Université de Montréal

Études sur le rôle d'IL-18 dans l'immunopathogénèse du SIDA

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Ce mémoire intitulé :

Études sur le rôle d'IL-18 dans l'immunopathogénèse du SIDA

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RÉSUMÉ

Le virus de l'immunodéficience humaine ou VIH est l'agent qui cause le SIDA. Le VIH donne lieu à une dérégulation dans la production de certaines cytokines qui ont un rôle immunologique très important chez les patients infectés. L'IL-18, autrement nommé facteur inducteur d'IFN-γ, est une cytokine pro-inflammatoire qui affecte le système immunitaire de façon importante. Son activité est régulée par l'"*IL-18 Binding Protein*" (IL-18BP), une autre cytokine qui se lie avec l'IL-18 et inhibe son activité biologique. Des études ultérieures ont montré des niveaux élevés d'Il-18 chez les patients infectés par le VIH par rapport aux personnes saines. Cependant, aucune étude n'a été réalisée concernant la production d'IL-18BP chez ces patients. Due à sa relevance dans la régulation de l'IL-18, nous avons étudié l'effet de l'infection par le VIH sur l'équilibre entre ces deux facteurs et l'impact de cet équilibre sur l'homéostasie des cellules NK. Nous avons mesuré les taux de l'IL-18 et de l'IL-18BP circulantes dans les sérums des patients infectés par le VIH en les comparants avec le même nombre de personnes saines et séronégatives. Nous avons aussi déterminé le nombre total des différents sous-types de cellules NK et analysé l'activité des cellules NK (*Natural Killer*). Finalement nous avons cherché à déterminer si l'IL-18 pouvait induire l'apoptose des cellules NK en activant l'expression de Fas ligand. Nos résultats nous démontrent que les patients infectés par le VIH ont trois fois plus d'IL-18 que les donneurs sains. Cependant les niveaux d'IL-18BP sont plus bas chez les patients infectés comparés aux donneurs sains. Alors, le ratio IL-18/IL-18BP est augmenté chez les patients infectés, ce qui entraîne une grande quantité d'IL-18 libre et biologiquement active circulante dans leur organisme. Nos études démontrent que chez ces patients, les concentrations d'IL-18 sont en corrélation négative avec l'activité cytotoxique de leurs cellules NK. Nos études in vitro démontrent que le traitement des cellules NK par l'IL-18 induit de façon fratricide leur apoptose en augmentant l'expression de Fas ligand. Finalement, cette production non coordonnée de ces deux facteurs pourrait contribuer à une

immunopathologie induite par l'IL-18 en entraînant une apoptose fratricide des cellules NK qui possèdent un rôle important dans la réponse antivirale. Le dérèglement de l'homéostasie des cellules NK pourrait donc contribuer à la pathogenèse induite par le VIH.

Mots-clés : HIV, Cellule NK, IL-18, IL-18BP, Fas, FasL, SIDA

ABSTRACT

HIV-1, the causative agent of AIDS, induces a deregulated production of several immunologically important cytokines in the infected persons. One of these cytokines is IL-18: a powerful proinflammatory cytokine that can regulate both innate and adaptive immune responses. In vivo, its activity is tightly regulated by IL-18 Binding Protein (IL-18BP), another cytokine that specifically binds and neutralizes IL-18 with high affinity. Previous studies have shown that IL-18 concentrations are significantly increased in the circulation of HIV-infected AIDS patients compared to those in healthy people. However, it is not yet clear how the increased levels of this cytokine affect the development of AIDS in HIV infected persons. Furthermore, little is known concerning the production of IL-18 antagonist (IL-18BP) in these patients. These issues were addressed in the studies presented in this thesis. We measured levels of IL-18 and IL-18BP in the sera of HIV-infected patients by using commercial ELISA kits and compared them with the values obtained from a similar number of healthy HIV-seronegative persons. We also determined the absolute and total number of different NK cell subsets and NK cell activity in the peripheral blood mononuclear cells (PBMC) of these individuals. Finally we determined the effects of recombinant human IL-18 as well as of IL-18-rich sera from AIDS patients on cytolytic activity and survival of human NK cells. Our results show that sera from HIV- infected patients contain up to 3 fold higher levels of IL-18 compared to the sera from healthy people. However, levels of IL-18BP were lower in the infected individuals compared to the healthy ones. Consequently, IL-18/IL-18BP ratio is increased in the patients resulting in a further increase in the concentrations of biologically active IL-18 in the circulation of these patients. Our results show that the concentrations of IL-18 correlated inversely with NK cell numbers as well as with their cytolytic activity in the infected persons. These results suggested the involvement of IL-18 in the disappearance of NK cells that prompted us to determine the potential cytocidal

effects of this cytokine on human NK cells. The results from our in vitro experiments show that recombinant human IL-18 and IL-18-rich sera from AIDS patients caused apoptosis in a human NK cell line as well as in primary human NK cells. Anti-FasL antagonist antibodies inhibited this cell death. In a series of experiments, we found that IL-18 enhances expression of FasL but does not affect the expression of Fas on human NK cells. In vitro IL-18 also stimulated transcription from human FasL promoter. Furthermore, the cytokine also enhanced susceptibility of NK cells to Fas-mediated death, as it decreased the expression of an anti-apoptotic protein Bcl-XL. Our study shows that enhanced IL-18 bioactivity in HIV-infected patients may contribute to the pathogenesis of AIDS by disrupting NK cell homoeostasis.

Key words: AIDS, HIV, NK Cell, IL-18, IL-18BP, FAS, FAS-L

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LISTE DES ABRÉVIATIONS

ADCC	Antibody-dependent cellular cytotoxicity
ADNc	Acide Desoxy nucléique complémentaire
ARN	Acide Ribonucleique
ARNm	Acide Ribonucleique messager
ART	Antiretroviral therapy
CA	Capside
CAF	$CD8^+$ T cell Anti-viral Factor
CDC	Centers for Disease Control
c-IAP	Cellular inhibitors of apoptosis protein
CMH	Complexe Majeure d'Histocompatibilité
CRFs	Circulating recombinant forms
CTL	$CD8^+$ T lymphocyte, lymphocyte T $CD8^+$
Env	Envelope
GM-CSF	Granulocyte-macrophage colony stimulating factor
GPCR	G protein coupled receptor
ICE	IL-1 converting enzyme
IL	Interleukine
IL-18BP	Inteleukine-18 Binding Protein
ILT	Immunoglobulin like transcripts
INF	Interféron
IN	Intégrase
KIR	Killer-cell Immunoglobuline-like Receptors

LTNP	Long Term Non Progressors
LTR	Long Terminal Repeat
MIP	Macrophage inflammatory protein
MTS	Maladie transmise sexuellement
NCR	Natural cytotoxixity receptors
Nef	Negatif effector
NK	Natural Killer
NU	Nucléocapside
PBMC	Peripheral blood mononuclear cells
PCP	Pneumocystis carinii pneumonia
Pol	Polymérase
PR	Protéase
RANTES	Regulated upon activation, normal T cell expressed and secreted
Rev	Regulator of viral gene expression
rhIL-18	Recombinante humaine de l'IL-18
RT	Reverse Transcriptase
SDF-1	α-chemokine stroma-derived factor-1
SHS	Secondary haemophagocytic syndrome
SIDA	Syndrome de l'immunodeficience acquise
SNC	Système Nerveux Central
SU	Protéine de surface
Tat	Transcriptional activator
Th1	Lymphocyte T helper de type 1

Th2	Lymphocyte T helper de type 2
TGF	Transforming growth factor
TM	Région Transmembranaire
TNF	Tumor necrosis factor
TRAF	TNF receptor-associated factors
VIH	Virus de l'immunodéficience humaine
Vpr	Viral protein R
Vpu	Viral protein U

1. CHAPITRE 1. INTRODUCTION ET REVUE DE LITTÉRATURE

Ce mémoire comporte les études faites pour comprendre et investiguer le rôle de l'IL-18 dans l'immunopathogénèse du SIDA.

1.1 INTRODUCTION

L'IL-18 autrement nommé facteur inducteur d'IFN-γ est une cytokine pléiotropique qui appartient à la famille de l'IL-1. Comme d'autres membres de cette famille, ce dernier est fortement pro-inflammatoire. Elle est produite comme une protéine précurseur qui est inactive biologiquement. Elle a besoin d'être clivée par la caspase-1 pour acquérir sa forme active. L'augmentation de la concentration de cette cytokine dans les maladies chroniques ou lors de l'infection par le VIH a été bien documentée. Dans le contexte de l'infection par le VIH, le rôle potentiel de cette cytokine n'est pas bien connu et élaboré. Il n'est pas très clair, jusqu'à date, si l'augmentation de la concentration de cette cytokine améliore ou affecte l'immunité innée antivirale. Cependant le rôle potentiel de cette cytokine sur la réplication virale a fourni des données contradictoires entre différents groupes de recherche. Il faut noter qu'in vivo, cette cytokine est toujours contrôlée par son antagoniste, « IL - 18 *Binding Protein* » (IL-18BP). En pratique, il n'y a rien qui est connu à propos de la production de cet antagoniste dans l'infection virale. Ce problème est expliqué dans ce mémoire. Concernant l'effet de l'IL-18 sur l'immunité innée, l'effet de cette cytokine sur la réponse et la fonction des cellules NK a été étudié.

1.2 REVUE DE LITTÉRATURE

1.2.1 VIH et SIDA

1.2.1.1 La découverte du SIDA

Le SIDA est l'acronyme du syndrome de l'immunodéficience acquise causé par une infection chronique du virus de l'immunodéficience humaine (VIH). C'est un phénomène qui a été reconnu au départ chez les homosexuels et les utilisateurs de drogues. L'été 1981, la CDC (*Center for Disease Control*) déclare l'infection des homosexuels par la bactérie PCP, la croissance des infections pulmonaires, l'apparition des tumeurs rares et la déplétion des lymphocytes T CD4 [1-3]. Ce virus a été découvert en 1983-1984 par deux groupes de chercheurs [1-3], et depuis ce temps le VIH est devenu une épidémie mondiale majeure. En 2007, le nombre d'adultes et d'enfants infectés à travers le monde est estimé à 32.3 millions, avec 2.5 millions d'individus qui ont été nouvellement infectés par le VIH et 2.1 millions de décès dus au SIDA [4]. Ce virus se répand dans plusieurs pays sous-développés et en voie de développement, comme la Chine, l'Inde, et les pays de l'Est de l'Europe (www.unaids.org). Les pauvres nations sub-sahariennes en Afrique sont les plus affectées. Dans plusieurs de ces pays le SIDA est une grande menace pour le développement des villes [5]. Le haut taux de mortalité observé est dû à un accès limité à la prévention et au traitement [5, 6].

Malgré tous les traitements et les améliorations contre le VIH, notamment dans la réduction de la mortalité et la morbidité, il reste beaucoup de variabilité dans la progression de la maladie [7].

1.2.1.2 Les sous-types ou Clades du VIH

Le VIH est l'agent qui cause le SIDA. La mutation rapide du VIH le rend de plus en plus diversifié, et ceci nous permet de détecter plusieurs variantes chez un patient infecté. En se basant sur la similarité génétique, le virus peut se diviser en types, groupes et sous types ou clades. En outre il existe deux types de VIH, le VIH-1 et le VIH-2 qui sont transmis par contact sexuel, par le sang et la transmission mère enfant [8, 9]. Le VIH-1 est l'agent le plus dominant et répandu dans le monde, aux États Unis, en Europe, en Afrique centrale et au Canada. Quant au VIH-2, il est moins virulent que le VIH-1. Il n'est pas transmis facilement et la période initiale entre l'infection et la maladie est plus longue. On le retrouve seulement en Afrique de l'Ouest et dans les Caraïbes [8, 9]. La variante VIH-1 est divisée en trois groupes majeurs : le groupe M pour" *Main* ", le groupe O pour "*Outlier*", et le groupe N pour "New, non M, et non O". Le groupe M est le responsable de la majorité des épidémies et il est divisé en neuf sous types ou "*clades" A, B, C, D, E, F, G, H, K,* et plusieurs *CRFs* "*circulating recombinant forms*" **(Figure 1**) [10, 11]. Cependant le groupe N qui est très rare a été découvert au Cameroun en 1998 et le groupe O apparaît seulement en Afrique de l'Est. Les CRFs sont des hybrides de deux ou plusieurs virus de différents sous types, comme exemple, CRFA/B est l'hybride des sous types A et B. Les CRFs sont nouvellement découverts et c'est le résultat d'une recombinaison génomique viral chez un individu infecté par de multiple sous-types [12]. La forme pure de sous-type E n'a jamais était découverte, c'est pour cela le CRFA/E prend son nom, mais c'est plus correct de l'appeler CRF01-AE. La désignation I n'est plus utilisée [10]. Les sous-types et les CRF sont répartis inégalement dans le monde, mais les sous-types A et C sont les plus répandus, et la clade B est le plus répandu en Amérique du nord, en Europe de l'Ouest, en Australie et au Japon. Cette dernière clade a été utilisée dans la découverte des médicaments antiviraux [8, 11]. Il est certain que d'autre sous-types et CRFs vont apparaître et seront répartis dans d'autres régions dans le futur. Finalement la prolifération de ces formes

virales entraîne un problème majeur dans le développement des vaccins et dans l'élaboration de nouveaux traitements.

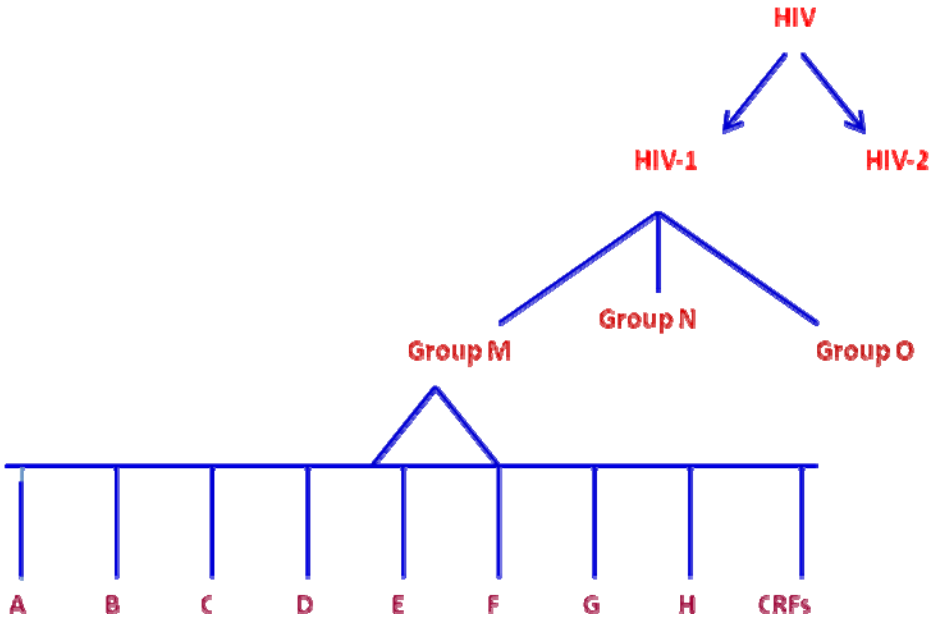

Figure 1 Sous-Types ou Clades du VIH

Ce diagramme illustre différents niveaux de la classification du VIH. Chaque type est divisé en groupe et chaque groupe est divisé en sous type ou *clades* et CRFs. *CRFs. Circulating recombinant forms.* Adapté de [13]

1.2.1.3 Organisation de la particule virale

Le VIH appartient à la classe des rétrovirus et aux sous-groupes des lentivirus "virus lents". La particule virale est formée de deux copies d'ARN gnomiques simple-brin et identiques, de la reverse transcriptase, et de deux enzymes: la protéase et l'intégrase [14]. Sur chaque région terminale de chaque brin existe une région appelée LTR "*Long Terminal Repeat*". Cette région agit comme un région promotrice pour contrôler la production de nouveaux virions et peut être activée par des protéines virales ou cellulaires [9, 15]. Le virus est formé d'une capside composée de plusieurs copies de la protéine virale p24, qui entoure les deux brins d'ARN, dont chacun code pour neuf gènes. Parmi ces gènes, *Gag*, *pol*, et *Env* qui ont toutes les informations nécessaires pour la fabrication des protéines structurales, et six autres gènes régulateurs *Tat, Nef*, *Rev*, *Vif*, *Vpr,* et *Vpu* qui expriment des protéines qui contrôlent la capacité de l'infection cellulaire, la réplication ou qui causent la maladie [9]. Le gène Gag code pour une protéine précurseur, qui est clivée par la protéase virale en quatre petites protéines : p24 (capside), p17 (matrice), p7 (nucléocapside) et p6. L'organisation de la particule virale du VIH avec la localisation de ses protéines et de ses deux brins d'ARN est démontrée à la **Figure 2**. Le gène *Pol,* code pour des précurseurs protéiques qui contiennent les quatre enzymes : la protéase, la reverse transcriptase, l'intégrase et le RNaseH. APOBEC3G est une protéine de défense antivirale qui agit comme un facteur de restriction post-entrée. *Vif* interfère avec cette dernière et cause l'inactivation de son effet antivirale pour augmenter la réplication virale. Cette interaction peut servir comme une nouvelle cible pour les médicaments antiviraux. Lors du bourgeonnement, l'enveloppe externe du virion est formée de deux couches lipidiques qui proviennent de la membrane cellulaire de l'hôte. Alors certains protéines sont d'origine cellulaire et d'autres des spicules (72 spikes) formés des glycoprotéines (gp), gp120 et gp41 [16], à la surface de cette enveloppe. *Env* encode le précurseur gp160, qui est clivé par la protéase virale en une unité cellulaire la gp120 et en une unité transmembranaire la gp41. Cependant gp120 est localisée à la surface des cellules

infectées et des virions à travers une interaction non covalente avec la protéine gp41 [17]. Le complexe gp120/41 existe sous forme de trimère à la surface du virion. Plusieurs chercheurs s'intéressent à ces protéines virales de l'enveloppe pour développer un vaccin contre le VIH [18]. Toutes les protéines encodées par le génome du VIH et leurs fonctions sont mentionnées brièvement dans le Tableau 1. Les protéines régulatrices comme la RT, la protéase et l'intégrase sont ciblées pour le développement des drogues contre le VIH.

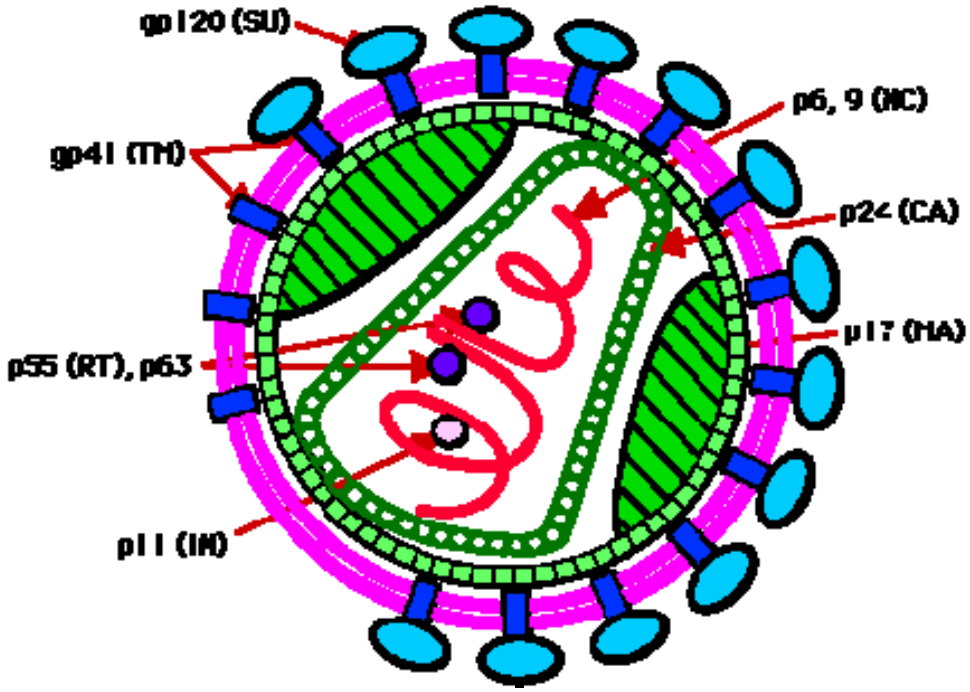

Figure 2 . Organisation de la particule virale.

