliminaire de la prolifération des lymphocytes T CD8+ (estimés par les populations cellulaires CD3+CD4-) des ganglions lymphatiques cervicaux en réponse aux Ag de *C. albicans* chez la souris Tg CD4C/HIV^{MutA} et non-Tg infectées à *C. albicans* comparativement à celle des souris Non-Tg non-infectées.

L'absence de réduction de la charge buccale chez les souris Tg **CD4C/HIV^{MutA}** transférées avec des DCs dérivés de la moelle osseuse provenant de souris non-Tg pourrait résulter d'un nombre insuffisant de DCs transférées bien que la même quantité de DCs protège les souris BALB/c contre une infection intra-veineuse à *C. albicans* (134). On ne peut pas exclure la possibilité que les DCs transférées n'aient pas migré à la muqueuse buccale pour endocyter le *C. albicans* ou dans les ganglions lymphatiques cervicaux pour présenter les Ag aux lymphocytes T CD4+. Il est également possible que le stade de maturation des DCs transférées puisse être à l'origine de cette absence de prolifération. Cette possibilité est suggérée par l'observation d'une meilleure migration dans les tissus des DCs dérivées de la moelle osseuse cultivées en présence de TNF-α, suite au transfert de ces cellules chez la souris BALB/c (606).

L'altération des DCs et des lymphocytes T CD4+ est donc impliquée dans l'initiation et la maintenance de la candidose buccale chronique chez les souris Tg, suggérant que des perturbations similaires pourraient rendre les patients infectés au VIH susceptibles à l'OPC. Néanmoins, aucune de ces cellules ne peut agir directement pour inhiber la prolifération de *C. albicans* dans les muqueuses buccales, du fait de leur localisation éloignée de la surface de l'épithélium où est circonscrit le champignon. Ainsi, des mécanismes effecteurs indirects impliquant les PMNs, les lymphocytes T CD8, les cellules épithéliales et/ou leur sécrétion de molécules anti-microbiennes pourraient circonscrire et inhiber la croissance de *C. albicans*.

Le rôle des PMNs dans la candidose buccale chez la souris Tg

Lors des expériences de déplétion des PMNs à l'aide de l'Ac monoclonal RB6-8C5 chez les souris Tg **CD4C/HIV^{MutA}**, nous avons observé aucune augmentation de la charge buccale de *C. albicans*, ni de dissémination systémique chez ces souris Tg tant au niveau de la phase précoce que tardive de la candidose buccale. Les PMNs sont donc dispensables pour le contrôle de la candidose buccale et systémique chez la souris Tg, en dépit de leur

activité anti-*Candida* bien caractérisée in vitro et in vivo (113, 188, 289, 290).

L'augmentation de la production d'IL-4 et d'IL-10 par les lymphocytes T CD4+ en réponse à une infection gastrique à *C. albicans* (527), pourrait moduler l'activité des PMNs à l'égard de *C. albicans.* Seules les souris SCID (289, 290), déplétées de leurs PMNs à l'aide d'un Ac monoclonal RB6-8C5, et les souris double mutantes bg/bg nu/nu (93, 94), dépourvues de lymphocytes T et de phagocytes, ont montré une augmentation soutenue de la charge buccale et un dissémination systémique de *C. albicans* aux organes profonds, suggérant que les phagocytes (PMNs et macrophages) circonscriraient le champignon à la surface de l'épithélium. L'absence de lymphocytes T CD8+ et l'altération complète des fonctions des macrophages chez la souris bg/bg nu/nu suggèrent que ces populations cellulaires pourraient prévenir l'augmentation de la charge buccale et de la dissémination systémique de *C. albicans* aux organes profonds chez la souris Tg **CD4C/HIV^{MutA}**.

Puisque les PMNs des souris Tg **CD4C/HIV^{MutA}** n'expriment pas le transgène, les variations de leur activité (augmentation de la flambée oxydative et diminution de l'activité anti-*Candida*) pourraient être induites indirectement par l'IL-4 (526) et l'effet suppresseur de l'IL-10 (522, 526). Ces deux cytokines ont la capacité de supprimer l'activité anti-*Candida* des PMNs chez les patients infectés au VIH (631). Néanmoins, les PMNs des souris Tg **CD4C/HIV^{MutA}** conservent leur activité anti-*Candida* au stade tardif de l'infection et sont augmentés en nombre chez ces souris Tg, démontrant que la candidose buccale chronique des souris Tg ne peut pas être expliquée par une déficience quantitative et/ou fonctionnelle des PMNs à l'égard de *C. albicans.*

Les lymphocytes T CD8+ sont-ils impliqués dans les défenses de l'hôte à l'égard de la candidose buccale?

Nous avons observé que le nombre absolu de lymphocytes T CD8+ était réduit chez la souris Tg **CD4C/HIV^{MutA}** tout au long du développement de la maladie apparentée au

SIDA, en accord avec la diminution du nombre de lymphocytes T CD8+ périphériques (261, 520) et de leur demi-vie (259) observée chez les patients infectés au VIH. Les proportions de lymphocytes T CD8+ naïfs (CD44-/^{lo}, CD62L+) et de phénotype mémoire (CD44^{hi}, CD62L-/^{lo}) (300) des ganglions lymphatiques cervicaux de souris Tg**CD4C/HIV^{MutA}** ont été observées modestement diminuées et augmentées, respectivement. Ces observations sont en accord avec la diminution progressive des lymphocytes T CD8+ naïfs concomitante à l'augmentation des lymphocytes CD8+ mémoires dans le sang périphérique des adultes (520) et des enfants (504) infectés au VIH. Bien que l'apoptose des lymphocytes T CD8+ peut être médiée par les macrophages via l'interaction de la gp120 avec le récepteur CXCR4 (261), aucune activation de l'apoptose a été observée chez les souris Tg **CD68/HIV**, exprimant le transgène dans les macrophages sans expression dans les lymphocytes T CD4+ (données non publiées, (496)). La diminution du nombre de lymphocytes T CD8+ observée dans les ganglions lymphatiques cervicaux de souris Tg **CD4C/HIV^{MutA}** pourrait résulter indirectement de l'expression de nef et de env dans les DCs, induisant ainsi l'apoptose des lymphocytes T CD8+. Cette hypothèse est renforcée par l'observation d'une diminution modeste de la proportion de lymphocytes T CD8+ des ganglions mésentériques de souris Tg **CD4C/HIV^{MutA}** exprimant les gènes *NEF*, *REV* et *ENV* comparativement à celle observée chez la souris Tg **CD4C/HIV^{MutG}** exprimant uniquement le gène *NEF* (248). La protéine Nef induit l'apoptose des lymphocytes CD8+ médiée par la caspase 8, en activant l'expression du TNF-α et de FasL par les DCs (500). Néanmoins, le FasL et le TNF-R1 sont dispensables pour le phénotype apparenté au SIDA de la souris Tg (496). Les protéines solubles nef et env sont exprimées chez la souris Tg **CD4C/HIV^{MutA}** (249), et sont toutes les deux impliquées dans la réduction de l'Ag CD8 à la surface des lymphocytes T (287), réduction observée également chez les souris Tg **CD4C/HIV^{MutA}**. La réduction de l'Ag CD8 pourrait être attribuée à l'expression du gène *ENV* chez la souris Tg **CD4C/HIV^{MutA}**. Cette hypothèse est suggérée par l'absence d'une diminution de l'expression de l'Ag CD8 à la surface des lymphocytes T CD4-CD8+ du thymus et de la rate de souris Tg **CD4C/HIV^{MutG}** exprimant uniquement le gène *NEF* (250).