CA. Capside, MA. Matrice, RT. Reverse Transcription, SU. Protéine de surface, TM. Protéine transmembranaire, NC. Nucléocapside, IN. Intégrase

Tableau 1. Les protéines du VIH et leurs fonctions

Gag	p24	Capside (CA), protéine structurale
	p17	Matrice (MA), protéine structurale
	p6	Rôle dans le bourgeonnement viral
	p7	Protéine de la Nucléocapside (NC) rôle dans la reverse transcription
Protéase (PR)	p10	Clivage protéolytique des protéines virales (Gag et Gag-Pol)
Polymérase (Pol)	p66, p51	Encode pour une variété d'enzymes virales, la reverse transcriptase et l'ARNase H, inclus P10 (PR), P32 (IN)
Integrase (IN)	p32	Intégration de l'ADNc viral dans le génome de l'hôte
Env, gp160	gp120	Protéine de surface (SU), initie le processus de l'infection en se liant à CD4
	gp41	Protéine transmembranaire (TM), rôle dans la fusion de l'enveloppe et la membrane cellulaire
Tat	p14	Élongation du transcrit virale, présente dans le virion
Rev	p19	Exporter l'ARN du virion épissé et épissé partiellement du noyau cellulaire
Nef	p27	"*Negatif effector*". Régule l'expression virale et l'activation des cellules de l'hôte," *down"* régulation du CD 4 et CMH de Classe I, bloque l'apoptose. Augmentation de la progression vers la maladie.
Vif	p23	Facteur qui augmente l'infection virale ; Rôle dans la synthèse de l'ADN proviral et l'assemblage du virion.vif interagit avec APOBEC3G et inhibe son activité antivirale.
Vpr	p15	Protéine virale R aide dans la réplication virale, transport du complexe pre-intégration au noyau, présent dans le virion, arrêt du cycle cellulaire en G2. Facilite l'infection des macrophages
Vpu	p16	Protéine virale U dissocie le complexe CD4/gp160; rôle dans le bourgeonnement, la dégradation du CD4, influence la relâche virale

1.2.1.4 Cycle de la réplication du VIH

Comme d'autres virus, le VIH peut se répliquer à l'intérieur de la cellule seulement. La première étape du cycle viral, est la liaison de la protéine gp120 au récepteur CD4 qui se trouve sur les lymphocytes T, les monocytes et les cellules dendritiques. Cette dernière subit des changements conformationels qui lui permettent de s'attacher à d'autres molécules connues comme les co-récepteurs CCR5 et CXCR4 qui sont très importants pour le processus de la fusion [17, 19]. Une fois que le virus fusionne avec les cellules, il relâche l'ARN viral et ses enzymes dans le cytoplasme. À ce moment la transcriptase inverse transcrit l'ARN en ADN viral en pleine longueur [20, 21]. Une fois l'ADN transporté dans le noyau, il sera intégré dans l'ADN génomique par l'intermédiaire de l'intégrase. Cette forme virale s'appelle provirus [20, 21] si les lymphocytes T $CD4^+$ ne sont pas actifs, le virus reste en état de latence ou quiescent pendant plusieurs années [22]. La capacité du virus de rester latent complique tous les essais pour l'éradiquer. Alors les patients qui sont VIH –positifs doivent rester indéfiniment sous traitement [22-24]. Une activation anormale de l'immunité pourrait être la cause majeure de la progression de la maladie. En outre, on remarque une corrélation positive entre le degré de cellules CD8 activés et le risque de la progression vers le SIDA [23]. Une fois que les cellules $CD4^+$ sont activées par les antigènes, les mitogènes et les cytokines, la réplication est activée. Alors, les cellules activées produisent des protéines virales par la transcription de l'ADN en ARN messager (ARNm), ceci est accompli en utilisant la machinerie cellulaire [20, 21]. L'ARNm quitte le noyau et sera transporté dans le cytoplasme. Une fois dans le cytoplasme, l'ARNm est traduite en des poly-protéines qui sont des précurseurs de la capside et de l'enveloppe. Finalement, la protéase clive les poly-protéines en protéines utilisables pour l'assemblage de la nouvelle particule virale ou virion [21]. Cette protéase est une cible pour une classe de médicaments utilisés pour le traitement contre le VIH [18]. Alors, les protéines de l'enveloppe fusionnent avec celle de la membrane cellulaire de l'hôte, avec la capside, l'ARN et les enzymes. Finalement le virion bourgeonne et

quitte la cellule [20, 21]. Une cellule peut faire des milliers des particules virales, ce qui résulte en la mort cellulaire. Toutes les étapes impliquées dans le cycle de réplication du VIH, dès l'infection du VIH et sa rentrée dans la cellule, sa réplication et le relâchement de nouveaux virions hors de la cellule sont démontrés schématiquement à la **Figure 3.**

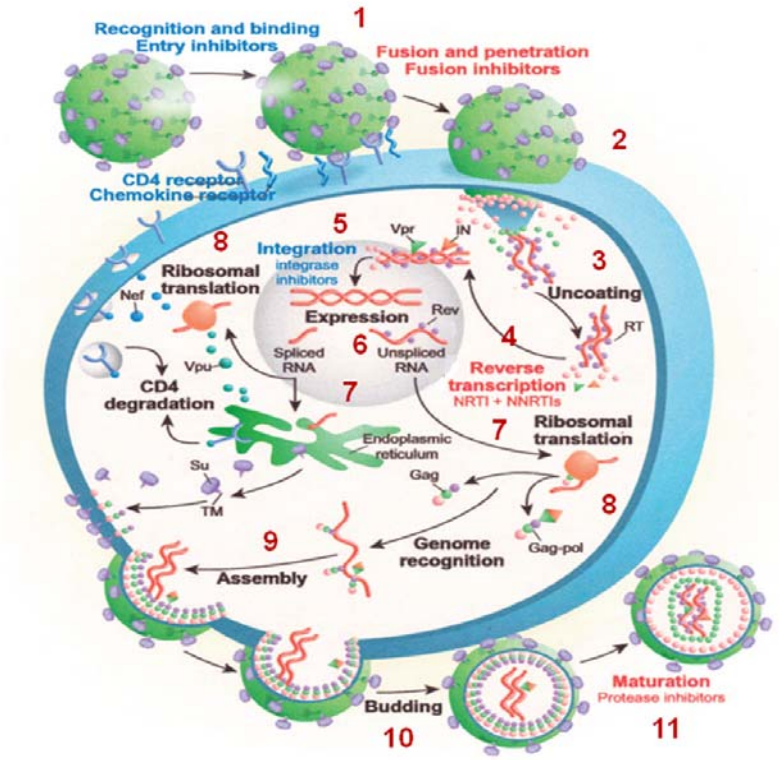

Figure 3. Le cycle de réplication du VIH.

1. L'attachement, 2. La fusion, 3.Le relâchement et la transcription par la reverse transcriptase, 4.Le transport de l'ADN dans le noyau, 5. L'intégration, 6. La transcription, 7. Le transport de l'ARNm vers le cytoplasme, 8.La traduction, 9. L'assemblage, 10.Le bourgeonnement, 11.La maturation.
RT. Reverse Transcriptase, IN. Intégrase, SU. Unité de surface, TM. Protéine Transmembranaire. [25]
Roger J. Pomerantz, David .L Horn, 2006

1.2.1.5 Infection et transmission du VIH-1

L'infection par le VIH dépend des cellules qui expriment le récepteur CD4 à leur surface et des co-récepteurs le CXCR4 ou le CCR5 (récepteur des chimiokines), comme les lymphocytes T $CD4^+$ activés, les monocytes, les macrophages, les thymocytes, et les cellules dendritiques [19]. Le CD4 est une glycoprotéine de surface, qui est membre de la superfamille des immunoglobulines. Ce récepteur assiste le TCR pour activer les cellules T après l'interaction avec les cellules présentatrices d'antigène (htt://users.rcn.com). Le CD4 amplifie le signal en recrutant une tyrosine kinase lck. De même cette molécule interagit avec le domaine extracellulaire des molécules du CMH de classe II qui se trouve sur la surface des cellules présentatrices d'antigènes (htt://users.rcn.com). Il est formé de 4 domaines d'immunoglobuline, et c'est le récepteur primaire utilisé par le VIH (www.mcld.co.uk./hiv/). Finalement la liaison du virus à ce récepteur crée un changement dans la conformation de gp120 qui permet au virus de se lier à d'autres récepteurs de chimiokines selon le type cellulaire infectés (www.mcld.co.uk./hiv/). Le corécepteur CCR5 est un récepteur couplé aux protéines G (GPCR: "*G protein coupled receptor*" pour les chimiokines-β (RANTES, MIP-1 α, MIP β), et se trouve principalement à la surface des macrophages, des cellules dendritiques et les cellules T activés[26]. Cependant CXCR4 est aussi un GPCR, qui agit aussi comme un récepteur pour le SDF-1 (*α-chemokine stroma-derived factor-1*). Il est exprimé sur presque toutes les cellules hématopoïétiques et principalement à la surface des cellules T $CD4^+$ [26]. Le VIH requiert que les cellules T soient activées lors de l'infection ce qui conduit à la mort cellulaire. Ainsi les virus utilisant CXCR4 émergent et infectent les cellules T $CD4^+$ qui sont beaucoup plus nombreuses par rapport aux cellules effectrices exprimant CCR5 [27]. Il existe des souches virales qui infectent plus efficacement les lymphocytes T, et sont appelées T-tropique, et normalement induisent des syncytiums (IS) [28]. Tandis que celles qui infectent plus efficacement les macrophages sont macrophages tropiques (M-tropique) et n'induisent pas des syncytiums (NSI) [28]. Durant la phase précoce de la maladie,

le virus utilise le corécepteur CCR5 [28], tandis qu'il utilise le corécepteur CXCR4 [19] dans la phase tardive de l'infection. Généralement, dans la phase terminale de la maladie, les virus sont typiquement double tropique (R5X4) [29-31] et peuvent utiliser ces deux types de co-récepteurs. Ceci est causé par la perte massive des cellules T, et la progression rapide vers la maladie de l'immunodéficience (SIDA) [32, 33]. Lors de la phase symptomatique de la maladie (SIDA), le tropisme du VIH change du CCR5 (R5) au CXCR4 (R4). Dans une culture de cellules T à long terme, le virus de VIH isolé tend à s'adapter à la culture et évolue comme une souche T-tropique. Les infections par le VIH-1 arrivent normalement avec les souches M-tropiques présentes dans l'inoculum naturel, et évoluent dans les dernières étapes de l'infection en des souches plus pathogènes T-tropique [27, 34]. Certaines souches utilisent le CD8 à la place de CD4 comme récepteur primaire durant l'infection [34]. Ces souches infectent les cellules T $CD8^+$ de manière très efficace et productive. Il y a une corrélation positive entre le degré de l'activation des cellules CD8 et le risque de progression en SIDA [23] . Si les cellules T CD4 ne sont pas activées, il est possible pour le virus de rester dans un état de latence pour plusieurs années [22]. L'infection des macrophages et des cellules dendritiques permet la réplication et la propagation du virus mais ne conduit pas à la mort des cellules réservoirs. Par exemple, les macrophages et les monocytes infectés passent dans plusieurs organes et les infectent , par exemple le cerveau, où le virus se cache [24]. Ce pool représente la cause des dommages dans le système nerveux et les tissus lymphoïdes sont des sites majeurs de la réplication virale.

Les facteurs qui influencent la charge virale et la progression de la maladie sont : le type de la réponse immunitaire, le patrimoine génétique de l'hôte, la co-infection avec d'autres maladies transmises sexuellement (MTS), l'âge et le changement de l'environnement [35, 36]. Les "non progresseurs à longs termes"(*long-term non-progressors*) sont des patients qui ont le virus depuis plus de dix ans et n'ont aucun symptôme. Ils ne prennent aucune médication, et démontrent certaines caractéristiques qui expriment une réponse antivirale

bénéfique. De même, la progression de la maladie dépend aussi d'un nombre de facteurs psychologiques et physiologiques incluant l'utilisation de la drogue, un comportement sexuel à haut risque, le stress, et la dépression [37-39] .

On trouve le virus dans le sang, et dans les liquides ou les fluides corporels comme le sperme, et le fluide vaginal. L'infection par le VIH survient principalement à cause des relations sexuelles (orale ou anale) sans aucune protection avec des partenaires infectés [9, 40, 41]. Le virus présent dans le sérum, le sang et dans d'autres liquides biologiques peut entrer à travers des membranes muqueuses traumatisées du vagin et l'anus [42]. Il y a d'autres voies d'infection, comme par les aiguilles contaminées, la transfusion sanguine, et la transmission mère enfant (le fœtus, le nouveau-né durant la grossesse, l'accouchement ou l'allaitement) [41-43].

1.2.1.6 Phase clinique et les symptômes de l'infection au VIH

L'infection primaire par le VIH présente normalement des symptômes de fièvre douce, douleur à la gorge, gonflement des ganglions lymphatique, des éruptions etc. et peut rester inaperçue [9, 15]. Ceci est suivi par une latence longue et variable. Quelques personnes infectées (environ 5%) peuvent rester asymptomatiques pour quinze ans ou plus sans aucun traitement. Ils sont référés comme des long termes non progresseurs (LTNP) et démontrent certaines caractéristiques pour une réponse immunitaire bénéfique anti-VIH [9, 36]. Durant cette période de latence clinique, le virus se réplique activement dans leurs organes lymphoïdes et les détruisent lentement, il y a une détérioration du système immunitaire accompagné d'une déplétion progressive des cellules T $CD4^+$ et une augmentation rapide de la charge virale dans le plasma prédit une progression rapide au SIDA [44]. Finalement les personnes infectées deviennent immunodéficients et développent la cachexie, des infections opportunistes des

tumeurs rares et/ou des dérangements du système nerveux central (SNC)[15]. Il est très important de noter que l'infection par le VIH ne signifie pas SIDA. Mais les personnes infectées peuvent finalement développer le SIDA si elles restent non traitées. C'est pour cela que l'infection par le VIH est divisée en trois phases [45] :

- Phase primaire aiguë : durant cette phase la réplication virale est massive dans le sang périphérique (une grande charge virale) [46]. Il y a une déplétion des lymphocytes T $CD4^+$. Le système immunitaire est actif en produisant des anticorps et des lymphocytes cytotoxiques ($CD4^+$ et $CD8^+$). Le processus s'appelle séroconversion [40, 47, 48].

- Phase chronique divisée en deux parties:

a) Phase clinique asymptomatique: Elle a une durée variable et comme son nom l'indique, il n'y a aucun symptôme. Il y a une diminution de la charge virale [46]. A cette étape, l'infection est caractérisée par un faible réplication et un faible charge virale ainsi que le parasitage des nodules lymphatiques. Le taux des lymphocytes T se rétablit en périphérie et reste faible, mais l'activation du système immunitaire augmente [18].

b) Phase clinique symptomatique : Durant des années, les nodules lymphoïdes et tissus deviennent endommagés, le virus devient plus varié et pathogène à cause des mutations produites. Le corps n'est plus capable de remplacer les lymphocytes T $CD4^+$ ($TCD4^+ < 500$ cellules $/mm^3$) et ceci mène à un système immunitaire très endommagé [18, 46]. Finalement il y a apparition des symptômes, la diarrhée, gonflement des ganglions lymphatiques, "*cold sores*", *Candyloma,,* symptôme dans le système gastro-intestinal, perte de poids rapide, perte d'appétit, une fatigue profonde, des symptômes respiratoires, une courte respiration, des toux sec, engourdissement, une neuropathie périphérique, des picotements, des infections fongiques et une dépression [18].

- Phase SIDA : C'est la phase de progression de l'infection du VIH vers l'immunodéficience. Le système immunitaire devient de plus en plus endommager et la maladie devient de plus en plus sévère. Le taux des lymphocytes T est plus petit que 200 cellules par mm^3 [18, 46]. L'activation du système immunitaire et la réplication virale augmente de façon marquée. Il y a souvent développement d'un cancer ou d'une ou plusieurs infections opportunistes spécifiques qui causent la mort de l'individu [15].

1.2.1.7 La Réponse immunitaire

L'infection par le VIH induit une forte réponse humorale et cellulaire chez les personnes infectées [49-51]. La réponse humorale comprend l'induction des anticorps qui peuvent neutraliser le virus, détruire les virions par l'activation de complément et tuer les cellules infectées par le phénomène de la neutralisation virale, et l'"*ADCC-mediating antibodies* "chez l'hôte. L' " ADCC" se réfère au « antibody-dependent cell-mediated cytotoxicity » [49]. Ces anticorps sont normalement spécifiques à la souche virale et ne reconnaissent pas des souches virales hétérologues. En outre, les souches primaires de VIH-1 étaient très résistantes à la neutralisation par des anticorps [49]. Ceci a été attribué aux grandes mutations, et à une grande glycosylation N qui empêchent la fixation des anticorps neutralisant. [49, 52]. Le virus induit aussi chez l'hôte une très forte réponse du CTL. Les épitopes pour le CTL ont été décrits dans presque toutes les protéines virales.[53]. On croit que les lymphocytes T $CD8^+$ contrôlent la super infection des lymphocytes T $CD4^+$ par le VIH (LTNP) *in vitro,* par un mécanisme indépendant de la productions des facteurs solubles, mais dépendant du contact de cellule à cellule et de la compatibilité HLA [54]. Le déclin de la virémie dans une infection primaire coïncide avec l'apparition du CTL antiviral dans la circulation. En outre, *in vivo,* la déplétion du CTL par un anticorps anti-CD8 cause une croissance immédiate de la charge virale [54]. Les évidences cumulatives

suggèrent qu'une réponse antivirale polyclonale à large spectre ralentisse la progression vers le SIDA [51]. MIP-1α, MIP-1β, et RANTES produits par les T $CD8^+$ en complexe avec les protéoglycanes sont des ligands de CCR5 qui limitent la réplication du VIH dans les cellules $TCD4^+$ au niveau de l'entrée [55]. Le facteur antiviral produit par les cellules T $CD8^+$ (*CAF* : *$CD8^+$ T cell Anti-viral Factor*), est non- cytotoxique et limite la réplication du VIH dans les cellules $TCD4^+$ [56] .

Cependant le CTL a échoué à contrôler la réplication virale [57-59]. Le taux élevé des mutants est une des raisons de cet échec, qui mène à l'apparition de leur évasion. Une autre raison est attribuée au manque d'aide de la part des cellules T $CD4^+$, ceci étant dû à leur déplétion progressive chez l'hôte infecté [58]. Elles ne sont pas totalement différenciées et il y a un manque dans leurs fonctions effectrices [58]. Certains chercheurs ont rapporté une expression élevée, et aberrante des récepteurs inhibiteurs spécifiques au CMH Classe I qui se trouve à la surface des CTL des individus infectés par le VIH [60]. Des études *in vitro* et *in vivo* ont montré que ces récepteurs apparaissent pour inhiber la fonction du CTL.

1.2.1.8 La production dérégulée des cytokines chez les personnes infectées par le VIH

Les cytokines et les chimiokines jouent un rôle important dans l'activation du système immunitaire, dans les fonctions des cellules immunitaires et dans la réponse inflammatoire contre les agents pathogènes. Ces cytokines sont impliquées dans la dérégulation, l'immunodéficience et dans le contrôle de la réplication virale. Dans la phase précoce, on remarque une augmentation rapide et transitoire de l'INF-α, du TNF-α et de l'IL-15 dont les cellules dendritiques plasmacytoïdes (CDp) sont la source principale [61]. Mais plus tard, les cellules dendritiques myéloïdes (CDm) seront responsables d'une sécrétion plus lente et prolongée de l'IL-18, du TNF-α, et de l'IL-10 [62]. En plus, la combinaison de l'IL-15 et l'IL-21 résulte en une expansion synergétique et une augmentation des

fonctions des cellules T $CD8^+$ chez les patients séropositifs [63]. L'IL12 avec l'IL-18 induit la production de l'INF-γ et active les fonctions des lymphocytes T $CD4^+$ [64]. De même, dans la phase précoce, l'IL-15 et l'IL-18 peuvent médier des effets immunostimulatoires sur le système immunitaire inné et adaptatif [65]. Durant la phase tardive, la capacité de produire l'IL-2, l'IL-12, l'IL-15 et l'IL-21 sera compromise, chez les personnes infectées par le VIH [66-70] . Toutefois, la production de ces cytokines est aussi dérégulée par le virus et ces protéines. En plus, il y a plusieurs autres cytokines qui contrôlent l'infection du VIH [71]. Pour plus d'information voir **tableau 2**.

Enfin le rôle des cytokines dans l'infection par le VIH devient une clé et une cible dans le développement des stratégies effectives pour la prévention contre le VIH et la reconstitution immunologique.

Tableau 2. Des cytokines qui se dérèglent lors d'infection par le VIH

IL-7	Augmente avec une haute virémie, et une diminution du nombre des cellules $TCD4^+$. Augmente l'activité des cellules NK, et réduit l'apoptose des cellules $TCD4^+$ et $TCD8^+$. Augmentation de la réplication virale du VIH dans les thymocytes et les cellules T
IL-4	Augmentation de sa concentration. Promouvoir la réponse T de type 2. Échec de contrôler, et éliminer la charge virale
IL-10, TGF-β	Un niveau élevé, augmentation de la réplication virale
IL-15	Un niveau élevé au début de l'infection, qui diminue dans la phase tardive de la maladie. Réduit l'apoptose chez les cellules $TCD8^+$. Augmente les fonctions des neutrophiles
IL-17	Une augmentation de la sécrétion par les cellules $TCD4^+$
IL-19, IL-20	Up régulé par Tat dans les cellules épithéliales
IL-21	Il est réduit et il y a une corrélation avec le nombre des cellules TCD4. Augmentation de la prolifération t le fonctionnement des cellules TCD8
IL-23, IL-27	Augmente les fonctions des cellules dendritiques. Inhibition de la réplication du virus du VIH

1.2.1.9 Traitement et vaccination

1.2.1.9.1 Thérapie antirétrovirale

Plusieurs antiviraux ont été développés depuis plusieurs années et ont été approuvés pour le traitement du SIDA. Il y a six classes des médicaments utilisés dans la thérapie antirétrovirale, et qui arrête la réplication virale en l'affectant à différente phase de son cycle cellulaire [18]. Tous les renseignements concernant les classes des médicaments et leurs fonctions sont discutées dans le **Tableau 3.** Le traitement qui se donne par la combinaison de trois médicaments, qui appartiennent à deux classes d'agents antirétrovirales et ceci inclut au moins un inhibiteur de PR [9, 72, 73]. Ceci consiste la thérapie antirétrovirale, " *highly active antiretroviral therapy*" (HAART). Ce traitement est très efficace dans l'inhibition de la réplication virale. Il augmente l'immunocompétence et, par conséquent, il ralentit la progression de cette infection vers le SIDA. Il a des effets bénéfiques directs sur les paramètres du système immunitaire inné et adaptatif [74]. C'est cette thérapie, qui a permis de nos jours, à cette maladie de devenir une infection chronique maniable. Cependant, ces médicaments coûtent très chers, ils ont aussi de très sérieux effets secondaires. C'est très difficile de se conformer à la stringence de ce régime. En outre, l'émergence des souches résistantes à ces médicaments reste un réel danger. Donc, la recherche pour des nouveaux médicaments plus efficaces ne s'arrête jamais.

1.2.1.9.2 Développement d'un vaccin

Il n'y a aucun vaccin prophylactique efficace valable pour cette infection. Cependant, le développement du vaccin progresse lentement à cause de la variation des souches, aussi bien de la capacité du virus d'échapper à la réponse immunitaire [9, 50]. Plusieurs concepts de vaccins se poursuivent, mais les essais n'ont montré aucune protection contre le virus. Jusqu'à présent, les études menées ont montrées que le vaccin augmentait le taux de l'infection par le VIH chez ces individus [75]. Finalement, le chemin reste assez long pour avoir un vaccin efficace.

Tableau 3 Médicaments antiviraux contre le SIDA

Adapté de Harris, A. and N.E. Bolus, HIV/AIDS: an update. Radiol Technol, 2008. **79**(3): p. 243-52.

Classe des médicaments antirétroviraux	Abréviations	Première utilisation comme traitement	Fonctions
Inhibiteurs de la Reverse Transcriptase nucléosides / nucléotides	NRTIs,	1987	Il interfère avec la Reverse Transcriptase
Inhibiteurs de la Rverse Transriptase Non-nucléosides	NNRTIs,	1997	Ils inhibent la Reverse Transcriptase
Inhibiteurs de Protéase	PIs	1995	Il inhibe la protéase, impliqué dans la réplication virale
Inhibiteurs de fusion ou d'entrée		2003	Ils inhibent la fusion ou l'entrée, Il prévient le VIH de se lier ou d'entrer dans les cellules immunitaires.
CCR5 Antagoniste		2009	Il inhibe l'entrée virale, en se liant au CCR5
Inhibiteurs d'Intégrase		2007	Il inhibe l'Intégrasse, dont le VIH a besoin pour intégrer son matériel génétique dans le génome humain

1.2.2 IL-18 et IL-18BP

Comme mentionné déjà, l'IL-18 est une cytokine pléiotropique de 18 kDa. Elle joue un rôle important dans l'induction des réponses immunitaires innées et adaptatives. IL-18 était connu précédemment comme un facteur induisant l'INF-γ [76] Cette cytokine a été découverte en 1989 [76, 77], et elle a été clonée et nommée IL-18 en 1995 [77, 78]. Elle est produite par une variété de type cellulaire et tissulaire, comme les macrophages activés, les cellules dendritiques, les cellules épithéliales, les cellules intestinales, et les kératinocytes [79]. Elle est exprimé aussi dans plusieurs tissus et organes comme le Foi, la rate, le placenta, le cœur, les reins, les os, le pancréas, et autre [80, 81]. La production de cette cytokine augmente constitutivement après une variété de stimuli et certaines infections virales et bactériennes [82].

IL-18 appartient à la famille de l'IL-1 [83]. Comme l'IL-1β, et l'IL-33, l'IL-18 est exprimé comme une protéine précurseur inactive de 24kD dans le cytoplasme des cellules. Elle contient 192 aa et n'est pas secrété par les voies conventionnelles [84]. Cette protéine précurseur est clivée en une cytokine mature et active de 18 kD par l'enzyme "*IL-1 converting enzyme*"(ICE) [85] .

Cette enzyme est une protéase cystéine avec l'acide asparatique dans le site active, aussi bien connu comme caspase-1 [85, 86]. Il est à noter que la caspase I se produit aussi comme un précurseur inactif de 45kD dans les cellules et elle a besoin de son propre clivage et activation avant de procéder au clivage du précurseur d'IL-18. Une fois que la caspase I est clivée, elle est mature, active biologiquement et elle est facilement secrétée à la surface cellulaire [83, 85, 87]. Il a été montré que le LPS peut activer aussi la caspase I dans les lignés cellulaires des macrophages chez la souris et l'humain [83, 87]. Dans certaines

cellules déficientes de la caspase I, d'autres protéases peuvent cliver le précurseur d'IL-18. Chez les kératinocytes, la protéinase 3 est impliquée dans le processus du précurseur [88, 89]. Comme la caspase I, la caspase 4 peut aussi cliver la forme du précurseur en une forme mature et secrété, mais ce processus est moins efficace [86]. La caspase 3 peut aussi cliver le précurseur et la forme mature de l'IL-18 en une protéine inactive biologiquement [88] . À noter que l'IL-18 et l'IL-18β ont une structure " *β-pleated folded*". L'IL-18 se lie a un seul et unique récepteur heterodimérique qui appartient à la superfamille de l'IL-1 et comprend IL-18Rα et β [84, 90, 91]. La chaîne α a été précédemment appelée comme "*IL-1R relate protein*"(IL-1Rrp) en considérant que la chaîne β a été connue comme "*IL-1R accessory protein like protein"(*IL- 1R-AcPL) [92, 93]. La chaîne α se lie avec une faible affinité à IL-18, cependant la chaîne β non, mais toute les deux forment une forte affinité à ce récepteur et transmettent les signaux intracellulaires à travers des voies partagés avec le récepteur de l'IL-1 [92, 93]. La portion cytoplasmique de la chaîne d'IL-18Rβ est homologue à ce qu'on appelle "*toll like receptor*" (TLR) qui reconnaît les structures moléculaires associées aux pathogènes et active NF- κB, MAPK et traduit des signaux via MyD88-IRAK-TRAF-6 [81, 90, 94]. Des multiples effets biologiques de l'IL-18 sont exercés sur les différentes cellules et tissues du corps. L'IL-18 potentialise la réponse immunitaire innée et adaptative contre les pathogènes bactériennes et virales [91, 95-98]. Il augmente l'activité cytolytique des deux cellules les NK et les CTL. Il est recommandé pour les fonctions effectrices normales des cellules NK mais pas pour leur développement et leur différentiation. Les souris IL-18KO, ont un nombre normal des cellules NK mais ces cellules sont sévèrement compromises dans leur capacité de tuer les cellules cibles sensible aux cellules NK [99]. IL-18 aussi augmente l'expression de la perforine et FasL dans ces cellules cytolytiques [97]. L'IL-18 joue un rôle important dans la régulation des réponses immune adaptative aux pathogènes. À cause de son habilité d'induire INF-γ à partir des cellules Th1, des cellules T non polarisés, des cellules NK, des cellules B et des cellules dendritiques, ceci est considéré pour induire la différentiation des cellules

T $CD4^+$ naïves en Th1 [97]. Toutefois, il a été bien clair que l'IL-18 et de l'IL-12 sont nécessaires pour cet effet [97]. IL-12 induit l'expression des récepteurs de l'IL-18 sur les cellules T $CD4^+$ naïves et les rend susceptible aux effets de l'IL-18. En retour, l'IL-18 augmente l'expression des récepteurs de l'IL-12, permettant un mécanisme moléculaire pour les effets synergiques de ces deux cytokines [100]. En plus de l'induction de l'INF-γ, l'IL-18 aussi augmente la cytotoxicité médié par FAS-L des cellules T $CD4^+$ TH1. En absence de l'IL-12, elle peut induire la production de l'IL-4 et l'IL-13 à partir des cellules NK et T et, ainsi actuellement l'IL-18 promouvait la différentiation des cellules T $CD4^+$ de type TH2 et l'induction des IgE et IgG1 [97, 101, 102]. Il est évident que les cellules T naïves expriment à leur surface IL-18Rα à bas niveau. L'INF-γ induit par les cellules B murines par l'IL-18 plus l'IL-12 prévient l'induction d'IgE et IgG1 par l'IL-4 à partir des cellules B et plutôt induit la production de l'IL-4, l'IL-13 et le relâchement des histamines à partir des basophiles et des mastocytes [103]. Ce qui est intéressant, c'est que l'histamine induit la production de l'IL-18 à partir des PBMC sans une induction concomitante avec l'induction de l'IL-12. Ainsi l'IL-18 peut stimuler les réponses médié par les deux cellules TH1 et TH2 dépendant de la disponibilité de IL-12. Les cellules NK expriment constitutivement le récepteur IL-18R. L'IL-18 et l'IL-12 augmentent indépendamment l'activité des cellules NK. Les activités cytotoxiques des lymphocytes T et les cellules NK sont augmentées par l'IL-18 à travers l'augmentation de l'expression de la perforine et FasL sur ces cellules [97] .

L'activité biologique de l'IL-18 est neutralisée par l'"*IL-18 Binding Protein*" (IL-18 BP) qui a été découvert Par Dinarello en 1997 [104, 105]. C'est une protéine de 40kDa, qui est secrétée sous forme soluble, car il lui manque un domaine transmembranaire. Elle est distincte des récepteurs de l'IL-18 et de la famille de l'IL-1 [106]. Elle n'a pas d'homologie avec aucune protéine transmembranaire et avec le domaine extracellulaire "extra cellular ligand binding

domain" du récepteur de l'IL-18 [106]. Il est un antagoniste potentiel de l'IL-18. Le gène de l'IL-18BP code pour quatre isoformes distinct [106]. L'expression de l'IL-18BP est induite par l'INF-γ. L'IL-18 et L'IL-18BP sont deux compétiteurs dans le réseau des cytokines et leurs concentrations déterminent les effets biologiques de l'IL-18 dans un contexte physiologique. Cette protéine bloque la liaison de l'IL-18 à son récepteur [107]. Ce qui est intéressant, c'est que plusieurs virus encode un homologue à l'IL-18BP pour bloquer l'effet antiviral de l'IL-18 [107]. Étant déjà une cytokine qui stimule l'immunité, alors ce n'est pas surprenant que l'IL-18 joue un rôle important dans la défense de l'hôte contre les pathogènes intracellulaires incluant les virus [84, 95, 98]. L'activation des lymphocytes T $CD8^+$ par cette cytokine seule, peut jouer un rôle dans la clairance virale. Le rôle protecteur de l'IL-18 dans les infections avec VHC et "*vaccinia virus*" a été démontré in vivo chez la souris [98, 108]. Cette protection a été entièrement attribuée pour les réponses de l'immunité innée. Habituellement, l'hôte répond à des infections en induisant la production d'IL-18. *In vitro* le virus de l'influenza A et "*Sendai virus*" induisent IL-18 à partir de la culture des monocytes-macrophages humaine [109, 110]. Afin d'éviter les effets antiviraux de l'IL-18, les virus se sont trouvés différentes stratégies, comme le poxivirus qui encode un homologue à IL-18BP qui peut neutraliser l'IL-18[107]. Étant une cytokine jouant un rôle anti angiogenique pour stimuler plus l'immunité, l'IL-18 réussit à supprimer la croissance tumorale. Différant de l'IL-12, la mort médié par l'IL-18 se fait par la voie Fas/FasL

Finalement l'IL-18 induit la production de TNF-α, GM-CSF (*granulocyte-macrophage colony stimulating*), et certaines chimiokines. Il peut avoir des effets multifonctionnels sur la réponse immunitaire toute dépendante de l'environnement [90, 111-113] . En somme l'IL-18 contribue à la défense cellulaire quand elle se produit en quantité optimale. Sa surproduction a été impliquée dans plusieurs maladies, le diabète dépendante de l'insuline, le multiple sclérose, l'arthrite rhumatoïde, la greffe versus la maladie de l'hôte, et la maladie de Crohn [97, 114-116]. À cause de sa capacité d'induire les réponses "*TH2-like*",

et promouvoir la synthèse d'IgE, l'IL-18 a été aussi impliqué dans les désordres allergiques, et dans l'asthme [117].

Toute information détaillée se trouve dans l'article 1 inclus dans ce document.

1.2.2.1 IL-18 et l'infection par le VIH

En dépit de l'habileté de l'IL-18 de renforcer les réponses immunitaires innées et adaptatives contre les infections virales, le rôle potentiel et la régulation de cette cytokine n'est pas bien étudié dans le contexte de l'infection par le VIH. Un cousin proche du VIH-1, le virus d'immunodéficience du simian (VIS) induit l'IL-18 in vivo et augmente l'activité des cellules NK dès la phase précoce de l'infection [118]. Tandis Kaizu el al démontre un taux élevé de l'IL-18 accompagné d'un rapide déclin des lymphocytes T, suggérant que durant les premières stades de la maladie, l'IL-18 ne cause pas une protection effective de l'infection par le SVIHs (simian/human chimeric immunodeficeincy virus), qui est un virus chimérique, crée pour la recherche par la combinaison d'une partie des génomes du SIV et du VIH. Il mimique mieux le HIV que le SIV [119-121]. Donc, ceci peut être un bon indicateur pour une infection virale pathogénique active, mais son rôle demeure inconnu et reste à être élucider [121]. En considérant son habileté, IL-18 a été utilisé comme un adjuvant chez les souris vaccinées avec de l'ADN exprimant *Nef, Gag/Tat/Nef* ou *Env* du VIH-1 [122]. Plusieurs expériences ont été effectuées *in vitro* sur différents types cellulaires, d'une part elle joue un rôle inhibiteur [123] ou un rôle stimulateur [124, 125]. Il a été démontré récemment que le niveau d'IL-18 dans le sérum est comparable chez les LTNP (long-term non-progressors), et les patients malades. Cependant, des études ultérieures ont indiqué que le taux d'IL-18 est plus élevé chez les patients symptomatiques par rapport aux contrôles séronégatifs et les individus asymptomatiques VIH positifs, dont ceci est relié à une progression de la maladie

[70, 126, 127]. Sailer et collaborateurs ont montré une augmentation du taux de l'IL-18 durant les premières phases de l'infection par le VIH, et peut mener probablement à l'inhibition du VIH-1 en augmentant la réponse immunitaire du Th1 et la réduction de l'expression du co-récepteur CXCR4 [128]. Toutefois, les patients ayant une forte concentration de cette cytokine démontre une réduction dans la capacité des PBMC stimulés ex vivo de sécréter cette cytokine. L'écart apparent entre ces deux rapports, l'augmentation du taux du plasma, en plus d'un épuisement fonctionnel des PBMC est expliqué par le fait que l'IL-18 peut être secrété par les plaquettes [69, 129] et les tissus adipeux [130]. En plus de stimuler la réponse immunitaire innée et adaptative, il a été rapporté que l'IL-18 augmente la réplication virale du VIH-1 dans les monocytes et les lymphocytes T en activant le P38 MAPK, le NF-κB et en stimulant la production de TNF-α et l'IL-6 provenant de ces types cellulaires. Ceci provoque la diminution de la réponse cytolytique et la réponse immunitaire de Th1 [124, 125, 131]. Cependant, quand on l'ajoute in vitro à une culture cellulaire des PBMC humaines infectés par le VIH, l'IL-18 probablement inhibe la réplication virale en augmentant l'activité cytolytique des cellules NK (down modulation des lymphocytes T CD4) et en induisant un état antiviral dans les cellules infectées à travers la production des cytokine pro inflammatoire comme l'INF-γ [123]. Cependant, ceci suggère que la variabilité de la réplication soit affectée par L'IL-18, qui dépend de plusieurs microenvironnements. Il est probable qu'on remarque l'implication de l'IL-18 dans la phase tardive de l'infection par le VIH. Ceci est causé par une augmentation du taux de l'IL -18 circulant et qui est associé à une diminution du décompte des lymphocytes T $CD4^+$ [127]. Finalement l'IL-18 joue un rôle à deux tranchants, d'un côté elle peut inhiber la réplication virale et jouer un rôle antiviral, et d'un autre côté elle peut avoir une activité pro virale en maintenant et aggravant l'infection de VIH-1 [132].

L'ensemble des travaux réalisés et publiés jusqu'à récemment sur l'IL-18 et l'IL-18P ont été révisé par notre groupe dans une nouvelle publication : " *Potential role of IL-18 in the immunopathogenesis of AIDS, HIV associated lipodystrophy and related clinical conditions* ".

1.2.2.2 Article 1

1.2.2.2.1 Potential role of IL-18 in the immunopathogenesis of AIDS, HIV associated lipodystrophy and related clinical conditions

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RÉSUMÉ

L'IL-18 est une cytokine pro inflammatoire. Elle appartient à la famille d'interleukine-1. Elle est produite suite à une infection virale. Cette cytokine est capable de développer et différencier les cellules T CD4 naïves en T helper de type 1 ou de type 2. Elle se lie à son récepteur, mais elle est neutralisée et contrôlée par son antagoniste l'IL-18BP. Comme déjà connue, la structure de son récepteur et de son antagoniste se diffère. Ceci protège les cellules et les tissus de son potentiel destructif et de ses effets pro inflammatoires. Selon plusieurs études y compris notre laboratoire, il y a une augmentation de la concentration et de l'activité biologique de cette cytokine dans la circulation des personnes infectées par le VIH. Contrairement à son antagoniste dont sa concentration diminue chez les patients. Alors ceci peut contribuer au développement de la pathogénèse de la maladie chez ses personnes infectées. De même, il y

a une différentiation des cellules T naïves en T helper de type 2, causé par la diminution de la sécrétion de l'INF-γ, et l'augmentation de la concentration de l'IL-4. Cet effet peut être plus prononcé chez les personnes infectées par le VIH dont la production de l'IL-12 et l'IL-15 est compromise. L'IL-18, augmente directement la réplication virale. À cause de son effet apoptotique, elle cause la mort de plusieurs cellules immunitaires et non immunes. Mais il y a toujours des affirmations, discutant de son rôle protecteur. Selon plusieurs études, incluant la notre, l'IL-18 cause une mort fratricide des cellules NK dans les maladies inflammatoires et chez les patients infectés par le VIH. Finalement cette revue couvre les effets de l'activité biologique de cette cytokine, et sa contribution dans l'immunopathogénèse de la maladie d'immunodéficience (SIDA). Tout en mentionnant l'association du VIH avec le syndrome de lipodystrophy, la démence, la sclérose en plaque et les problèmes métaboliques. En somme, ceci nous montre l'importance de cette cytokine comme cible thérapeutique.

Contribution

J'ai contribué dans la rédaction de cet article, à donner mes idées, à la recherche des nouvelles idées et informations et finalement à chercher des nouvelles références

Potential role of IL-18 in the immunopathogenesis of AIDS, HIV-associated lipodystrophy and related clinical conditions

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Running Title: IL-18 and AIDS pathogenesis

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Summary:
IL-18 is a pleiotropic and multifunctional proinflammatory cytokine that is produced in response to a viral infection. The biological activities of the cytokine are tightly controlled by its natural antagonist IL-18 binding protein (IL-18BP) as well as by activation of caspase 1, which cleaves precursor IL-18 into its biologically mature form. The cytokine plays an important role in both innate and adaptive antiviral immune responses. Depending upon the context, it can promote TH1, TH2 or TH17 responses. Increased serum concentrations of IL-18 and concomitantly decreased concentrations of its natural antagonist have been described in HIV-infected persons as compared to HIV-seronegative healthy subjects. We discuss in this review article how increased biological activities of IL-18 contribute towards immunopathogenesis of AIDS, HIV-associated lipodystrophy syndrome and metabolic disturbances. While the advent of potent anti-HIV drugs has significantly enhanced life-span of HIV-infected patients, it has increased the number of these patients suffering from metablic disrders. The cytokine may prove to be a useful target for therapeutic intervention in these patients.

Key words:
Adipokines, Adipose tissue, AIDS, Cytokines, HIV-1, IL-18, IL-18BP, Lipodystrophy.

Introduction:

Viral infections usually induce production of several cytokines from the host cells. They do so by stimulating one or more so-called pattern recognition receptors (PRRs), which recognize certain pathogen-associated molecular patterns (PAMPs). The PRRs like Toll-like receptors (TLRs) occur on the cell surface and/or intracellularly in endosomal membranes and monitor extracellular environment and endosomes for the presence of PAMPs (reviewed in [1, 2]). The PRRs like nucleotide-binding and oligomerization domain (NOD)-like receptors (NLR), and Dex/DH box and CARD containing RIG-1 like RNA helicases (RHL) occur mainly in the cell cytoplasm and act as intracellular sensors for the PAMPs that have gained access to the interior of the host cells (reviewed in [3, 4]). A wide variety of bacterial and viral proteins, lipoproteins, nucleic acids, cell wall constituents, endo- and exotoxins, etc may act as PAMPs for one or more of the PRRs. After binding with their cognate PAMPs, the PRRs become activated and activate signaling cascades that culminate in the induction of several proinflammatory cytokines and chemokines from the host cells. The virus-induced cytokines and chemokines activate and chemoattract neutrophils, macrophages and natural killer (NK) cells in an attempt to kill and eliminate the infected cells. At the same time, they activate dendritic cells (DC) and initiate inflammatory and pathogen-specific immune responses ([5, 6]). If the host immune responses successfully eliminate the pathogen, the cytokine production falls back to their base levels. However, pathogens like Human Immunodeficiency Virus type 1 (HIV) have evolved many immune escape mechanisms. Consequently, the infection persists and the host continues to produce cytokines, which may cause chronic inflammation, tissue destruction and overactive but ineffective immune responses (reviewed in [7, 8]). An optimal production of these cytokines is essential not only for the innate host resistance to pathogens but also for the induction, amplification and maintenance of the pathogen-specific immunity. The type of the cytokines induced in the host also determines the qualitative nature of the pathogen-specific immune response. It may induce differentiation of naïve $CD4^+$ T cells into predominantly TH1, TH2, TH17 or Treg type effector/memory cells (Figure 1; reviewed in [9]). It has been well documented that the responses mediated by TH1 type cells are more effective in protecting the host from intracellular pathogens like viruses, whereas those mediated by TH2 type cells promote allergic responses to expel extracellular parasites and allergens. TH17 cells provide additional protection against extracellular bacteria and are potent inducers of inflammation, tissue destruction and autoimmunity.

A predominance of TH2 type cytokines was observed in HIV-infected/AIDS patients long ago and was postulated to be responsible for the inability of the host to eliminate and control the viral infection. Recent studies at single cell level also are in conformity with these earlier observations ([9, 10]). It is believed that many of the immune abnormalities that occur in HIV-infected patients result from a dysregulated production of several immunologically important cytokines. For example, several researchers have reported decreased production of IL-12, IL-15 and IL-21, and increased production of TGF-β and IL-10 in HIV-infected/AIDS patients ([11-14]; reviewed in [15]). Several researchers including us have reported increased concentrations of IL-18 in the sera of HIV-infected/AIDS

patients ([16], reviewed in [17]). This review discusses potential implications of the increased concentrations of this cytokine for anti-viral immune responses, pathogenesis and immunotherapy in HIV-infected AIDS patients. The review will begin with an overview of the IL-18 immunobiology.

IL-18, its production and secretion:

IL-18 was first discovered in 1989 as the "IFN-γ-inducing factor" present in the serum of mice infected with the BCG strain of Mycobacterium bovis, and challenged with lipopolysaccharide (LPS). The factor induced IFN-γ production in IL-2-treated splenocytes taken from normal mice ([18]). The cytokine was rediscovered, cloned and named as IL-18 in 1995 ([19, 20]). It is a multifunctional and pleiotropic proinflammatory cytokine that is produced by activated macrophages, myeloid type dendritic cells, Kupffer cells, keratinocytes, articular chondrocytes, intestinal and airway epithelial cells, microglial and ependymal cells of brain as well as by certain neurons in the medial habenular neucleus. The gene is expressed in many tissues and organs including thymus, liver, lung, spleen, intestines, placenta, heart, kidney, brain, prostate, pancreas, adipose tissue, skeletal muscles, bone, cartilage, adrenal cortex and neurohypophysis (reviewed in [21, 22]). These cells and tissues usually express IL-18 gene and protein constitutively and increase their expression in response to infection, cold and LPS. Physical and mental stress can also stimulate IL-18 production via hypothalamus-pituitary-adrenal axis by releasing adrenal corticostimulating hormone ([23]). Dendritic cells produce this cytokine costitutively and increase its secretion when they interact with other cells involving CD40/CD40L ([24]).