En dépit de cette déplétion, les lymphocytes T CD8+ maintiennent leur capacité à répondre à l'infection à *C. albicans* en augmentant quantitativement dans la muqueuse buccale et dans les ganglions lymphatiques cervicaux de souris Tg **CD4C/HIV^{MutA}**, tant au niveau de la primo-infection que dans la phase de porteur chronique (141). Chez les patients infectés au VIH présentant une OPC, les lymphocytes T CD8+ s'accumulent dans la couche basale de l'épithélium des muqueuses buccales, démontrant que ces populations cellulaires peuvent être activement recrutées dans la muqueuse en réponse à la candidose (433, 523). Lors des expériences d'infection à *C. albicans*, nous avons observé une augmentation significative de la charge buccale de *C. albicans* chez les souris Tg **CD4C/HIV^{MutG}** CD8 homozygotes KO comparativement aux souris Tg **CD4C/HIV^{MutG}** tout au long de la phase de porteur chronique. Les souris CD8 KO et non-Tg ont éliminé le *C. albicans* des muqueuses buccales immédiatement après la primo-infection, démontrant que l'augmentation de la charge buccale à *C. albicans* chez la souris CD8 KO apparaît uniquement lorsqu'elle exprime le transgène CD4C/HIV^{MutG}. Ces résultats suggèrent que les lymphocytes T CD8+ deviennent critiques pour les défenses de l'hôte à l'égard de *C. albicans* seulement lorsque les lymphocytes T CD4+ et/ou les APCs sont perturbés par l'expression du gène *NEF* chez la souris Tg **CD4C/HIV^{MutG}**. La protéine Nef induit la diminution du CMH I à la surface des DCs humaines (11, 500) et des DCs des ganglions lymphatiques provenant de souris **CD4C/HIV^{MutA}** (491), altère la présentation des Ag par les DCs aux lymphocytes T CD8+ (11), et affecte les fonctions des lymphocytes T CD8+ (500, 582). Les souris KO pour la β2-microglobuline, déficientes en lymphocytes T CD8+ et perdant l'expression du CMH I, sont susceptibles à la candidose systémique à *C. albicans* d'origine endogène, mais ont montré seulement une infection superficielle et transitoire de la langue et de l'oesophage (33). Les résultats obtenus chez les souris Tg démontrent que les lymphocytes T CD8+ participent in vivo aux défenses de l'hôte à l'égard de la candidose buccale, spécifiquement dans le contexte de l'expression de nef dans les populations cellulaires définies.

Les lymphocytes T CD8+ exercent une activité directe d'inhibition de la prolifération

de *C. albicans* (194, 433). Néanmoins, le rôle précis des lymphocytes T CD8+ dans le confinement de *C. albicans* dans la couche superficielle des muqueuses buccales d'individus infectés au VIH, soit par une activité anti-*Candida* directe ou indirectement par la sécrétion de cytokines augmentant l'activité anti-microbienne des macrophages et des PMNs à l'égard de *C. albicans*, est encore mal défini et pourrait être caractérisé plus précisément.

Conclusion

L'objectif de ces études de doctorat était de définir l'implication de certaines populations cellulaires dans la pathogénèse de la candidose buccale chez une souris transgénique (Tg) exprimant le génome du VIH-1 qui reproduit les principales manifestations cliniques et biologiques de l'infection au VIH (248, 249). Nous avons démontré que l'altération du phénotype et des fonctions des DCs et des lymphocytes T CD4+ détermine la susceptibilité à la candidose buccale chez la souris Tg et que les lymphocytes T CD8+ et non les PMNs sont requis pour limiter la charge buccale chronique de *C. albicans* chez cette souris Tg. Ces études suggèrent que des perturbations similaires pourraient induire la susceptibilité à l'OPC des patients infectés au VIH et démontrent pour la première fois que les lymphocytes T CD8+ pourraient participer aux défenses de l'hôte contre *C. albicans* in vivo.

Au terme de ces études et en tenant compte de la littérature, nous avons schématisé les altérations des défenses de l'hôte qui pourraient induire la susceptibilité à l'OPC dans l'infection au VIH ***(Figure 2)***. Une déplétion progressive et une dérégulation fonctionnelle des cellules de Langerhans de la muqueuse buccale pourraient altérer l'apprêtement et la présentation des Ag de *C. albicans* aux lymphocytes T CD4+, qui sont eux-mêmes déplétés dans l'infection au VIH, résultant en une diminution du nombre de lymphocytes CD4+ Th1 et en la perturbation de l'immunité adaptée à l'égard de *C. albicans*. Des mécanismes préservés de défense innée (calprotectine, PMNs, cellules γ/δ) et les lymphocytes T CD8+ cytotoxiques pourraient prévenir la dissémination de *C. albicans* aux organes profonds.