IL-18 belongs to the IL-1 family of cytokines (reviewed in [25]). All members of the family fold into a similar β-pleated trefoil structure. Like IL-1β and IL-33, IL-18 is produced as an inactive 24 kD precursor protein containing 192 amino acids. It has an unusual signal peptide of 35 amino acids and hence is not secreted via conventional secretory pathway. Within the cytoplasm, the precursor is cleaved on the carboxyl side of its aspartate 36 by the IL-1β converting enzyme (ICE) into mature biologically active18 kD cytokine ([26]). ICE-KO mice synthesize but do not process IL-18 precursor ([27]). ICE is a member of a family of cysteine-aspartate proteases called caspases, and is more commonly known as caspase 1. The caspase itself is also produced in the cells as an inactive 45 kD precursor, which must be activated before it could cleave the cytokine precursor. In addition to ICE; certain other proteases produced by mast cells (chymase) and neutrophils (proteinase 3, elastase, MMP-9, etc) can also cleave the extracellular IL-18 precursor into biologically active forms. Some proteases (e.g., caspase-3) may cleave the precursor into biologically inactive components. Thus, the secretion of biologically active mature IL-18 from macrophages requires two signals emanating from two distinct families of PRRs. (reviewed in [28, 29]). The first signal comes from TLRs upon binding with their cognate ligands, and enhances transcription and translation of the precursor form. The second signal comes from a variety of stimuli and results in the assembly of certain NLRs into a multiunit complex called "inflammosome", which acts as platform for activating caspase-1, processing of IL-18 precursor and its secretion from the cells (reviewed in [28, 29]). The requirement of the two distinct signals for the secretion of biologically active IL-18 is probably a safeguard mechanism to prevent excessive production of this cytokine that may cause tissue damage. A well-studied model of the second signal is binding of ATP to

an adenergic receptor P2X7R, an ATP-gated ion channel, expressed on the surface of IL-18 producing cells, e.g., macrophages (see below). The second signal also causes secretion of the cytokine from the cells in exosomes as mutivesicular bodies ([30]).

A typical NLR (also known as Caterpiller, NOD, NOD-LRR or NACHT-LRR) is characterized by the presence of a C-terminal ligand-binding LLR, a centrally located nucleotide binding and oligomerization (NOD) domain, and an N-terminal effector (Pyrin, CARD or BIR) domain. To date more than twenty distinct members of the NLR family have been identified (reviewed in [3], [31]). Only a few members of the family assemble into inflammosomes, while others form multiprotein complexes called apoptosomes to regulate ell death, and nodosomes to activate NF-κB. Still others may act as accessory proteins to scaffold the multiunit NLR complexes. Three NLR members, NALP-1, NALP-2 and NALP-3 assemble into inflammosomes and recruite precursor caspase-1 into these complexes via ASC and related proteins, which help in the oligomerization and protein-protein interactions within the complexes. Normally NLRs exist in auto-inhibited monomeric forms. A variety of stimuli cause their assembly into inflammosome accompanied by activation and auto-cleavage of caspase-1 into its mature form, which then cleaves the precursor forms of IL-18 and related cytokines (IL-1β and IL-33). It is not yet clear how NALP proteins recognize their ligands via their LRR domains and how these ligands get access to these receptors with the cytoplasm of the cells. Recent studies have shown that a diverse array of substanses, e.g., phagocytosed urate and silica crystals, aluminum compounds, amyloid β crystals viral DNA, intracellular bacteria and their products, bacterial and viral nucleic acids, etc can cause activation of caspase-1 and secretion of active IL-1β ([32-36]). Some pore-forming bacterial toxins, e.g., listeriolysin O, nigericin and maitotoxin can also cause activation of caspase-1. It is believed that these toxins open channels within the plasma membrane of the cell giving access to various ligands to NLRs and/or cause efflux of K^+ from the cells. The binding of extracellular ATP to P2X7R causes translocation of a protein pannexin-1 (a hemichannel) from the cytoplasm to the receptor. The channel provides access to extracellular ATP to the cell interior. It also allows efflux of K^+ from the cell. NLRs are believed to sense this efflux. It appears that some infammosomes may sense cell integrity via rupture of lysosomal membranes, as the rupture may allow lysosomal constituents like cathepsin B to gain access to the cell interior. Direct intracellular injection of cathepsin B and rupture of lysomal membranes have also been shown to cause inflammosome assembly and activation of caspase-1 ([29], [32]). The recognition of a wide array of structurally diverse ligands via structurally similar LRR domains of various NALPs remains enigmatic. The assembly of certain "nodosomes" could also trigger oligomerization of certain "inflammosomes" and hene processing and secretion of mature IL-18 and relared cytokines. For example, muramyl dipeptide (MDP), derived from the bacterial cell wall proteaglycan, binds NOD-2 in the host cell cytoplasm and activates caspase-1 via an inflammosome that contains NALP-1 ([37]).

The exact molecular mechanisms that couple the cleavage and secretion of the mature IL-18 (and related cytokines) are not completely understood. It has been shown that cellular oxidation via NADPH oxidase plays an essential role in the ATP-mediated secretion of mature cytokines. Thus, the reactive oxygen and nitrogen species that increase NADPH oxidase activity also result in the processing and secretion of IL-18. Antioxidants only partially block this oxidation. Since free ATP does not exist outside cells and only

stressed and injured cells release it, IL-18-producing cells sense it as a "danger signal" and respond by secreting proinflammatory cytokines. It is noteworthy that along with the cleaved cytokine, some precursor cytokine is also secreted. This may be degraded or activated depending upon the type of the proteases present in the extracellular milieu. In the presence of inhibitors of caspase-1, ATP causes secretion of only precursor IL-18. Gain-of-function mutations in certain NALPs have been documented. As expected, the individuals with these mutations suffer from a variety of chronic autoinflammatory diseases (reviewed in [3, 22, 31]).

IL-18 Receptor:

The cytokine exerts its biological effects on target cells via IL-18 receptor (IL-18R), which is expressed on a wide variety of body cells, e.g., vascular endothelial cells, NK, NKT, B, dendritic and TH1 type $CD4^+$ T cells, basophils, mast cells, synovial fibroblasts, adipocytes, etc. The receptor comprises two units, α and β chains, whose extracelluar and intracellular regions contain Ig-like and Toll/IL-1R (TIR) domains, respectively. Extensive mutagenesis studies have shown that IL-18 has three binding sites for its receptor: Two bind with the IL-18Rα chain and the third binds with the IL-Rβ chain ([38]). Upon binding of IL-18 to the receptor, the TIR domains transduce signals via MyD88-IRAK-TRAF-6, and activate NF-κB and MAPK (p38 and JNK; Figure 2; reviewed in [22]).

Control of IL-18 activity in the body:

IL-18 is a powerful proiflammatory cytokine. Its biological activity is tightly controlled in the body by a natural antagonist, IL-18 binding protein (IL-18BP; [39, 40]). The antagonist-bound IL-18 can bind to the α chain of the IL-18R but cannot recruit its β chain and transduce signals. The agonist is produced constitutively in the human body by a wide variety of cells and tissues. The protein is produced as a negative feed back mechanism in response to IL-18. In this regard, IL-18-induced IFN-γ acts as a powerful stimulus for the production of this protein. Gamma Interferon-activated sequences (GAS) and interferon regulatory factor-response elements (IRF-RE) are present in the promoter region of the gene for IL-18BP ([41, 42]), which exists in four isoforms designated "a", "b", "c" and "d". The isoforms result from alternate splicing of the mRNA ([39]). Of these isoforms, only "a" and "c" bind with, and inactivate IL-18. Furthermore, the "a" isoform binds IL-18 with 10-fold higher affinity than the "c" isoform, and accounts for most of the IL-18 neutralizing activity in the circulation of human body. IL-18BP bears no homology with IL-18R. It binds with IL-18 with an affinity that is several orders of magnitude higher than that of IL-18 for its receptor, forming a high affinity (Kd=400pM) complex with a very low dissociation rate and 1:1 stoichiometry. At equimolar concentrations, the "a" isoform can neutralize 50% IL-18, reaching upto 95% at a molar excess of 2. The circulating levels of IL-18 increase sharply in sepsis; however, most of it exists in inactive state, bound with IL-18BP ([43]). In *in vitro* experiments, IL-18BP impairs TH1 responses by inhibiting IL-18-mediated production of IFN-γ and activation of NF-κB ([40, 44]). By inhibiting IL-18-induced IFN-γ production, IL-18BP promotes prostaglandin production from human PBMC. IL-18 may promote protect host from viral infections. Therefore, it is not surprising that human poxviruses, ectromelia, vaccinia and cowpox have usurped the IL-18BP gene and have incorporated it into their genomes. The gene acts as a virulence factor, as its expression inactivates host's IL-18 response and a

mutant virus becomes relatively avirulent in mice ([45, 46]). The viral IL-18BP is immunosuppressive and has anti-inflammatory properties ([45]).
Another member of the IL-1 superfamily, IL-1F7, is also processed by casapse-1. Its splice variant, IL-1F7b, complexes with IL-18BP and enhances its IL-18-neutralizing activity. It can also bind the IL-18Rα chain weakly but cannot recruit the β chain and transduce signals ([47];reviewed in [48]).

Biological activities of IL-18:
IL-18 exerts many biological effects: it induces production of IFN-γ, TNF-α, IL-8, IL-1β, MIP-1α, NO and matrix metalloproteases from several cell types including T and NK cells; induces expression of adhesion molecules on vascular endothelial cells; promotes maturation of NK and T cells and increases their cytotoxicity via increased expression of FasL and perforin; regulates pathogen-specific immune responses; and plays a role in the homeostasis of energy (see Table 1). The cytokine increases expression of perforin without affecting expression of perforin mRNA. The neutralization of IL-18 prevents accumulation of neutrophils in the lungs and liver of mice in otherwise lethal endotoxaemia induced by LPS from Salmonella typhimurium and Escherichia coli ([49]). Professional antigen presenting dendritic cells (DC) produce IL-18 as well as express its receptors. IL-18 chemoattracts DC to the site of viral infection and induces their maturation directly and indirectly ([50]). IL-18 plays an important role in intercellular communication among DC, NK, T, B and endothelial cells. It induces expression of OX40L (CD134L) on DC. The CD134L-expressing dendritic cells induce differentiation and expansion of IFN-γ producing antigen-specific T cells ([51]). The cytokine is the most powerful stimulus for IL-12-induced production of IFN-γ from NK and T cells. A coordinate secretion of these two cytokines is very effective in invoking TH1 type immune responses. IL-18 and IL-12-induced IFN-γ causes expression of IL-12Rβ1 chain on naïve T cells and makes them responsive to IL-12 and differentiation into TH1 type CD4+ effector/memory T cells. Recent studies have shown that IFN-γ also prevents differentiation of TH17 type $CD4^+$ T cells, which are the most powerful inducers of inflammatory conditions and tissue destruction in the host (reviewed in [52, 53]). This may explain protective effects of IL-18-induced interferon from certain models of autoimmune and chronic inflammatory diseases. The interferon also induces antiviral state in the host cells. It can also induce apoptosis of virus-infected cells by modulating expression of TRAIL and TRAIL receptors on human cells ([54]). The protective effects of IL-18-induced IFN-γ have been demonstrated in mice from HSV-1 infection ([55]).
IL-18 can effectively promote TH1 type responses by inducing proliferation of TH1 type $CD4^+$ T cells. However, the cytokine *per se* does not induce differentiation of TH1 cells. Naïve T cells only express the ligand-binding IL-18Rα chain. The ability of IL-18 to amplify TH1 type responses depends upon IL-12, which induces expression of IL-18Rβ chain on naïve T cells during their differentiation into TH1 type $CD4^+$ T cells ([56]). Interestingly, in the absence of IL-12, IL-18 promotes TH2 type immune responses. The cytokine does so by inducing production of IL-4, IL-5, IL-9 and IL-13 from basophils and mast cells (reviewed in [57]). These cells constitutively express IL-18R and produce these cytokines in response to IL-18. It is noteworthy that IL-4 promotes differentiation and development of TH2 type $CD4^+$ T cells. IL-13 induces goblet cell hyperplasia and mucus secretion, whereas IL-5 causes eosinophilia. IL-18 also induces secretion of IL-4,

IL-9 and IL-13 from ligand-activated NKT cells. Even fully differentiated TH1 type $CD4^+$ T cells secrete TH2 type cytokines in addition to IFN-γ, when they undergo antigen-specific stimulation in the presence of IL-18. Under these conditions, B cells undergo differentiation and produce IgE ([57]). Thus, IL-18 has the potential to induce TH2 type responses, which promote allergic immediate hypersensitivity type responses needed for expulsion of multicellular parasites. These effects of IL-18 cannot be inhibited even by the presence of IL-12. This explains why viral infections are generally accompanied by both TH1 and TH2 type responses and exacerbate allergic diseases like asthma. The ability of IL-18 to promote both TH1 and TH2 type cytokine responses in a context-dependent manner has earned it the name of a "switch" cytokine.

Despite a protective role of IL-18 against viral, bacterial and fungal infections, chronic and excessive production of the cytokine may cause tissue destruction and contribute towards virus-induced immunopathology. It is important to note that increased levels of IL-18 have been reported in the circulation of patients suffering chronic inflammatory and autoimmune diseases like multiple sclerosis, hepatitis, Crohn'disease, graft versus host disease, psoriasis, rheumatoid arthritis, etc. The cytokine plays an important role in the etiopathogenesis of these diseases (reviewed in [58]).

As the cytokine is produced in brain, pituitary and adrenal glands in response to stress and cold, it mediates communication between CNS, endocrine and immune systems. The cells in the adrenal cortex that produce glucocortoids also produce IL-18. Furthermore, being produced in AT, IL-18 could play a role in energy and lipid homeostasis (see below).

IL-18 production in HIV infection:

As mentioned above, IL-18 is an integral part of the cytokine profile that is produced in response to a viral infection. Many viruses and intracellular pathogens induce its production from host cells and consequently increase IFN-γ production from NK and T cells (reviewed in [59, 60]). This early induction of IFN-γ is important for inducing a TH1 type antiviral response and inhibiting TH17 type strong inflammatory responses. The induction of IL-18, in response to a viral infection, is at least in part responsible for virus-induced activation of NK cells in the host. It appears that like many other viruses, HIV-1 also induces IL-18 response in the host. A close cousin of HIV-1, SIV, induces the expression of this and other cytokine genes in experimentally infected Rhesus monkeys ([61]). We could not find any study that investigated a direct effect of the virus on IL-18 production from human PBMC, dendritic cells or macrophages. However several researchers have documented increased concentrations of this cytokine in the circulation of HIV-infected persons. Torre *et a*l first described increased concentrations of IL-18 in the sera of HIV-infected symptomatic and AIDS patients but not in asymptomatic persons as compared with these levels in healthy subjects. Furthermore they found no correlation of the cytokine levels with viral load, CD4+ T cell counts or opportunistic infections in AIDS patients ([62]). These results have been verified and extended by several researchers including us ([16, 63]; reviewed in [17]). We showed that the patient sera contained on the average three-fold higher concentrations of this cytokine as compared to their control counterparts. The increased IL-18 concentrations were associated with their increased biological activities when they were tested for their ability to induce IFN-γ from human T cells. We could not find any correlation of these elevated serum

concentrations with $CD4^+$ T cell counts, $CD8^+$ T cell counts, the ratio between these two counts or with the plasma viral loads of these patients. However, Stylianou *et al* ([63]) found a significant correlation between the concentration of this cytokine and viral load of the patients. They also found that anti-retroviral therapy decreased viral load as well as the levels of this cytokine, however, they always remained above normal values. The workers suggested that increased concentrations of the cytokine despite ART might indicate treatment failure. Collectively, these results suggest that HIV, its gene products plays and/or nucleic acids may induce enhanced production of this cytokine in HIV-infected individuals. In keeping with this notion, Sailer et al reported higher levels of the cytokine in HIV-infected persons early in the course of the infection ([64]).

Source of IL-18 in HIV-infected persons:

Despite increased levels of IL-18 in the circulation of HIV-infected persons, their PBMC were reported to produce less amounts of this cytokine constitutively as well as in response to LPS and Staphylococcal bacteria ([16, 65, 66]). The decreased production occured both at the protein and mRNA levels. David et al. noted correlation between the decreased IL-18 and IL-2 mRNAs in the PBMC of HIV-infected patients ([65]). Interestingly, the production of IL-18 from the PBMC in response to LPS correlated with the serum levels of the cytokine in HIV-seronegative healthy subjects but not in HIV-infected individuals. The expression of caspse-1, which processes IL-18 precursor into its biologically active form, was also reduced in the PBMC of HIV-infected persons as compared to those in HIV-seronegative healthy control subjects ([16]). The reduction was seen both in the precusor and activated forms of the protease, and was not due to reduced numbers of monocyte-macrophages in HIV-infected persons. In this connection, we, and others have showed that a decreased production of IL-18 from the PBMC of HIV-infected persons was due to high concentrations of TGF-β in the circulation of these patients ([16, 66]). We also found a significant negative correlation between IL-18 production from the PBMC of HIV-infected persons and their serum TGF-β levels. Neutralizing this cytokine with cytokine-specific antibodies significantly augmented their ability to produce IL-18 constitutively as well as in response to LPS ([16]). It was also shown that IL-10 could play a similar role in suppressing IL-18 and IL-1 β production from the PBMC in these patients ([66]). Interestingly, mononuclear cells from the placenta of HIV-infected women were also compromised in their ability to produce this cytokine ([67]). It may be relevant to mention that the concentrations of both IL-10 and TGF-β1 are increased in the circulation of HIV-infected persons ([15]).

Despite decreased production of IL-18 from the PBMC of HIV-infected persons, it is not clear what contributes to the increased concentrations of this cytokine in the circulation. In an attempt to find a source of increased IL-18 in the circulation of HIV-infected persons, we discovered that human platelets contain abundant amounts of this cytokine and release it upon activation into the circulation. Platelet activation occurs in HIV-infected persons and this activation contributes to increased IL-18 levels present in the circulation of these individuals. In this regard, we observed a significant positive correlation between platelet activation and circulating IL-18 levels in HIV-infected persons ([68]). It is noteworthy that IL-18 is also induced in response to anxiety, physical and psychological stress via hypothalamus-pituitary-adrenal (HPA) axis in animal models. Given that HIV-infected persons undergo enormous amounts of emotional and

psychological stress, these organs could potentially contribute to increased IL-18 levels in the circulation. Furthermore, intestinal epithelial cells also contain preformed IL-18 and release them in response to a variety of stimuli ([58, 69]). A wide spread infection of $CD4^+$ T cells and macrophages present in the gastrointestinal tract could also stimulate the release of this cytokine from the intestinal epithelial cells.

IL-18BP in HIV-infection:

As stated above most of the circulating IL-18 is bound with IL-18BP and is biologically inactive. This protects the body from potentially harmful proinflammatory effects of the cytokine. We found a severe imbalance in the production of IL-18 and IL-18BP in HIV-infected persons (101). The serum concentrations of these two soluble mediators correlate with each other in HIV-seronegative healthy subjects. However no such correlation existed between them in HIV-infected persons. Contrary to the concentrations of IL-18, those of IL-18BP were significantly decreased in HIV-infected persons as compared to their healthy counterparts. An increase in the serum concentrations of IL-18 was not accompanied by a corresponding increase in its antagonist. Rather the concentrations of the antagonist were decreased in these sera. Consequently, the concentrations of biologically active free IL-18 were further increased in the sera of HIV-infected persons. Indeed we have earlier reported increased biological activity of IL-18 in the sera of HIV-infected AIDS patients ([16]). The caveat is that these results were based upon the measurement of only "a" isoform of IL-18BP. However, since this isoform is mainly responsible for neutralization of IL-18 in humans, it is reasonable to conclude that IL-18BP is decreased in these patients. As mentioned above, this protein is produced as a negative feed back mechanism in response to IL-18-induced IFN-γ. Decreased production of IL-12, IL-15 and IL-21 has been reported in HIV-infected persons (reviewed in [15]). In the absence/deficiency of these cytokines, IL-18 has little capacity to induce IFN-γ production. Indeed a decreased production of IFN-γ has been documented in the circulation of HIV-infected persons (). The decreased production of this interferon may be at least in part responsible for decreased production of IL-18BP in these patients. Imbalance in the production of IL-18 and its natural antagonist has also been reported in secondary haemophagocytic syndrome (SHS), hepatic cirrhosis, Crohn's disease, Lupus Nephritis and sepsis in humans ([43, 70-73]). In these conditions, the levels of IL-18 increase in the circulation, but these increases are not accompanied by corresponding increases in the production of IL-18BP. Consequently, the levels of free biologically active cytokine are more increased than those indicated by measurements of the total cytokine. For example, Novick *et al* [43] have reported that the levels of IL-18 are increased by 20-fold as compared with a 10-fold increase in those of IL-18BP in sepsis patients. This imbalance in the production of these two soluble mediators results in increased levels of free biologically active IL-18.

Effects on antiviral immunity:

As mentioned above, the ability of IL-18 to promote TH1 responses depends upon IL-12, which induces expression of IL-18R on TH1 type $CD4^+$ T cells. In the absence/deficiency of IL-12, IL-18 acts on mast cells, basophils, and NKT cells, and induces production of IL-4, which promotes TH2 type responses in the body (reviewed in [57]). A decreased production of IL-12 has been well documented in HIV-infected persons. The defective production occurs early in the course of the infection ([13, 15]). In addition to IL-12,

decreased production of IL-15 and IL-21 has been well documented in HIV-infected persons ([11, 14, 15]). These are the cytokines that work in synergy with IL-18 for inducing IFN-γ and other cytokines from NK and T cells. Therefore it is very unlikely that IL-18 could promote increased IFN-γ production and consequently decrease HIV replication in these patients. Decreased numbers of IFN-γ producing, but not of IL-4-producing, T cells as well as decreased levels of IFN-γ observed in the circulation of HIV-infected persons ([54, 74]) support this notion. A decreased production of this interferon could also occur due to increased production of other immunosuppressive cytokines e.g., TGF-β and/or IL-10, in these patients (reviewed in [10, 15]). A decreased production of IFN-γ and a predominance of TH2-like responses in this infection have been incriminated in the pathogenesis of AIDS (reviewed in [75]).

Increased IL-18 concentrations have been reported in the circulation of several chronic inflammatory conditions and infections. These conditions are often accompanied by decreased numbers of NK cells as well as by their reduced cytotoxic activities ([73, 76]). Interestingly, researchers have discovered an inverse correlation between serum IL-18 levels and NK cell numbers in these patients. It appears that IL-18 somehow resulted in the depletion of NK cells in these patients ([73, 76]). We found a similar inverse correlation between serum IL-18 concentrations and NK cell numbers in HIV-infected persons (101). This correlation existed between the cytokine and all major subsets of NK cells: $CD3^-CD56^+$, $CD3^-CD16^+$ and $CD56^+CD16^+$. Furthermore, we found that the cytokine induced fratricidal killing of NK cells by a mechanism that involved Fas-FasL interactions and TNF-α. Recombinant human IL-18 induced expression of FasL on the cell surface in primary human NK cells as well as in an established human NK cell line. The cytokine stimulates the FasL gene promoter and hence increases transcription from the gene. Furthermore, the cytokine increases sensitivity of human NK cells to apoptotic stimuli by decreasing expression of an anti-apoptotic protein Bcl-XL, without affecting the expression of Bcl-2. Although IL-18-treated cells may increase killing of Fas-expressing target cells, they may kill other NK cells, which constitutively express Fas on their surface. It is noteworthy that decreased NK cell numbers and their compromised functional activities have been well documented in HIV-literature (reviewed in 1). Thus, increased IL-18 concentrations may play a role in causing defects in the NK cell compartment.

Effects on HIV replication:

The effects of IL-18 on HIV replication remain controversial (see ref [17] for a review). The cytokine has been shown to enhance HIV replication *in vitro* in chronically infected human monocytic and T cell lines ([77, 78]) as well as in an acutely infected T cell line ([79]). The increased viral replication in the monocytic cell line was ascribed to the cytokine-induced activation of NF-κB and p38 MAPK, and involved IL-6 and TNF-α [77, 79]. Contrary to the results from these studies, one group of researchers reported that the cytokine inhibits, and its antagonist IL-18BP promotes HIV replication in human PBMC. The inhibition of the viral replication was reportedly due to increased production of IFN-γ in the cell cultures [80]. The authors also documented a decrease in the expression of CD4 in IL-18-treated PBMC cultures. In this regard we have shown that in *in vitro* experiments recombinant human (rh) IL-18 alone has minimal effects on HIV replication in human PBMC and in purified $CD4^+$ T cells. However, the cytokine increases HIV replication, in combination with TGF-β or IL-10 (101). These cytokine combinations are

relevant to HIV infections, as increased levels of these two cytokines have been reported in HIV-infected persons [15, 16, 75, 81, 82]. Although IL-18 could potentially decrease HIV replication via inducing IFN-γ from NK and T cells, it most likely does not happen in these persons. In a relatively more recent study (), the researchers noted a close association between IL-18 and IFN-γ levels of whole blood after stimulation with LPS in HIV-seronegative subjects. Despite observing enhanced levels of IL-18 in the whole blood in HIV-infected persons, they unexpectedly observed decreased levels of IFN-γ in the circulation of these patients as compared with those in HIV-seronegative healthy control subjects. These observations suggest that increased IL-18 concentration in the circulation of HIV-infected persons do not result in enhanced IFN-γ levels. The probable reasons could be decreased concentrations of IL-12, IL-21 and IL-15, and increased production of TGF-β and IL-10. Therefore IL-18-mediated inhibition of HIV replication via this interferon may be compromised in HIV-infected persons. The authors ([64]) have also observed that increased IL-18 levels correlated with decreased expression of CXCR4 on the patients' PBMC. The authors suggest an antiviral role of IL-18 due to its association with a decreased expression of the viral co-receptor CXCR4. However there is no direct experimental proof of the ability of IL-18 to decrease the expression of CXCR4 on human cells. In animal models of Rhesus macaques and Cynomolgous monkeys, pathogenic, but not non-pathogenic chimeric SIV/HIV-1 (SHIV) viruses caused increased IL-18 levels, which were associated with more rapid disease progression (loss of $CD4^+$ T cells), and a higher viral load set point ([83]). Thus increased IL-18 concentrations *in vivo* seem to contribute towards disease progression. Nevertheless, it has been argued ([84]) that these increased levels of the cytokine represent a protective host response. They argue that neutralizing this IL-18 could have worsened the disease condition in these animals. Given that this response does not protect the infected animals, a counterargument that neutralizing IL-18 may benefit the host would seem more logical. Therefore, it would be reasonable to conclude that raised IL-18 concentrations in HIV-infected persons contribute towards enhanced HIV replication. In line with this conclusion, raised serum levels of IL-18 in HIV-infected subjects have been associated with AIDS progression. The levels are higher in progressors but tend to remain stable in non-progressors [63]. In this regard anti-retroviral therapy, especially HAART, decreases the cytokine concentrations. However the levels always remain above normal and do not correlate with CD4+ T cell counts. It may be interesting to note that a recombinant infectious clone of SIV that carried the simian IL-18 gene in the sense or anti-sense orientation had no effect on any virological or immunological parameters when injected in Rhesus macaques ([85]). The clone with the IL-18 gene in the sense orientation induced only a slight increase in serum IFN-γ levels in early phase of the infection. No effect of the IL-18 gene expression was observed on viral replication ([85]). Therefore the collective evidence suggests that increased IL-18 concentrations in the sera of HIV-infected persons may not inhibit HIV replication; they may rather enhance it both directly in combination with other immunosuppressive cytokines, and indirectly by blunting antiviral immunity.

Before discussing the potential role of IL-18 in the pathogenesis of HIV-associated lipodystrophy syndrome, it would be important to describe some basic concepts about the biology of adipose tissue.

Basic concepts in biology of adipose tissue:

Depending upon their anatomical locations, AT tissues can be divided into subcutaneous (or peripheral) and visceral (or central) adipose tissues (SAT and VAT, respectively; [86]). The latter occurs around our internal organs. The two tissues differ from each other in many biological characteristics: ability to produce cytokines, insulin-mediated suppression of adipolysis, mitochondrial activities, etc. The visceral fat releases more free fatty acids (FFA) and contributes more to lipidemia. It has more infiltration of macrophages and produces more pro-inflammatory mediators, e.g., TNF-α, IL-6, IL-18, and MCP-1. For this reason, visceral adiposity is more relevant to metabolic disorders, e.g., insulin resistance (IR), type 2 diabetes (T2D), hypertension and cardiovascular manifestations. In obesity, both VAT and SAT are enlarged due to an increase in the size of invidual adipocytes (hyperplasia) as well as in the number of adipocytes (hypertrophy).

Adipocytes (fat cells), the main cellular elements of adipose tissue, belong to two functionally distinct types: white and brown adipocytes (reviewed in [86, 87]). White adipocytes mainly store excess energy as lipids and produce a wide array of cytokines, chemokines and hormones often referred to as adipokines (see below) that affect functions of many other systems in the body. Each white adipocyte possesses a single large lipid vacuole (unilocular) and usually does not undergo proliferation. New adipocytes develop from the recruitment and differentiation of preadipocytes into fuly differentiated adipocytes. The preadipocytes are precursors of fat cells and reside in vascular stroma of adipose tissues. They undergo proliferation, secrete proinflammatory mediators before becoming adipocytes. Brown adipocytes specialize in burning lipids for the production of heat. Each cell has many small lipid vacuoles (multilocular) and often expresses markers of cell proliferation (e.g., PCNA). The cells are rich in mitochondria, whose iron-rich proteins confer characteristic brown apprarance to these cells. Another feature of these cells is expression of the mitochondrial uncoupling protein (UCP)-1 (also known as thermogenin), a proton transporter that short-circuits oxidative phosphorylation to energy production in the form of heat. BAT does not exist as a distinct mass in adult humans; however, brown adipocytes may be found scattered among white ones in the WAT depots. They may also occur as small pockets at defined anatomical locations in most, if not all, adult individuals ([87]). BAT has greater vascular supply as compared to WAT and produces more angiogenic factors (e.g., VEGF) and less pro-inflammatory mediators (e.g., IL-6, TNF-α, resistin, angiotensinogen, etc). It is noteworthy that WAT promotes, and BAT protects individuals from obesity and related metabolic abnormalities. Therefore, converting WAT into BAT may provide a cure from obesity epidemic in humans.

It is noteworthy that an excess of fat (obesity) and a lack of it both lead to insulin resistance, dyslpidemia and metabolic disorders. An optimal amount of fat is necessary for energy and lipid homeostasis in the body. It provides the needed soluble mediators to regulate functions of other body systems (reviewed in [88]).

Differentiation of adipocytes:

Most of our knowledge about differentiation of adipocytes has come from *in vitro* differentiation of 3T3-L1 preadipocytes. Two transcription factors, C/EBP-α and PPAR-γ, act as master regulators of adipogenesis (reviewed in ref [89]; see Figure 3). PPAR- γ is necessary for the development of both BAT and WAT. It is a ligand-activated receptor

that heterodimerizes with the retinoid x receptor (RXR)-α. The heterodimer induces expression of several adipogenic genes. The ligands for PPAR-γ are produced endogenously. C/EBP-α is involved in terminal differentiation of adipocytes. It is essential for the development of WAT but not for BAT. Recent studies have shown that white and brown adipocytes arise from two distinct precursor cells. The BMP-2 and 4 drive differentiation of white, and BMP-7 drives differentiation of brown adipocytes. The expression of a transcription factor PRDM-16 is crucial for the differentiation of brown adipocytes ([87, 90, 91]). In concert with PGC-1α, and β, it induces expression of BAT-specific genes and suppresses the expression of several WAT-specific genes by interacting with CtBP-1 and -2. The forced expression of PRDM-16 in developing white adipocytes confers them characteristics of brown adipocytes. Silencing expression of PRDM-16 from the precursors of brown adipocytes promotes their development into muscle cells.

HALS:

HALS, first described in 1998 ([92]), is characterized by redistribution of fat, dyslipidemia, hypercholesterolemia, hyperlactatemia and systemic insulin resistance. The patients usually lose subcutaneous fat on face ("empty cheek syndrome"), arms, legs and upper trunk, and accumulate around viscera, breast and at ectopic locations, e.g., in the dorsocervial region giving rise to "buffalo hump" condition. It has been estimated that more than one third of HIV-infected patients undergoing anti-retroviral therapy manifest HALS. The syndrome may occur in infected persons without receiving any therapy. However, anti-retroviral drugs especially reverse transcriptase inhibitors (RTI) and protease inhibitors (PI) hasten and exacerbate the condition ([93]; reviewed in [94]). It is noteworthy that both SAT and VAT behave quite differently in HALS; the former undergoes atrophy whereas the latter becomes enlarged.

Experimental studies have shown that wasting of the SAT (lipoatrophy) occurs in HIV-infected patients with HALS due to inhibition of differentiation of pre-adipocytes as well as due to increased lipolysis in adipocytes ([49, 94]). The number of cells undergoing apoptosis is markedly increased in the SAT of HALS patients. Increased lipolysis causes release of glycerol and non-esterified free fatty acids (FAA) that causes hyperlipidemia. FAAs increase IR, induce production of proinflammatory mediators (e.g., IL-6 and TNF-α) from macrophages, and inhibit lipoprotein lipase activity. They are also toxic to insulin-producing β-cells of pancreas.

The expression of C/EBP-α and PPAR-γ, which are necessary for adipogenesis, is reduced in lipoatrophic adipose tissue in HALS. The expression of the PPAR-γ Co-activator protein (PGC)-1, which regulates biogenesis of mitochondria, is also reduced in the adipose tissue in this syndrome. Furthermore, the respiratory chain in mitochondria of the adipose tissue becomes defective. Due to reduced expression of UCP-1 (a thermogenic protein) and UCP-2 (that controls ROS production), the mitochondria produce more reactive oxygen species (ROS), which cause oxidative stress to AT. ROS also induce increased production of proinflammatory mediators (see below).

Studies have also shown that accumulation of fat at ectopic places, e.g., dorsal region of neck (the "buffalo hump" condition), pubis and trunk, represents a distinct disturbance of adipose tissue. It results from accumulation of adipocytes, which express markers of brown adipocytes, e.g., smaller size, multilocularity, expression of UCP-1 and PCNA

(reviewed in [95]). The "buffalo hump" adipose tissue, however, has lower levels of mitochondrial DNA and lack local inflammatory response. It is not yet clear whether this tissue develops from a selective amplification of pre-existing brown adipocytes or results from a "browning" of white adipocytes. If transplanted to other areas, these adipocytes give rise to fat pads. It is noteworthy that external signals associated with thermnogenic requirements cause enlarged brown AT in rodents (reviewed in [96]). There is some evidence to suggest that certain PI drugs and defective mitochondrial function may induce expression of UCP-1 and development of "buffalo hump" in HIV-infected persons ([95]). It is also not yet known whether the adipocytes that accumulate in the VAT in HIV-infected patients are of white, brown or mixed characteristics.

Anti-retroviral drugs and HALS:

As stated above anti-retroviral drugs hasten and exacerbate the development of HALS in HIV-infected patients. Both protease inhibitors (PI) and reverse transcriptase inhibitors (RTI) play a role in the development of this syndrome. These drugs inhibit differentiation of pre-adipocytes, promote apoptosis of adipocytes and increase lipolysis within these cells. They do so by inhibiting expression of SERBP-1; a transcription factor needed for the expression of genes for several enzymes and factors involved in adipogenesis. PI may also induce expression of anti-adipogenic factors like Pref-1. Furthermore, Nelfinavir, a PI, has been shown to increase lipolysis by promoting proteasome-mediated degradation of perilipin, which is a droplet-associated protein ([97]).

RTI drugs also promote HALS by a distinct mechanism; by inhibiting the activity of the mitochondrial DNA polymerase-γ. This inhibition results in the depletion of mitochondrial DNA and hence reduced biogenesis of mitochondria. The depletion of mitochondrial DNA has been documented in the patients receiving these drugs ([98]). Different RTI may vary in their inhibitory effect on the activity of the DNA polymerase ([99, 100]). It is noteworthy that AZT, an NRTI, causes loss of mitochondria, impairs oxidative capacity and induces site-specific changes in adipose tissues in rats ([101, 102]).

Role of pro-inflammatory cytokines in HALS:

Adipose tissue is considered as an active endocrine organ. It is infiltrated with macrophages, vascular endothelial cells, fibroblasts, and other types of cells. The tissue produces a wide variety of soluble mediators (cytokines, chemokines and hormones), which include IL-18, TNF-α, IL-6, IL-8, MCP-1, chemerin, PAI-1, angiotensinogen, leptin, resistin, visfatin, lipin, adiponectin, etc. They are often referred to as adipokines to underline their origin from the adipose tissue (reviewed in [103]). Via these adipokines, adipose tissue has the ability to regulate body metabolism, inflammation, reproduction, immune responses and life span of an individual.

A characteristic finding in HALS is the occurrence of increased levels of several proinflammatory cytokines (IL-6, TNF-α, IL-18) in the circulation as well as in the adipose tissue of the patients ([94]). The patients also have increased levels of circulating CRP; a general indicator of inflammation. Furthermore, the levels of soluble(s) TNFR-I and sTNFR-II, which indicate overall activation of the TNF system, are also increased. In contrast to the levels of proinflammatory cytokines, the concentrations of adiponectin and lipin are decreased in the circulation of these patients. They correlate inversely with those of IL-18, IL-6 and TNF-α in these patients. It is noteworthy that adiponectin is an anti-

inflammatory cytokine that enhances insulin sensitivity, and lipin plays a role in adipocyte differentiation. The concentrations of leptin, a regulator of energy homeostasis, however, usually remain within physiological limits ([49, 94, 104, 105]). The increased levels of the proinflammatory cytokines may result also from direct effects of HIV and/or of its gene products on various human cells ([106-108]). Anti-HIV drugs also induce production of these cytokines from human cells and tissues including adipocytes. It is widely believed that proinflammatory cytokines play an important role in the causation of HALS. They inhibit adipocyte differentiation and have catabolic effects on adipocytes. Furthermore, they inhibit lipoprotein lipase activity causing lipidemia. For example, increased levels of TNF-α in the circulation of HIV-infected HALS patients with HALS are accompanied with glycerol release and decreased ASP production ([49]). *In vitro*, the cytokine inhibits expression of several genes involved in adipogenesis, e.g., C/EBP-α and PPAR-γ. It also causes lipolysis, de-differentiation and apoptosis in adipocytes ([109-111]). The cytokine increases net oxidation of lipids, and attenuates insulin-mediated suppression of free fatty acids and glucose uptake via inhibiting expression of the glucose transporter GLUT-4 in HALS patients ([112]). Another proinflammatory cytokine IL-15 also reduces hepatic lipogenesis, promotes fat oxidation and decreases both brown and white adipose tissues without affecting food intake. The cytokine inhibits lipoprotein lipase activity and uptake of lipids by AT. Furthermore, it induces the expression of several genes, whose products are involved in thermogenesis, e.g., UPC-1 and UCP-3, PPAR-δ and PPAR-α ([113]). The cytokines with anti-inflammatory effects may antagonize the effects of pro-inflammatory cytokines. For example, IL-4 promotes adipogenesis, and increases lipid biosynthesis by increasing the expression of SERBP-1 in vascular endothelial cells ([114, 115]).

Role of IL-18 in HALS:

It is noteworthy that IL-18 is one of several pro-inflammatory cytokines, whose concentrations are increased in the circulation as well as in the adipose tissues in HIV-infecred HALS patients. The potential role of this cytokine in the etiopathogenesis of HALS is beginning to emerge. IL-18 mRNA and protein as well as IL-18R transcripts have been detected in the adipose tissue, including adipocytes, and TNF-α increases this expression ([116]). As mentioned above, concentrations of IL-18 increase in the sera of HIV-infected persons. Interestingly, these levels are significantly higher in HALS patients as compared to those of the patients without HALS. These levels correlate with those of TNF-α, but not with those of IL-6. Furthermore, an inverse correlation was found between IL-18 levels and the limb fat quantity, implicating this cytokine with lipoatrophy ([117]). IL-18 gene is expressed at higher levels in the adipose tissue of the patients with HALS as compared to the patients without HALS. The level of expression in the femoral gluteal adipose tissue correlated with the degree of lipoatrophy ([118]). It was also observed that in the subcutaneous tissue, from where fat disappears, IL-18 gene expression is increased. Taken together these studies suggest that IL-18 is implicated in the depletion of adipocytes from these areas. As mentionrd above, an important finding in the HLAS patients is decreased levels of adiponectin in their circulation. This anti-inflammatory mediator is produced by adipocytes, but ironically its concentrations decrese in obese individuals probably due to biological effects of pro-iflammatory cytokines. In this regard, IL-18 has been shown to inhibit transcription and mRNA expression as well as protein secretion from adiponectin gene by phosphorylating NFAT-

c4 via EKR1 and 2 ([119]). Taken together these observations suggest that IL-18 plays an important role in ipoatrophy seen in HALS patients.

IL-18 and insulin resistance:

Production of proinflammatory cytkines from adipose tissues provides a link between obesity and metabolic disorders, e.g, insulin resistance (IR). In fact it is the inflammation induced by these cytokines rather than obesity *per se* that underlies obesity-associated IR, T2D and T2D-associated nephropathy and retinopathy ([120]). These cytokines may increase IR in many ways: They interfere with insulin-mediated signaling, induce expression of SOCS proteins that increase insulin resistance, disturb adipocyte metabolism and decrease ability of the adipose tissue to store lipids. Consequently, there is dyslipidemia and lipids start accumulating in ectopic places, e.g., liver, pacrease, heart and skeletal muscles and increase insulin resistance in these organs. Increased IL-18 concentrations in HIV-infected individuals are likely to contribute to insulin resistance and metabolic disorders in all these fashions. Hypercholesterolemia, dyslipidemia (low HDL, hypertriglyceridemia, decreased VLDL), insulin resistance, increased fasting levels of glucose and insulin in the blood, were reported in HIV-infected persons even before the advent of HAART era ([121]). The implication of IL-18 in these manifestations is supported by the reports that the serum levels of this cytokine are significantly higher in HIV-infected patients with hypertriglyceridemia as compared to the patients with normal triglyceride levels ([122]). It is noteworthy that increased concentrations of this cytokine have also been reported in the circulation of HIV-seronegative T2D patients either diagnosed recently or with a long history of the disease. The levels decrease with high high fiber diet ([123]). Increased levels of the cytokine predict nephropathy in these patients ([124]). These levels correlate with obesity, glucose intolerance and could predict the onset of T2D in obese patients. The levels decrease with weight loss in obese individuals ([123, 125, 126]; reviewed in [127]). Interestingly, the cytokine and its recptor are expressed in pancreas in humans. The cytokine, alone as well as in combination with IL-12, causes acute pancreatitis and destruction of insulin-producing cells ([128, 129]). Moreover, administration of exogenous IL-18 promotes development of diabetes in young NOD mice ([130]). The cytokine is also responsible for streptozotocin-induced diabetes in mice, and IL-BP alleviates the disease in these mice ([131]).

Cardiovasculr (CV) Diseases:

IL-18 has been implicated in the development and progression of atherosclerosis. Functional IL-18Rs are expressed in atheroma-associated vascular endothelial cells and smooth muscles, which are augmented by LPS, TNF-α, IL-1β and other proinflammatory stimuli (reviewed in [22]). Mononuclear phagocytes in the lesions express mature IL-18. IL-18 induces several biological effects in the vascular endothelial cells that are reminiscent of atherogenesis: induction of IL-6, IL-8, ICAM-1 and various matrix metalloproteinases (MMP). Unexpectedly the cytokine also induced IFN-γ in smooth muscle cells ([132]). Increased levels of IL-18 expression in atherosclerotic plaques have been associated with plaque instability that may lead to acute ischemic syndromes ([133]). Increased levels of the cytokine also occur in the circulation of atherosclerosis patients. The serum levels of the cytokine correlate with thickness of the intima-media of coronary artery ([134, 135]). The thickness reflects overall burden of systemic atherosclerosis. Increased levels of the

cytokine have also been reported in patients having unstable angina pectoris as well as in acute coronary disease ([136, 137]). More importantly, *in vivo* neutralization of IL-18 by its natural antagonist (IL-18BP) slows down the development of atherosclerosis in a mouse model of the disease ([138]). In a prospective study, Blankeberg et al ([139]) have shown that increased IL-18 levels predict poor prognosis and increased risk of deaths in the patients with established coronary heart disease. IL-18 induces secretion of PAI-1 from adipocytes and promotes thrombogenic events ([119]). Increased levels of this thrombogenic mediator in the circulation of HALS patients have been documented ([140]). The IL-18-induced secretion of PAI-1 suggests that IL-18 may be causally involved in the enhanced platelet activation. Indeed, we have demonstrated a positive correlation between serum IL-18 levels and platelet activation in HIV-infected persons ([68]). Heart failures with increased IL-18 concentrations in the circulation cause more deaths than the heart failures with physiological levels of the cytokine (reviewed in [141]). The cytokine is expressed in heart itself: many cell types in this organ e.g., cardiomyocytes, smooth muscle cells, endothelial cells, resident macrophages and infiltrated neutrophils, etc, produce this cytokine ([141]). CRP, which is produced in liver in response to IL-6 and whose concentrations increase in the circulation in iflammatotry conditions, induces IL-18 production from vascular endothelial cells ([103]).