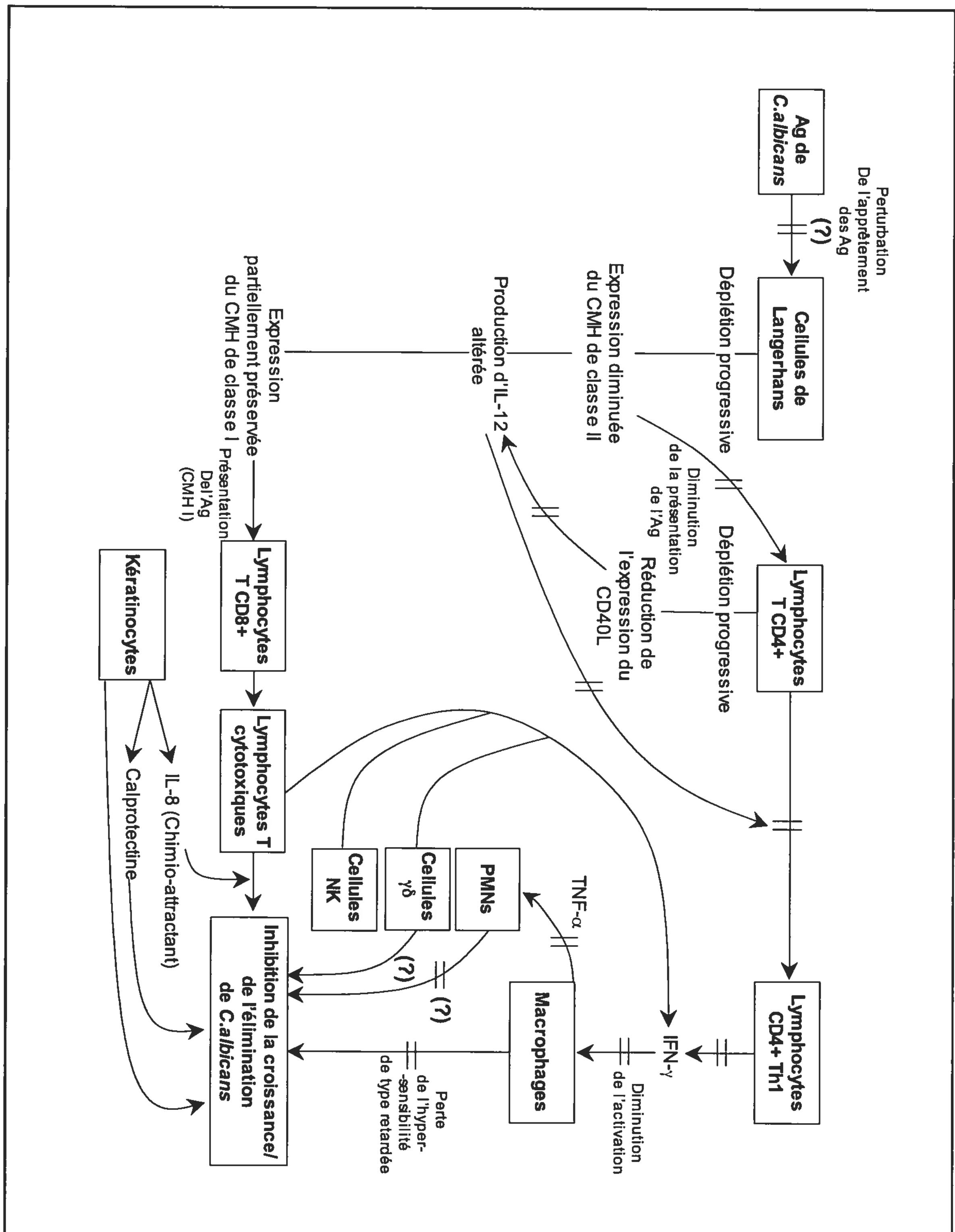

Figure 2: Hypothèse des altérations des défenses de l'hôte à l'égard de la candidose oropharyngée dans l'infection au VIH. Adaptée de *de Repentigny et al., Clin Microbiol. Rev. (2004).*

Chapitre 4

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