More than 60% of the patients receiving anti-HIV drugs develop hyperlipidemia, hyperglycemia, and central obesity ([142]). Several studies have documented occurrence of increased carotid intima media thickness and biomarkers of cardiovascular risk (cholesterolemia, triglyceridemia, dyslipidemia, lower homocysteinemia, increased CRP levels, etc) in HIV-infected children and adults as compared to their HIV-seronegative counterparts ([143-145]). Occurrence of cardiovascular events is a real risk in HIV-infected patients undergoing ART. Use of lipid-lowering treatments may have been useful in stabilising this risk. It is highly likely that increased IL-18 concentrations in the circulation of in these patients play a role in the development of this risk. Neutralizing these concentrations with IL-18BP could prove to be a beneficial strategy in these patients.

HIV-associated dementia (HAD):

HAD is a severe neurological complication in HIV-infected persons. It is characterized by development of sever cognitive, behavioral and motor dysfunctions. In the btain, both HIV-infected and non-infected macrophages and microglia produce proinflammatory cytokines like TNF-α, IL-1β and IL-18. These proinflammatory cytokines are believed to play a major role the pathogenesis of HAD (reviewed in [146]). These cytokines increase permeability of the blood-brain barrier that allows infiltration of HIV-infected macrophages into brain. The cytokines cause neuronal death by a variety of mechanisms, e.g., by overstimulating N-methyl-D-aspartate (NMDA) receptors, and inducing NO and RONS. Increased concentrations of IL-18 occur in the circulation and CSF in patients suffering from psychic disoerders. Gp120 induces caspase-1 activation and IL-1β secretion from microglia and astrocytes in the brain ([146]). These cells also express IL-18 and likely to produce this cytokine in response to HIV infection. Indeed, increased levels of IL-18 occur in the CSF of HIV-infected persons suffering from opportunistic infections of the CNS ([147]). It is noteworthy that a significant correlation was found between IL-18 production and cognitive decline in Alzheimer disease patients ([148]).

Given that HIV-infected patients have increased concentrations of biologically active IL-18, the cytokine is likey to contribute towards the development of HAD in these patients.

HIV and IL-18 gene polymorphism:

The human IL-18 gene is maps to chromosome 11q22.2 to 11q23.3. It contains 6 exons and 5 introns spread over 20.8 kb region in 11q23.1. Two TATA-less promoters, located upstream exons 1 (Promoter 1) and 2 (Promoter 2), have been described for the gene. The promoter 1 is constitutively active in cell lines like THP-1 and U937, while the promoter 2 is induced by LPS and infectious agents ([149]). It has been shown that promter 1 is exclusively used in adrenal gland constitutively as well as in response to neurogenic stimuli, while promoter 2 is used by cells of the immune system ([150]). Despite frequent sequencing of the gene in diverse human populations, no non-synonoymous SNP could be found in the exons, and exon-intron boundaries of the gene. Furthermore, no SNP could be detected in the mRNA splice sites. A limited degree of polymorphism has been reported in the promoter region, 3′ and 5′ untranslated regions (UTR) of the IL-18 gene (reviewed in [151]). Three synonymous SNP have been described in the proximal prompter region of the IL-18 gene: G-656T, C-607A and G-137A. Of these SNP, replacing C at -607 with A abrogates a cAMP response element-binding site, whereas replacing G at -137 with C abrogates a DNA binding site for the nuclear factor H4TF1. The haplotype CG is associated with an increased production of IL-18 protein ([152]). Interestingly, -607C was recently shown to be a risk factor for vertical transmission of HIV infection in a cohort of Brazillian children born to HIV-infected mothers, who took no antiretroviral therapy during pregnancy and did not undergo Caesarian section ([153]). This study suggests that the children with genetic ability to produce more IL-18 may be at a higher risk of perinatal infection. However, it is noteworthy that –607C alone has not been associated with increased production of the cytokine. It would have been interesting to determine the IL-18 promoter genotype of the mothers. It is quite likely that the mothers may have a high IL-18 producer genotype of their IL-18 promoter. Such mothers may be simply having higher viral loads and hence may transmit HIV to their newborns more efficiently.

Several studies have reported varying degrees of association of IL-18 gene SNP and their haplotypes with atherosclerosis, Multiple sclerosis, coronary heart disease, acute graft versus host disease, Crohn's disease and ulcerative colitis. Unfortunately, no clear-cut correlations could be demonstrated between predisposing genotypes and enhanced IL-18. Furthermore, many of the results have been contradicted by other studies (reviewed in [151]). As mentioned above, the biological activity of this cytokine is mainly controlled by IL-18BP and activation of caspase 1. The genetic variations that lead to a diminished production of IL-18BP and/or enhanced activation of caspase 1 could result in enhanced biological activities of this proinflammatory cytokine, and hence may predispose humans to proinflammatory diseases.

Conclusions and Future Directions:

The fact that certain viruses have incorporated the genes for IL-18BP and ICE inhibitor (e.g., the Cytokine Response Modifier gene in certain Pox viruses) into their genomes, suggests the importance of IL-18 and related cytokines as an impediment to viral replication. However, HIV has probably developed alternate strategies to evade antiviral

effects of this cytokine. It compromises the production of other cytokines (e.g., IL-12, IL-15, IL-21, etc) without which IL-18-induces responses blunt antiviral immune responses. Furthermore, increased production of immunosuppressive cytokines like TGF-β and IL-10 attenuates IL-18-induced IFN-γ production. Indded, a large body of data collected from clinical studies suggests that increasing concentrations of biologically active IL-18 in the circulation of HIV-infected individuals undermine innate and adaptive antiviral immune responses especially in later stages of the infection. They may also enhance HIV replication, especially in combination with other cytokines. Because of its ability to induce other inflammatory cytokines and amplify TH17 type $CD4^+$ effector/memory T cells, it might be involved in tissue destruction in this infection.

IL-18 is a cytokine produced by adipose tissue and it regulates lipid metabolism, energy homeostasis, insulin resistance and cardiovascular manifestations. It is noteworthy that studies in IL-18 and in IL-18R KO mice suggest a protective role of this cytokine in obesity, as these mice develop obesity, insulin resistance and atherosclerotic lesions with age ([154]). They also exhibit defective control of food intake by the hypothalamic satiety center and become hyperphagic. The plasma IL-6 levels are not affected in these mice. In accord with these results, IL-18BP-over-expressing mice also overate and became hyperglycemic. IL-18 deficiency caused decreased levels of constitutively activated STAT-3 in these mice ([154]). Interestingly, intracerebral but not intravenous or intraperitoneal injections of recombinant murine IL-18 reversed these effects ([154]). These data suggest that effects of IL-18 on insulin resistance and adiposity may be quite different depending upon its route of administration: it may promote insulin sensitivity and energy expenditures when acting on brain and may prote adiposity and insulin resistance when acting in the periphery (organs). Similar results were reported for another proinflammatory cytokine IL-6. IL-6 KO mice develop mature-onset obesity, IR and diabetes. Its peripheral administration promotes insulin resistance and dyslipidemia, whereas its administration into CNS protects mice from these manifestations ([88, 124]). Clearly further studies are needed to learn more on the effects of IL-18 and its natural antagonist on energy and lipid homeostasis in animal models. Furthermore, while IL-18 gene knock-out and transgenic mice provide valuable information, such studies must be verified by neutralization of the cytokine by siRNA or antagonist to avoid potential effects of compensatory mechanisms. It may be relevant to mention here that it has been argued that obese individuals as well as T2D patients develop resistance to IL-18, as their leukocytes respond poorly to this cytokine in *ex vivo* studies due to reduced expression of IL-18Rα and β chains on their surface ([155]). Due to a compromised production of IL-12, which induces expression of IL-18Rβ on naïve T cells, it is highly likely that the number of T cells responding to IL-18 may decline especially in later stages in the course of HIV infection. Unfortunately no studies have been reported on the expression of IL-18R or responsiveness of human cells to this cytokine in HIV-infected cells. Such studies are highly desirable for fully understanding the role of this cytokine in controlling HIV infection and its role in antiviral immunity and immunopathogenesis.

In the context of HIV infection, increased concentrations of IL-18 in HIV-infected patients blunt antiviral immunity, contribute towards immunopathogenesis of AIDS and promote disturbances in fat metabolism, IR, atherogenesis and cardiovascular manifestations. Therefore targeting IL-18 may be a therapeutic option. Fortunately, a natural antagonist with strong neutralizing activity exists for this cytokine and may be the

best option. Moreover, small molecular inhibitors of ICE have been developed. They inhibit ICE-mediated processing of IL-18 and IL-1β precursors into their biologically active forms. They may be used to reduce activities of these proinflammatory cytokines in chronic infectious and inflammatory conditions. Still P2X7R may be another target. Small molecular inhibitors of the receptor may be used to modulate inflammatory responses mediated by IL-18 and related cytokines (IL-1β and IL-33). These therapeutic options may be exercised in HIV-infected patients under close monitoring for unpredictable side effects.

Pro-inflammatory cytokines are known to regulate fat metabolism, induce IR and promote atherogenic process. Some of these effects are mediated by their direct effects on adipocytes. Little is known about potential effects of IL-18 on adipocytes and insulin-mediated signaling in human cells. It is noteworthy that human adipocytes express this cytokine and its receptor as well. Future studies shoud address these issues. Given that brown and white adipocytes arise from distict precursors and undergo distict programs of differentiation, such studies should be conducted on both types of cells separately. A potential role of IL-18 or its antagonist in the differential behavior of SAT and VAT also needs to be carefully examined. The results may be important for understanding the etiopathogenesis of HALS. They will also have implications for obesity, diabetes and atherosclerosis.

Experimental studies have shown that IFN-γ induces IL-12Rβ1 chain on naïve $CD4^+$ T cells in an IRF-1 dependent pathway that is required for IL-12-mediated differentiation of these cells into TH1 type cells. An early production of IFN-γ, therefore, is important for inducing effective antiviral immune responses. By its ability to induce this cytokine, IL-18 could serve as an efective adjuvant for anti-HIV vaccination. In vaccination studies, the injection of IL-18-encoding DNA as an adjuvant has been shown to induce protective TH1 type immunity. In this regard, co-injection of IL-18 and IL-12 encoding DNA may be more effective (reviewed in [58]). Future studies on anti-HIV vaccination using this cytokine as an adjuvant are highly desirable.

Abbreviations:

AT: Adipose tissue; ART: Anti-retrovial therapy; BMP: Bone morphogenetic protein; ASC/Pycard: Apoptosis-associated speck-like protein; BAT: Brown adipose tissue; BCG: Bacillus Calmette-Guerin; BIR: Baculovirus inhibitor of apoptosis repeat domain; CARD: Caspase recruitment domain; C/EBP: CCAAT/Enhancer binding protein; CHOP: C/EBP-homologous protein; CRP: C-reactive protein; FFA: Free fatty acids; HALS: HIV-associated lipodystrophy syndrome; ICE: IL-1β converting enzyme; JNK: Jun N-terminal Kinase; LRR: Leucine-rich repeat; MAPK: Mitogen-activated protein kinase; MMP: Matrix metalloproteinases; NACHT: Oligomerization module present in NAIP, CIITA, HET-E and TP-1; NALP: NACHT, LRR and Pyrin domain-containing protein; NADPH: NALP/NLRP: NOD: Nucleotide-binding and oligomerization domain; PAMP: Pathogen-associated molecular patterns; PCNA: Proliferating cell nuclear antigen; PGC-1: PPAR-γ co-activator-1; PRDM-16: PR-domain containing 16; PREF-1: Preadipocyte effector-1; PRR: Pattern-recognizing receptors; RIG-1: Retinoic acid induced gene-1; RONS: Reactive oxygen and nitrogen species; SAT: Subcutaneous adipose tissue; SIV: Simian immunodeficiency virus; SREBP-1: Sterol response element binding protein-1;

TIR: TLR-IL-1 receptor; Treg: Regulatory T cells; VAT: Visceral or central adipose tissue; WAT: White adipose tissue.

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Table 1. Biological effects of IL-18

IL-18:

1. Induces, togerher with IL-12, production of IFN-γ from NK and T cells.
2. Plays a role in the interactions of DC with other cell types.
3. With IL-12, suppresses, and without it promotes IgE synthesis.
4. Induces proliferation of TH1 and TH17 type $CD4^+$ effector/memory cells.
5. Activates and induces production of IL-4, IL-13 and histamine from mast cells and basophils.
6. Converts TH1 type CD4+ T cells into TH2 type cytokine-producing cells upon antigenic stimulation.
7. Induces FasL expression on NK cells
8. Enhances perforin expression in NK and T cells without affecting transcription.
9. Induces TNF-α, IL-6, IL-1β, NO, MCP-1 and PAI-1 production from a variety of human cells.
10. Induces ICAM-1, VCAM-1 on vascular endothelial cells and synovial membrane fibroblasts.
11. Causes accumulation of neutrophils at inflammatory sites.
12. Affects energy homeostasis and lipid metabolism.
13. Stimulates production of matrix metalloproteinases and tumor metastasis.
14. Plays a role in tissue destruction in chronic inflammatory conditions.
15. Induces IL-4 from ligand-activated NKT cells.
16. Induces HIF and modulates angiogenesis.
17. Expands IFN-γ producing antigen-specific T cells via CD134 pathway.
18. Promotes cartilage degradation but inhibits bone erosion.

DC: Dendritic cells; HIF: Hypoxia-induced factor; NO: Nitric oxide; PAI-1: Type 1 plasminogen activator inhibitor.

Table 2. How IL-18 contributes towards AIDS pathogenesis

1. By promoting TH2 type immune responses in the absence/deficiency of IL-12
2. By amplifying TH17 cells and causing chronic inflammation and tissue destruction.
3. By enhancing HIV replication especially in concert with other cytokines.
4. By causing NK cell loss by fratricidal killing of NK cells via FasL and TNF-α
5. By promoting the development of HALS and other metabolic disorders in HIV-infected patients

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1.2.3 Les cellules NK et l'infection par le VIH

Les cellules "*Natural Killer*" (NK) constituent un élément bien reconnu et important du système immunitaire inné. Elle joue un rôle important dans la défense de l'hôte contre les pathogènes, les rejections des greffes, des tumeurs, et des cellules allogéniques [133-136]. C'est une population des lymphocytes de faible densité, de large granulosité, elles constituent 10 à 15% des cellules mononucléaires du sang périphérique (PBMC). Elles se trouvent, aussi bien, dans les tissus et dans les organes [137-139]. Les cellules NK se distinguent des monocytes ($CD14^+$), des cellules B ($CD19^+$), et des cellules T ($CD3^+$) par un phénotype $CD3^-$, $CD14^-$, $CD19^-$, $CD16^+$, $CD56^+$[137, 138]. Les cellules NK tuent les cellules qui n'expriment pas à leur surface l'antigène du soi du CMH classe I, contrairement au CTL. Ces observations ont mené Karre et collaborateurs a proposé l'hypothèse du "*missing self hypothesis*", dans lequel ils ont postulé que les cellules NK reconnaissent les molécules du CMH du soi. La réduction ou la déficience de ces molécules du CMH du soi rend les cellules cibles plus susceptible à l'attaque des cellules NK [140-144]. Comme c'est déjà connu, les molécules du CMH de classe I sont les ligands des récepteurs inhibiteurs. Une fois liée à leur ligand, ces récepteurs inhibent la cytotoxicité des cellules NK et la sécrétion des cytokines [140, 144]. Les cellules NK sont régulées par une balance entre les récepteurs inhibiteurs et activateurs qui reconnaissent et se lient à la fois aux molécules classiques et on classiques du CMH classe I, aussi à d'autre molécules [145-147]. Les cellules NK ont deux types de recepteurs: " *Non-MHC Class–I Binding Receptors*" dont la famille des *"natural cytotoxicity receptors* "(NCR) et les " *MHC-Class-I Binding*" dont il es divisé en trois groupes : la famille des NKG2-CD94, la famille des Immunoglobulines" *like transcripts*" (ILT) et la famille des "*killer-cell immunoglobuline-like receptors*" ou (KIR) [137-139, 147, 148].

Finalement, les cellules NK peuvent médier la lyse de leur cible par un mécanisme qui implique la sécrétion des perforines et des granzymes, et elles peuvent causer l'apoptose par l'interaction Fas/FasL et TRAIL aussi [137, 138].

D'autres informations bien détaillées sur les cellules NK et leurs fonctions dans l'infection par le VIH, ont été présentées dans deux articles de revue, qui ont été rédigés par notre équipe et sont déjà publiés dans le "Journal of Leukocyte Biology". Ces articles sont présentés dans les pages suivantes.

1.2.3.1 Article 2:

1.2.3.1.1 Antiviral NK cell responses in HIV infection: I. NK cell receptor genes as determinants of HIV resistance and progression to AIDS

Alexandre Iannello, Olfa Debbech, Suzanne Samarani, and Ali Ahmad. 2008. Journal of Leukocyte Biology, 84 (1):1-26.

RÉSUMÉ

Les cellules NK constituent un élément bien connu et important du système immunitaire. Elles jouent un rôle important dans la défense contre les pathogènes et les tumeurs. C'est une population de lymphocyte de faible densité et de forte granulosité. Elles constituent 10% à 15% des cellules mononuclées du sang périphérique. Elles ont été caractérisées par leur habileté de tuer les tumeurs et les cellules infectées par des virus. Leur cytotoxicité dépend d'un anticorps (ADCC) et sous la restriction du CMH. Elles attaquent les cellules cibles qui sont déficientes ou l'expression du CMH est réduite. Leur fonction est régulée par une délicate balance entre

les récepteurs activateurs et inhibiteurs et des corécepteurs dont leurs ligands sont exprimés à la surface des cellules cibles. Cet article élabore l'importance de la famille des récepteurs KIR et leur interaction avec les gènes du CMH classe I peut déterminer une résistance du système immunitaire innée de l'hôte en réponse à une infection virale, ceci inclus l'infection par le VIH. Alors cet article de revue donne une idée sur l'immunobiologie, les récepteurs et leurs ligands. Finalement, il explique comment le polymorphisme des gènes qui codent pour les récepteurs KIR et leurs ligands HLA déterminent la résistance ou la susceptibilité à l'infection par le VIH et le développement vers la maladie de l'immunodéficience (SIDA).

Contribution

J'ai contribué dans la rédaction de cet article, de donner mes idées, à la recherche des nouvelles idées et informations et finalement à chercher des nouvelles références.

Antiviral NK cell responses in HIV infection: I. NK cell receptor genes as determinants of HIV resistance and progression to AIDS

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Abstract: NK cells play an important role in controlling viral infections. They can kill virus-infected cells directly as well as indirectly via antibody-dependent, cell-mediated cytotoxicity. They need no prior sensitization and expansion for this killing. NK cells are also considered as important regulators of antiviral immune responses. They do so by secreting a multitude of soluble mediators and by directly interacting with other immune cells, e.g., dendritic cells. NK cells do not possess a single well-defined receptor to recognize antigens on target cells. Instead, they express an array of inhibitory and activating receptors and coreceptors, which bind to their cognate ligands expressed on the surface of target cells. These ligands include classical and nonclassical MHC class I antigens, MHC-like proteins, and a variety of other self- and virus-derived molecules. They may be expressed constitutively and/or de novo on the surface of virus-infected cells. NK cell receptors (NKRs) of the killer-cell Ig-like receptor (KIR) family, like their MHC class I ligands, are highly polymorphic. Several recent studies suggest that epistatic interactions between certain *KIR* and *MHC class I* genes may determine innate resistance of the host to viral infections, including HIV. In the first part of this review article, we provide an overview of the current state of knowledge of NK cell immunobiology and describe how NKR genes, alone and in combination with HLA genes, may determine genetic resistance/susceptibilty to HIV infection and the development of AIDS in humans. *J. Leukoc. Biol.* 84: 1–26; 2008.

Key Words: *CD94/NKG2 · chemokines · cytokines · HIV-1 · HLA · KIR · KIR haplotypes · MHC class I · MICA · MICB · NKG2D · ULBP*

INTRODUCTION

HIV is the causative agent of AIDS: a dreadful and socially stigmatizing disease syndrome of the 20th century that inevitably leads to death. The AIDS epidemic has killed more than 25 million humans since 1981. At present, close to 40 million people are infected with the virus in the world. It has been estimated that in 2006, more than 4 million new infections occurred with the virus, and 3 million people died of AIDS in the world. It is widely believed that the viral infection is spreading in several underdeveloped and developing countries, e.g., China, India, and Eastern European countries (www.unaids.org). This situation underlines the need for developing an effective anti-HIV vaccine. However, at present, the prospects of developing an effective anti-HIV vaccine do not seem very promising. Although, anti-HIV drugs have significantly prolonged lifespans of HIV-infected patients and have reduced deaths, they do not cure the infection. Furthermore, sooner or later, the virus develops resistance to these drugs. Researchers have made spectacular advances in studying and analyzing immune responses to the virus. Most of these studies have largely focused on virus-specific immunity, i.e., HIV-specific antibodies, $CD4^+$ and $CD8^+$ T cell responses. It is noteworthy that the inductive and effector mechanisms of these responses depend on the recognition of small viral peptides by the immune cells. Unfortunately, small RNA viruses (such as HIV) are quite apt in neutralizing these responses by their remarkable ability to mutate (reviewed in ref. [1]). On the other hand, innate immune responses do not limit themselves to the recognition of small viral peptides. Instead, innate immune cells recognize so-called molecular patterns associated with pathogens (e.g., dsRNA, CpG motifs in viral DNA), altered self (reduced expression of MHC antigens), and/or induced self (de novo expression of pathogen-induced host proteins) on the body's own cells. Activation of innate immune cells is also a prerequisite for mounting an effective, pathogen-specific adaptive immunity. Understanding these responses may provide better ways of controlling viral infections.

NK cells constitute an important component of the host's innate immune system. Once considered as relatively unimportant and nonspecific killers of tumor cells, NK cells are now recognized as important cells with ready-to-go effector and regulatory functions. For long, NK cells have been known to

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kill virus-infected cells, and NK cell-deficient individuals have been known to suffer from repeated viral and bacterial infections [2, 3]. However, a lack of understanding of NK cell immunobiology until recent years has been an impediment in appreciating the role of these cells in controlling these infections. Today, scientists have made significant advances in understanding how NK cells function and regulate innate and adaptive immune responses. Consequently, we have learned a lot about the role of these cells in HIV and other viral infections. The review focuses on NK cell responses in HIV infections and their relevance to anti-HIV resistance, immunotherapy, and vaccination. We also underline some of the important unresolved issues with respect to these cells in HIV infection that need to be addressed in future research. Understandably, it will have to begin with an overview of the current immunobiology of NK cells.

NK CELLS

NK cells are a population of low-density, large granular lymphocytes, which mainly develop and differentiate in bone marrow and then enter into the circulation. Significant numbers of NK cells also develop and differentiate in thymus, spleen, tonsils, and lymph nodes [4, 5]. As a result of their different sites and pathways of development, NK cells are heterogeneous with respect to their phenotypic characteristics and functional capabilities. They make up 10–15% of the PBMC. In response to proinflammatory stimuli, which may be induced by a viral infection, NK cells migrate to various tissues and organs of the body. Significant numbers of NK cells occur in thymus, lymph nodes, gut, liver, peritoneum, and other organs and tissues of the body. NK cells represent a cell lineage distinct from those of monocytes, granulocytes, and B cells. They share a common hematopoietic progenitor with T cells. However, they are less specialized than T cells and retain certain ancestral characteristics of plasticity and versatility. Phenotypically, NK cells are $CD3^-$, $CD2^+$, $CD16^+$, $CD56^+$, $CD14^-$, and $CD19^-$. Unlike T and B cells, they do not express a well-defined single antigen receptor. They also do not express recombinase activating genes (RAG) and retain their TCR and BCR genes in germ-line configurations. See **Table 1** for commonly used NK cell markers. It is noteworthy that most of these markers are not NK cell-specific, as cells of other types may also express them. However, NKp46 and NKp30 may be considered as NK cell-specific markers, as non-NK cells rarely express them.

NK cells should not be confused with NKT cells. The two cell types are quite distinct from each other with respect to their origin, phenotype, antigen recognition, and certain effector functions (reviewed in refs. [6, 7]). As their name implies, NKT cells share characteristics with NK and T cells; i.e., they are $CD3^+$, bear clonotypic αβ TCR and certain NK cell markers ($CD56^+$, $CD161^+$), and secrete IFN-γ and/or IL-4. They recognize self and foreign glycolipids in association with a MHC class I-related glycoprotein CD1d. Activation of NKT cells usually leads to activation of NK cells and dendritic cells (DC) in the body.

Human NK cells can be divided into two major subsets based on the level of expression of CD56 and the presence or absence of CD16. The two markers are usually expressed reciprocally on these cells. The two subsets $CD56^{high}CD16^-$ and $CD56^{low}CD16^+$ represent ~10% and 90% of NK cells present in the peripheral blood, respectively [8, 9]. The cells in the two subsets differ in their proliferative potential, homing characteristics, functional capabilities, and responses to different cytokines (listed in **Table 2**). The cells in the former subset express high-affinity receptors for IL-2, proliferate in response to picomolar concentrations of the cytokine, produce mainly cytokines upon activation, and have low cytotoxic potential. They express little KIR and preferentially migrate to secondary lymphoid organs, e.g., lymph nodes, tonsils. Most of the NK cells in lymph nodes are $CD56^{high}$. The $CD56^{low}$ $CD16^+$ NK cells express low-affinity IL-2Rs, proliferate in response to nanomolar concentrations of IL-2, express KIR, and are highly cytotoxic. These NK cells migrate to inflamed tissues in response to chemotactic stimuli. By virtue of expression of CD16, they are also efficient mediators of ADCC. The $CD56^{high}$ subset is less cytotoxic as compared with the $CD56^{low}$ subset, probably as a result of their lower expression of perforin and ζ-chain [9, 10]. The ζ-chain is a signal-transducing component of the high-affinity receptor for IgE (FcεRI) and acts as a signaling partner for several activating NK cell receptors

TABLE 1. Common Markers Used for Phenotypic Characterization of Human NK Cells

Marker	Structure	Ligand	Prevalence	Function
CD2	Ig-like	CD58	All	Adhesion, Conjugate formation
CD3	Ig-like	TCR	Absent	–
CD19	Ig-like	BCR	Absent	–
CD14	G-LRR	LPS	Absent	–
CD16A	Ig-like	IgG[a]	Subset	ADCC, Degranulation
CD56	Ig-like	Self	Subset	Homotypic adhesion
NKp46[b]	Ig-like	HA	All	Activation
NKp44[b]	Ig-like	HA	All	Activation
NKp30[b]	Ig-like	?	All	Activation
CD161	C-type Lectin	LLT-1	All	Activation?
CD8	Ig-like	MHC class I	Subset	Costimulation, Conjugate formation

Prevalence refers to whether all or a subset of NK expresses the marker. G-LRR, GPI-anchored leucine-rich repeat-containing glycoprotein; HA, hemagglutinin of influenza virus; LLT-1, lectin-like transcript-1; ADCC, antibody-dependent, cell-mediated cytotoxicity. [a] IgG, IgG aggregated or found in immune complexes. [b] The marker is specific to NK cells. ? indicates unknown or controversial.

TABLE 2. Characteristics of Two Major NK Cell Subsets

Characteristic	$CD56^{hi}CD16^{low}$	$CD56^{low}CD16^{hi}$
IL-2R	High affinity	Low affinity
Cytotoxicity	+	+++
Perforin	Low	High
Cytokine production[a]	+++	+
Main location	Lymph nodes[b]	Blood
KIR	Low	High
NKG2 Expression	High	Low
ADCC Function	Inefficient	Efficient
LFA-1	Low	High
CD62L[c]	High	Low
CCR7[d]	High	Low
SHIP-1[e]	High	Low
CD3ζ-chain[f]	Low	High
IL-7R	High	Low
c-Kit[g]	High	Low
ICAM-3	High	Low
Lysozyme production	Yes	No

High and low refer to levels of expression. [a] Cytokines include IFN-γ, TNF-α, TNF-β, IL-5, IL-13, and GM-CSF. [b] In T cell-rich areas of lymph nodes and other secondary lymphoid organs and in body tissues and organs such as liver. [c,d] Lymph node homing receptors. [e] Needed for IFN-γ production. [f] Signaling partner for activating receptors such as CD16a. [g] c-Kit receptor tyrosine kinase needed for IL-mediated proliferation. KIR, killer-cell Ig-like receptors; NKG2, NK cell group 2; CD62L, CD62 ligand.

(NKRs), e.g., CD16a, NKp30, NKp46, etc. Both NK cell subsets become potent killer cells upon incubation with cytokines and are called lymphokine-activated killer cells. Some workers also differentiate between $CD16^{high}$ and $CD16^{dim}$ subsets of NK cells. Furthermore, NK cells expressing CD56 and CD16 have also been described. They may represent different activation and differentiation states of NK cells. It is noteworthy that incubation with different cytokines may change phenotypic, functional, as well as homing characteristics of NK cells. For example, IL-2, IL-12, and IL-15 can convert $CD56^{high}CD16^{-}$ and $CD56^{low}CD16^{+}$ NK cell subsets into $CD56^{bright}CD16^{+}$ cells. On the other hand, TGF-β1 converts $CD16^{+}$ NK cells into $CD16^{-}$ NK cells [11–13].

NK CELL FUNCTIONS

Although NK cells have been traditionally known for their ability to kill certain tumor and virus-infected cells, they also perform other important roles (detailed in **Table 3**). Like neutrophils, NK cells can kill bacteria by secreting α-defensins: the antimicrobial peptides that can disrupt bacterial cell walls. They act as important immunoregulatory cells by secreting cytokines and chemokines. It is noteworthy that NK cells are the only known source of IFN-γ other than activated T cells. The cytokine is known to activate macrophages and drive $CD4^{+}$ T cell differentiation into type 1 (TH1) cells. It also induces expression of TRAIL on T cells. An immediate release of this cytokine from NK cells in early stages of an infection is crucial for inducing virus-specific immunity. In addition to IFN-γ, NK cells have been documented to secrete TNF-α, GM-CSF, IL-5, IL-13, IL-10, TGF-β, MIP-1α, MIP-1β, RANTES, NO, etc. (reviewed in ref. [14]). In addition to their immunoregulatory properties, IFN-γ and TNF-α can induce an antiviral state in the host cells and inhibit virus replication by noncytopathic mechanisms. This virtual curing of the infected cells is increasingly being appreciated in controlling viral infections [15].

NK cells regulate immune responses by interacting physically with other cells in the body. Their interactions with monocyte-macrophages, DC, and T, B, NKT, and vascular endothelial cells are beginning to be understood. These interactions bear important implications for viral infections, malignancy, and autoimmunity. Under appropriate conditions, NK cells can cause differentiation of monocytes into macrophages [16]. They also interact with activated macrophages. These interactions result into activation of interacting NK cells via 2B4 and CD48 interactions. NK cells also kill macrophages that become overactive (with high doses of LPS) and express de novo ligands for certain activating NKRs. They also kill macrophages, which produce immunosuppressive, cytokine-like TGF-β and IL-10. Consequently, NK cells promote TH1 responses needed for the induction of an effective immune response against intracellular pathogens [17, 18]. Interestingly, NK cells themselves have the potential to act as APC. It may be relevant to mention here that a special kind of cell was described in mice with DC and NK cell-type characteristics. It was named as IFN-producing killer DC (IKDC), which kill virus-infected cells as well as tumor cells, produce IFNs, and present antigens to T cells [19–21]. The IKDC, in fact, represent a distinct activation stage of NK cells [22]. Such cells have not been found in humans. However, it is argued that the human immune system is more developed than that of mice, and all human NK cells can act as APC under appropriate conditions. Activated human NK cells express several molecules, which enable them to interact with T cells, e.g., CD11c, MHC class II, CD80, CD86, CCR7, CD70, etc. Incubation of NK cells with IL-18 has been shown to enhance their helping functions [23]. This NK cell "help" function is supported by the reports that in the presence of activated NK cells, antigen-specific CTL may be generated without help from $CD4^{+}$ T cells [24].

NK cell–DC interactions are complex and are important for innate and adaptive immune responses against viral infections. They will be discussed in a later section in this article.

NK cell interactions with other immune cells are important for the induction of effective immune responses. It has been shown that NK cells can regulate adaptive $CD4^{+}$ memory T

TABLE 3. NK Cell Functions

1. Natural cytotoxicity against hazardous (infected or malignant) cells.
2. ADCC against hazardous cells in the presence of antibodies.
3. Immune regulation via secretion of chemokines and cytokines.
4. Immune regulation via physical interaction with other immune cells, e.g., monocyte/macrophages, T and B cells, and DC.
5. Regulate hematopoiesis via secreting GM-CSF.
6. Regulate angiogenesis via interaction with vascular endothelial cells.
7. Uterine NK cells play a role in placentation and pregnancy.
8. Role in contact hypersensitivity.
9. Rejection of bone marrow allografts.

cells. More specifically, adoptive transfer of $CD4^+$ memory T cells specific for myelin oligodendrocyte glycoprotein (MOG; an autoantigen) is able to expand and cause experimental autoimmune encephalitis (EAE; a mouse model of multiple sclerosis) in RAG-2-deficient mice, which lack T and B cells but have functional NK cells. However, NK cells in these mice are inhibited from killing the $CD4^+$ memory T cells, as the latter expresses Qa-1 (the mouse equivalent of human HLA-E). The transferred cells do not cause EAE in Qa-1 lacking mice. NK cells kill MOG-specific $CD4^+$ memory T cells in these mice. These results show that NK cells play an important role in the homeostasis of memory T cells and may also eliminate autoreactive $CD4^+$ memory T cells under appropriate conditions. The results suggest that blocking Qa-1/NKG2A interactions may represent a better clinical strategy to eliminate autoreactive T cells than using anti-CD3 antibodies [25].

In certain mouse models, NK cell activation has been shown to be indispensable for inducing antitumor antibody and CTL responses [17, 26]. In addition to producing IFN-γ, physical interactions between NK cells and other immunocytes are needed for these responses. For example, via CD40/CD40L interactions, NK cells can induce transcription of activation-induced cytidine deaminase and switch recombinations in B cells [27].

NK cells are plastic and may differentiate themselves into cell types that produce predominantly IFN-γ or IL-5. It is not known what causes this polarization in NK cells. It has also been suggested that these differences in cytokine production may result from their different differentiation states. Nevertheless, this polarized production of cytokines from NK cells may be correlated with certain disease conditions. For example, IL-5- and IFN-γ-producing NK cells were shown to correlate with remissions and relapses in multiple sclerosis in humans, respectively [28]. The NK cells producing predominantly IL-5 also expand and play a role in asthma in humans [29].

In a provocative study, NK cells were shown to mediate memory-type responses. In RAG−/− mice, which lack T and B cells, O'Leary et al. [30] demonstrated NK cell-dependent, anamnestic responses to a hapten in mediating contact hypersensitivity. Although the molecular mechanisms behind this NK cell-dependent memory are not clear, the results may have important implications for development of vaccines.

NK cells play an important role in successful pregnancy and reproductive efficiency. The KIR2DL4/HLA-G interactions and consequent secretion of IFN-γ from uterine NK cells are needed for placentation [31, 32].

NK CELL ACTIVATION

NK cells exist in the body in a semi-activated state and are ready to kill susceptible target cells. However, they may undergo activation in vitro and in vivo, which enhances their functional capabilities. Various stimuli that activate NK cells are listed in **Table 4**. A variety of cytokines activates NK cells and induces their proliferation as well. Activated NK cells have increased expression of activating receptors, adhesion molecules, and intracellular effector molecules, e.g., perforin. They secrete higher amounts of cytokines and have higher cytolytic potential and a reduced threshold to trigger their killing mechanism. Virus-induced cytokines usually result in activation of NK cells of the host. These cytokines include IFN-α/β, IL-2, IL-12, IL-15, IL-18, IL-21, and others [14, 33]. Of these cytokines, IL-15, IL-21, and fms-like tyrosine kinase 3 ligand have been found to be essential for the development, differentiation, and homeostasis of NK cells. IL-15 knockout (KO) mice are deficient in NK cells. Furthermore, NK cells from normal mice undergo apoptosis when transfused into IL-15 KO mice (reviewed in ref. [34]).

TABLE 4. How NK Cells Become Activated in Viral Infections

NK cells may become activated and undergo expansion in a viral infection in several ways:

1. Recognizing viral proteins and/or virus-induced proteins in the host cells via activating receptors.
2. Monitoring the expression of MHC or MHC-like ligands for NKRs.
3. Recognizing viral proteins, DNA, or RNA via so-called pattern recognizing receptors, e.g., TLR, but may require accessory cell help.
4. Virus-induced cytokines from DC, monocyte-macrophages, e.g., IFN-α/β, IL-12, IL-15, IL-18, IL-21, IL-23, etc.
5. By interaction with other cell types, e.g., DC, macrophages.
6. Recognizing genotoxic stress-induced, *de novo*-expressed ligands for the NKG2D receptor.

NK cells also express TLRs. Therefore, they can undergo activation upon binding with certain viral proteins and nucleic acids via these receptors [35, 36]. However, NK cells require accessory cell function (presence of $CD14^+$ monocytes or plasmacytoid DC) for TLR-mediated activation [37–39]. Certain viral proteins may act as ligands for one or more activating NKRs and therefore, may directly activate NK cells; e.g., HA of the influenza virus activates NK cells via NKp46 and NKp44 [40]. NK cells also become activated when they contact a susceptible target cell (e.g., K562, which has reduced expression of MHC class I and/or has increased expression of ligands for certain activating NKRs) and trigger their cytolytic machinery to kill the target cell.

NK cells mainly kill their target cells by releasing cytotoxic molecules (perforin, granzymes, and granulysin), which are normally contained in their granules. These molecules are released within the immune synapse (IS) onto the surface of the target cells. NK cells can also kill target cells by FasL, TRAIL, and TNF-α if the target cells express appropriate receptors [41, 42]. NK-cell mediated lysis is usually determined in microcytotoxicity assays by measuring the release of ^{51}chromium, lactate dehydrogenase, or perforin in culture supernatants. Individual NK cells mediating the lysis can be recognized and counted by detecting the expression of lysosomal protein lysosome-associated membrane protein-1 (CD107a) on their surface [43, 44].

REARRANGEMENT OF CYTOSKELETON IN NK CELL-MEDIATED KILLING

As mentioned above, NK cells mainly kill their target cells via releasing cytotoxic granules. To avoid unintended damage to

neighboring cells, NK cells release these effector molecules in a well-defined and restricted area of contact with the target cells (reviewed in ref. [45]). This area of contact between the two cells is called IS. The synapse is an activating one if it is formed between a NK cell and a susceptible target cell. The formation of the synapse is initiated by interaction between different activating receptors and adhesion molecules (e.g., CD2, LFA-1, CD11a, CD11b) on NK cells and their cognate ligands on the target cell. The adhesion molecules aggregate in the form of a ring at the periphery of the synapse (**Fig. 1**). The engagement of integrins causes their activation and results in talin-mediated actin polymerization. The close contact between NK and target cell membranes induces reorientation of the MTOC of the NK cell toward the synapse. The microtubule-associated motor proteins (kinesins) shuttle granules containing cytotoxic mediators toward the center of the synapse. Within the center of the synapse, different activating NKRs interact with their cognate ligands on the target cell. The glycosphingolipid-rich membrane microdomains (lipid rafts) rapidly accumulate in the center of the synapse in a cytoskeleton-dependent manner. The lipid rafts are rich in effector signaling molecules. Their recruitment to the synapse requires activities of several kinases (Src, Syk, and the serine-threonine kinase protein kinase Cε). A ring of F actin surrounds the center of the synapse and prevents spillover of the cytotoxic mediators from the synapse. Within the synapse, NK and target cells interact with each other via membranous protrusions, which end at coated pits on the surface of opposing cells [46]. The Src homology 2 (SH2) domain-containing phosphatase (SHP)-1 is recruited to the periphery of the synapse within 1 min. It limits the activation event to the center of the synapse. Actin polymerization and MTOC reorganization are key events needed to trigger NK cell cytotoxicity (degranulation). The pharmacological agents that inhibit actin polymerization also inhibit NK cell-mediated killing [47]. Another phosphatase, SHIP, is also recruited to the synapse within minutes to terminate the triggering of the NK cell. After discharging its cytotoxic mediators to the membrane of the target cell, the NK cell separates itself and is ready to kill another target cell. A single NK cell can kill several target cells in a sequence one after the other. The inhibitory IS is formed between an NK cell and a resistant target cell. Its formation is prevented at an early stage by inhibitory receptor-recruited phosphatases. The inhibitory receptors cluster in discrete microdomains in the center of the inhibitory synapse and interact with their cognate ligands.

A characteristic feature of the activating IS is exchange of membrane proteins (receptors, ligands, adhesion molecules, etc.) between the interacting cells. For example, NK cells may transfer inhibitory KIR to target cells. The latter cell types may transfer MHC-class I chain-related protein A (MICA) to NK cells, which in turn, may transfer these acquired proteins to other NK cells via homotypic, intercellular contacts [48, 49].

NK cells, like other immune cells, can also communicate with other cells at a distance by forming nanotubules and transfer molecules and Ca^{++} fluxes to them (reviewed in ref. [50]).

NKRs

NKRs and coreceptors regulate NK cell-mediated killing. They include a variety of molecular structures expressed on the surface of NK cells. They bind specific ligands on target cells. NKRs can be divided into inhibitory and stimulatory types depending on the nature of the signal they send to NK cells

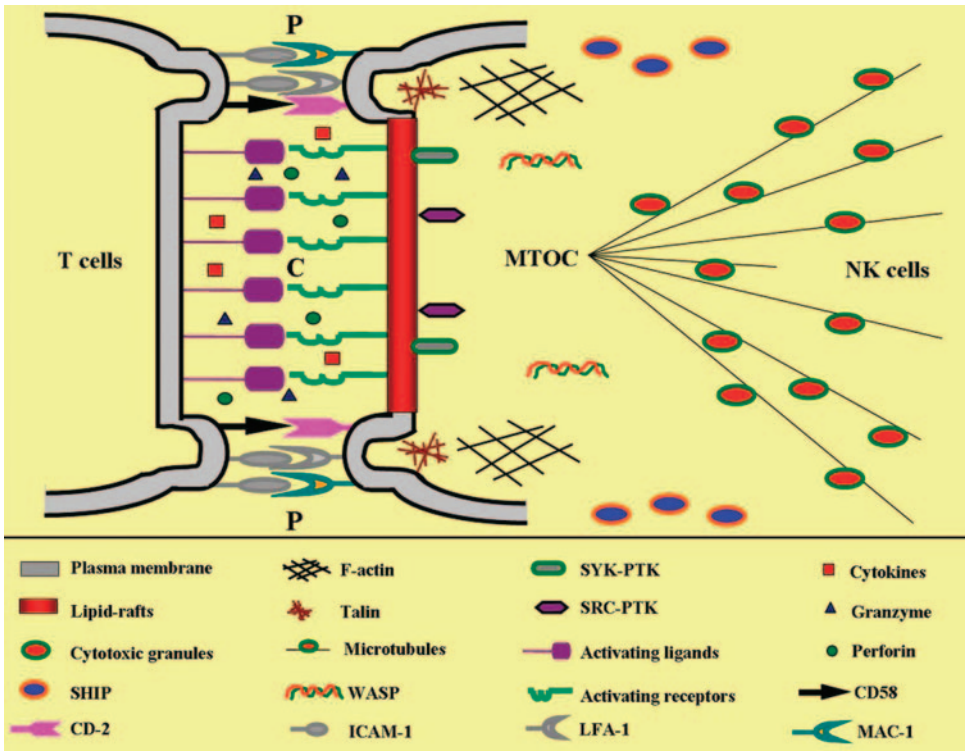

Fig. 1. Formation of IS between NK and target cells. Various NKRs interact with their cognate ligands on the target cell in the center of the synapse, which contains lipid rafts and activating NKRs. The adhesion molecules [CD2, LFA-1, membrane-activated complex 1 (MAC-1)] bind to their cognate ligands on the target cell and aggregate in the form of a ring. Activation of integrins leads to talin-mediated actin polymerization. The close contact between NK and target cell membranes induces reorientation of the microtubule-organizing center (MTOC) of the NK cell toward the synapse. The microtubule-associated motor proteins (kinesins) shuttle granules containing cytotoxic mediators toward the center of the synapse. Within the center of the synapse, different activating NKRs interact with their cognate ligands on the target cells. A ring of F actin surrounds the center of the synapse, preventing the spillover of the cytotoxic mediators from the synapse. T and NK indicate target cell and NK cell, respectively. C and P represent center and periphery of the synapse. WASP, Wiskott-Aldrich syndrome protein; PTK, protein tyrosine kinase; SYK, spleen tyrosine kinase; SRC, sarcoma tyrosine kinase.

after binding to their specific ligands. Each NK cell expresses inhibitory and stimulatory NKRs. Target cells may vary from one another with respect to the level of expression of ligands for different NKRs. According to the prevalent wisdom, triggering of an NK cell depends on the balance between inhibitory and stimulatory signals that it receives from the target cell via NKR (see **Fig. 2**). A NK-sensitive target cell usually would express one or more ligands for activating NKR, whereas a NK-resistant target cell would express more ligands for inhibitory receptors and/or may lack ligands for activating NKR. The human NKR can be divided into MHC and non-MHC-binding receptors.

MHC-binding receptors

They include KIR, NKG2, and Ig-like transcript (ILT) families. They are discussed below.

KIR (CD158) family

The KIR are type I integral membrane glycoproteins that are usually expressed as monomers on the cell surface [51–53]. At present, 14 distinct *KIR* genes and two pseudogenes have been described (see http://www.ebi.ac.uk/ipd/kir for an update on *KIR* genes and alleles). They are located on human chromosome 19q13.4 in a tandem head-to-tail manner in a short, 150-kb region, called leukocyte receptor complex (LRC). *KIR* genes show extensive allelic polymorphism. For example, *KIR2DL1* and *KIR3DL1* genes have at least 14 and 47 alleles, respectively. Their transcripts also undergo alternate splicing, giving rise to distinct receptor variants. It has been estimated that after MHC, KIR is the most polymorphic locus in humans. *KIR* genes are not present in mice, suggesting their recent evolution after divergence of the two species 5 million years ago. They are undergoing rapid evolution in humans under pressure from pathogens, malignancy, and autoimmunity. Two human populations living next to each other and having similar *HLA* genes frequently differ with respect to their *KIR* genes. This observation suggests that the latter genes are evolving faster than the former ones.

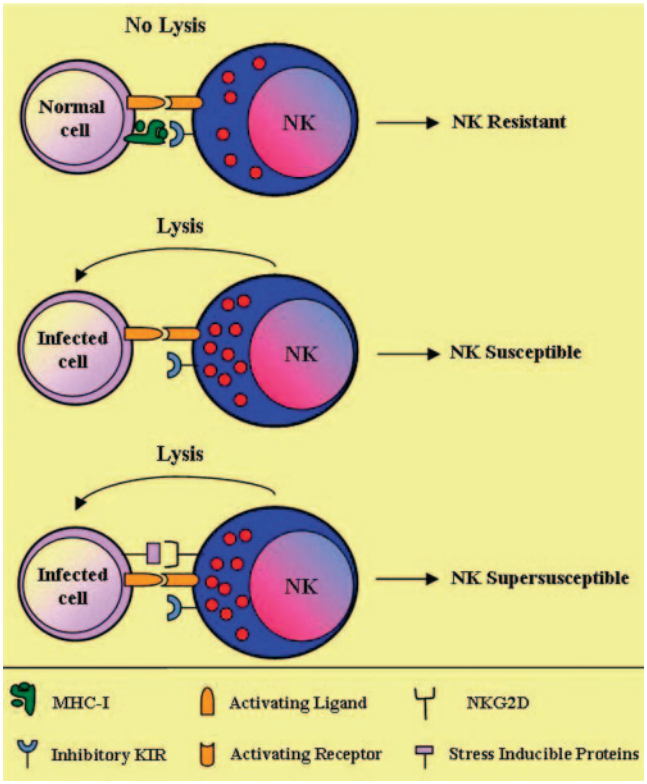

Fig. 2. Model for NK cell-mediated killing of virus-infected cells. Normal cells express ligands (usually MHC class I molecules) for inhibitory receptors of NK cells and are resistant to NK cell-mediated killing (top panel). Viral infections may reduce the expression of these inhibitory ligands on the infected cells and make them susceptible to killing by NK cells (middle panel). They may further induce expression of ligands for activating NKRs (e.g., for NKG2D) and make them super-susceptible to the killing (bottom panel).

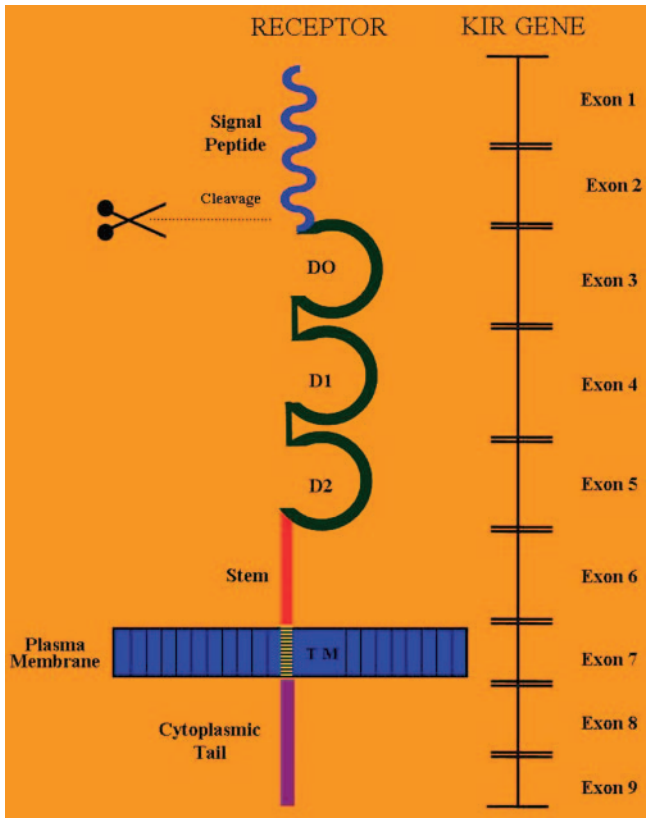

Fig. 3. Structure of a typical KIR gene and its encoded receptor. A typical KIR gene comprises nine exons shown here on the right side of the figure. Double horizontal lines in the gene indicate introns. The schematic structure of the encoded receptor is shown on the left. The part of the receptor encoded by each individual exon is also indicated. The scissor in the figure indicates cleavage site for the signal peptide. The letters N and C designate N- and C-terminals of the protein, respectively; not drawn to the scale. D0, -1, -2, Extracellular Ig-like domains; TM, transmembrane region.

KIR structure. A typical *KIR* gene contains nine exons as illustrated in **Figure 3**. The exons encode leader sequence (exons 1 and 2), extracellular Ig-like domains (D0, D1, and D2; exons 3–5), stem (exon 6), transmembrane region (exon 7), and cytoplasmic tail (exons 8 and 9) of the KIR. The two-domain KIR lack an extracellular domain (D0 or D1). The ones lacking D0 (KIR2L1, KIR2DL2/3) are called type I KIR, whereas the ones lacking D1 (KIR2DL4 and KIR2DL5; see below) are

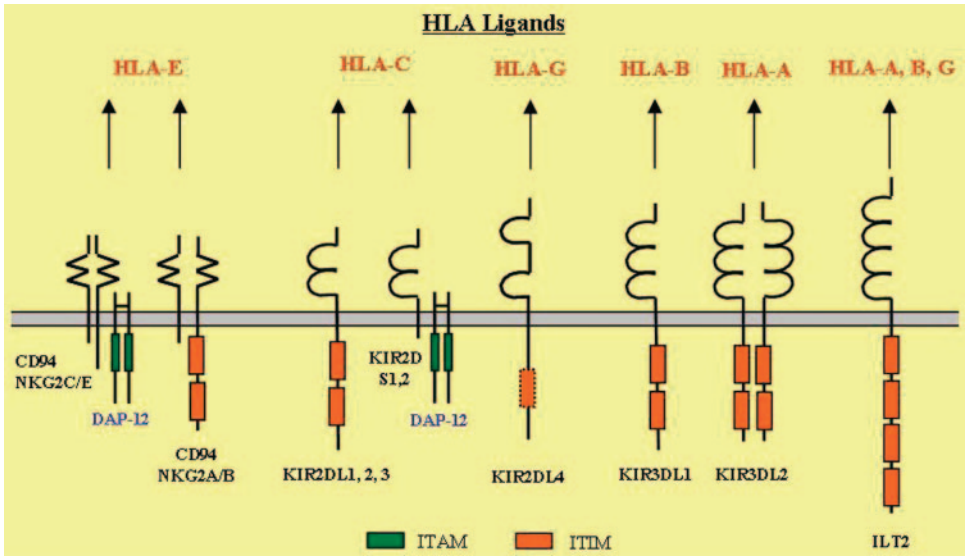

Fig. 4. MHC-binding NKRs. The figure shows the schematic structures of main MHC-binding receptors: KIR, ILT-2, and CD94/NKG2. The ligands for the receptors are also shown on the top of each receptor; not drawn to the scale. DAP-12, Dynax activation protein 12.

called type II KIR. The structure of a typical *KIR* gene along with the receptor is shown in Figure 3. The extracellular region of the receptor binds with its ligand and consists of two or three Ig-like domains. The cytoplasmic tail transduces receptor-initiated signals. Depending on the length of the cytoplasmic tail, a KIR could be short-tailed (S) or long-tailed (L). The L forms are usually inhibitory KIR and have two ITIMs (with canonical sequence I/VxYxxL) in their cytoplasmic tails, as depicted in **Figure 4**. Upon binding to their ligands, the tyrosine residues in the ITIMs become phosphorylated and recruit SHP-1 and -2. These phosphatases dephosphorylate several substrates involved in the NK cell activation cascade, e.g., Vav, ζ-associated protein 70 (Zap-70), Syk, phospholipase C (PLC)-γ1, Shc, and SH2 domain-containing leukocyte protein of 76 kDa, and inhibit the NK cell from triggering its cytotylic machinery and secreting cytokines. The receptors with a short (S) cytoplasmic tail are stimulatory. They lack ITIMs but possess a positively charged amino acid (lysine) in their transmembrane regions. Via this amino acid, they associate noncovalently with a dimer of an adaptor protein, killer cell-activating receptor-associated protein (KARAP) or DAP-12 [54]. Each DAP-12 carries ITAMs [with canonical sequence D/Ex(0–2)YxxL/Ix(6–8)YxxL/I] in its cytoplasmic tail. When an activating KIR binds to its ligand, the tyrosine residues in the ITAMs are phosphorylated and recruit various tyrosine kinases, e.g., Syk and Zap-70, and send activating signals to NK cells to kill target cells and secrete cytokines. The signals converge to phosphorylate Vav, which is a guanine nucleotide exchange factor (GEF) and activates the Rho family of small GTPases. Upon activation, the GTPases cause actin polymerization, cytoskeleton rearrangement, and triggerring of degranulation (reviewed in ref. [45]).

The KIR are named, depending on the number of Ig-like domains (2 or 3) present in the extracellular region as well as the length of the cytoplasmic tail (L or S). Each *KIR* gene and its alleles are given different numbers. The KIR expressed in humans, their signaling partners, and ligands are given in **Table 5**.

TABLE 5. Human KIR (CD158) and Their Ligands

Receptor	Signaling partner	Distribution	Ligand
A. Inhibitory KIR			
1. KIR2DL1 (p58.1)	SHP-1, 2	NK, CTL	HLA-C II
2. KIR2DL2/3 (p58.2)	SHP-1, 2	NK, CTL	HLA-C I
3. KIR3DL1 (p70; NKB1)	SHP-1, 2	NK, CTL	HLA-B Bw4
4. KIR3DL2 (p140)	SHP-1, 2	NK, CTL	HLA-A3, A11
5. KIR2DL4[a]	FcεR1γ-chain	NK	HLA-G
6. KIR2DL5	SHP-2	NK, CTL	HLA-G?
B. Activating KIR			
1. KIR2DS1 (p50.1)	DAP-12	NK	HLA-C II, ?
2. KIR2DS2 (p50.2)	DAP-12	NK	HLA-C I, ?
3. KIR2DS4 (p50.3)	DAP-12	NK	HLA-Cw4, ?
4. KIR2DS3, 5	DAP-12	NK	?
5. KIR3DS1 (p70)	DAP-12	NK	HLA-Bw4-I, ?

All KIR are expressed clonally on overlapping subsets of NK cells except KIR2DL4, which is expressed on all NK cells. Inhibitory KIR are also expressed on CTL of the effector/memory phenotype. Activating KIR may be expressed on CD4+ T cells in some disease conditions but are rarely expressed on CTL. HLA-Bw4-I designates Bw4 allotypes having isoleucine at position 80. The question mark (?) indicates unknown and/or controversial ligands. [a] Despite having an ITIM-containing, long cytoplasmic tail, it functions as an activating receptor.

Two-domain KIR and their ligands. KIR bind to supertypic public determinants expressed by a subset of closely related HLA or MHC class I antigens. The two-domain receptors (e.g., KIR2DL1, KIR2DL2/3) bind to HLA-C antigens. It is noteworthy that all HLA-C antigens show a dimorphism at position (p)80 in the amino-acid sequences of their α-2 helices: They have asparagine or lysine. The HLA-C with an asparagine at p80 (e.g., HLA-Cw2, -4, -5, -6, -17, and -18) are called group I HLA-C, whereas the ones with a lysine at p80 (e.g., HLA-Cw1, -3, -7, -8, -13, and -14) are called group II HLA-C. The KIR2DL1 binds group II HLA-C, and KIR2DL2 and its allelic variant (KIR2DL3) bind group I HLA-C [53, 55–58] (reviewed in refs. [53, 54]). Thus, each HLA-C allotype is recognized by KIR2DL1 or by KIR2DL2/3. Interestingly, the amino acid at p44 of the protein sequence of a KIR determines its specificity for a given HLA-C group: If it is methionine (as in the case of KIR2DL1), the KIR binds group II HLA-C, and if it is lysine (as in the case of KIR2DL2/3), the KIR binds group I HLA-C [56]. It may be relevant to mention here that these ligand specificities are not absolute: KIR2DL1 and KR2DL2/3 may bind to group I and group II HLA-C ligands, respectively. However, they do so with tenfold lower affinities.

KIR2DL4 (CD158d) is unusual in several respects. First, it binds HLA-G: a nonclassical HLA antigen. Second, it is expressed mainly in endocytic vesicles and binds internalized, soluble HLA-G. Third, the receptor is expressed usually by all human NK cells. Its expression can also be increased by cytokines, e.g., IL-2. Fourthly, the receptor has an ITIM-containing, long cytoplasmic tail (a typical feature of an inhibitory KIR) as well as a charged amino acid (arginine) in its transmembrane region (a feature of an activating KIR). The presence of a charged amino acid in its transmembrane region allows it to associate noncovalently with the signaling adaptor molecule γ chain of the FcεRI. Cross-linking of the receptor with HLA-G-expressing target cells induces secretion of IFN-γ from NK cells but does not trigger cytotoxicity [59–64]. It is noteworthy that HLA-G is expressed in the female reproductive tract, invading placental trophoblasts and thymic epithelial cells. The HLA-G/KIR2DL4 interactions may be important in generating local immune responses against invading pathogens in the female reproductive tract. Uterine NK cells express KIR2DL4, and secretion of IFN-γ from uterine NK cells has been shown to be crucial for vascularization of placenta (reviewed in ref. [65]). KIR2DL4 is expressed at lower levels on uterine NK cells in women undergoing spontaneous, recurrent abortions [32]. Gomez-Lozano et al. [66] have described a multiparous woman lacking *KIR2DL4* in her genome. It is probable that a KIR2DL4-like receptor may have compensated the function of this receptor in this woman.

The *KIR2DL5A* and *KIR2DL5B* were considered as allelic forms of the same gene. However, now, it has become quite obvious that they represent two closely related but distinct genes, which are present in telomeric and centromeric halves of the *KIR* gene cluster, respectively [67]. The KIR haplotypes may have both, none, or one of the two genes. In fact, the *KIR* genes are evolving so rapidly that our conventional ways of distinguishing between alleles and genes are being challenged. *KIR2DL5A* (previously known as *KIR2DL5*001* allele) is expressed, like other clonally expressed *KIR* genes, as a monomer on $CD56^{low}$ NK cells in a variegated manner. It is also expressed on a subset of T cells [68]. Individuals (52–80%) may express this receptor, depending on the population group. *KIR2DL5B* is a hybrid gene that arose from recombination between *KIR2DL5A* and *KIR3DP1* genes. It has three alleles, *KIR2DL5B*002*, **003*, and **004*, of which only **003* is expressed. The *KIR2DL5 A* and *B* genes have 99.58% sequence similarity in their exons. Each KI2DL5 receptor has two ITIMs, of which one is atypical. The receptor has inhibitory function and preferentially recruits SHP-2 [69]. The ligand for KIR2DL5 is not known. However, their extracellular regions are similar to that of KIR2DL4. Therefore, it is believed that KIR2DL5 receptors also bind HLA-G. Interestingly, *KIR2DL5* genes are conserved among primates.

Three-domain KIR and their ligands. Of the three-domain KIR, KIR3DL1 (p70) is expressed as a monomer on the surface of NK cells. It binds to HLA-B and HLA-A allotypes bearing the HLA-Bw4 serospecificity. It is noteworthy that all HLA-B allotypes can be divided into two mutually exclusive Bw4 or Bw6 serospecificities depending on residues 77–83. About one-third of all HLA-B and some HLA-A allotypes are $Bw4^{+}$. The remaining two-thirds of the HLA-B allotypes is $Bw6^{+}$. It may be relevant to mention here that HLA-Bw4 allotypes also show dimorphism at p80 of their amino-acid sequence: They may have isoleucine (HLA-Bw4-I) or threonine (HLA-Bw4-T) at this position. It was demonstrated that KIR3DL1 receptors bind the former HLA-Bw4 allotypes with higher affinity [70, 71]. As mentioned above, the *KIR3DL1* gene exists in 47 allelic forms, encoding 41 distinct allotypes, which differ in their ability to bind their MHC ligands. Interestingly, this binding is dependent on the peptide bound to the peptide-binding groove of the MHC class I ligand [72–74]. Some KIR3DL1 allotypes (*001, *002, *008, *015, *009) are expressed at relatively higher levels on the surface of NK cells, and others (*005, *007) are expressed at lower levels. Epidemiological data from Martin et al. [75] suggest that the highly expressed KIR3DL1 allotypes bind HLA-Bw4-I allotypes with high affinity, as their coinheritance gives the highest protection from AIDS in HIV-infected individuals. Furthermore, it also suggests that the lowly expressed KIR3DL1 allotypes bind with HLA-Bw4-T allotypes better than with HLA-Bw4-I ones. This assumption explains the better protection provided by these KIR allotypes, when they are coinherited with HLA-Bw-T as compared with HLA-Bw4-I allotypes. However, these findings need to be tested by direct-binding assays. One KIR3DL1 allotype (*004) is not expressed on the cell surface and remains intracellular. Still, it appears to be of some functional significance [74–76].

Although *KIR3DS1* encodes an activating KIR, it segregates as an allelic variant of *KIR3DL1*. To date, 12 allelic variants have been described, which encode 10 distinct allotypes of KIR3DS1. It is noteworthy that KIR3DS1 and KIR3DL1 show more than 95% sequence homology in their extracellular domains. Genetic epidemiological data strongly suggest HLA-Bw4-I allotypes as ligands for KIR3DS1 [77]. Nevertheless, KIR3DS1 failed to bind HLA-Bw4 tetramers as well as HLA-Bw4 ligands when expressed in EBV-transformed human cells

[78]. It is likely that the receptor binds to HLA-Bw4 ligands when the latter has bound a certain foreign peptide.

KIR3DL2 (NKAT4; p140) is expressed as homodimers on the surface of NK cells. It binds different HLA-A antigens when complexed with certain peptides derived from the viral protein EBV nuclear antigen 1 (reviewed in ref. [53]). The ligands for other KIR are not yet known (see Table 5).

Affinities of KIR for their ligands. It is important to note that KIR differ in their affinities for their MHC ligands. For example, the KIR2DL1 binds with group II HLA-C with higher affinities than does KIR2DL2 for its respective MHC ligands (group I HLA-C). Furthermore, KIR2DL2 has higher affinity for its ligands than its allelic variant KIR2DL3. Similarly different KIR3DL1 allotypes bind to HLA-Bw4 allotypes with different affinities. These differences in affinities of different KIR for their respective MHC ligands are important from the functional point of view as they translate into different levels of inhibition of the NK cells. The differences in affinities are more pronounced between activating and inhibitory KIR.

The S KIR have activating functions. They may represent an allelic variant of an inhibitory *KIR* gene (e.g., *KIR3DS1* is an allelic form of the *KIR3DL1*) or may represent a distinct activating KIR gene (e.g., *KIR2DS4*). It is believed that activating KIR bind the same HLA antigens as do their inhibitory counterparts but with several orders of magnitude lower affinities. In fact, many authors believe that these receptors may bind some unknown ligands expressed by human pathogens, malignant cells, and/or they may bind their cognate MHC ligands that have bound foreign pathogen-derived peptides [52, 53, 79]. It is noteworthy that KIR2DS4 was reported to bind an unknown ligand present on the surface of melanoma cells [80]. The idea is supported further by the fact that certain activating forms of LY49, which are functional homologues of KIR expressed on murine NK cells, bind certain viral proteins. For example, an activating receptor LY49H, which is present on NK cells in C57BL/6 mice, binds a murine CMV (MCMV)-encoded glycoprotein m157 and protects the host from the virus [81]. The virus-susceptible mouse strain 129/SvJ lacks this receptor and instead expresses an inhibitory receptor LY49I for the viral glycoprotein on NK cells. The viral glycoprotein m157 is an MHC class I homologue encoded by MCMV to evade the host's NK cell responses [1]. Another activating receptor LY49P recognizes MHC class I antigens ($H\text{-}2D^K$) in mice when bound with a viral peptide [82].

A functional consequence of the different affinities of the inhibitory and activating KIR for their MHC ligands is that under physiological conditions, inhibitory KIR act as dominant-negative regulators for NK cell functions. They are the main receptors that regulate NK cell functions in humans and maintain tolerance of NK cells toward one's own cells.

KIR-binding and MHC-bound peptides. The binding of two-domain KIR to their MHC ligands is sensitive to the nature of the bound peptide. Certain amino-acid side-chains at p7 and p8 of the peptide may interfere with the binding [83]. Otherwise, these KIR do not distinguish between self and nonself peptides bound to their MHC ligands. However, the three-domain KIR are sensitive to the peptide bound to Bw4. Usually, they can bind to their MHC class I ligands complexed with endogenous peptides. This prevents NK cells from killing the body's own cells. The binding of a foreign peptide to their MHC ligands may abrogate their binding with these KIR. This may result in loss of the KIR-imposed inhibition on the NK cell. For example, the binding of a neomycin-derived peptide to HLA-B27 abrogates its recognition by KIR3DL1 [84]. Neomycin-expressing cells are no longer recognized by NK cells expressing this KIR and hence, may be killed. Such interference with KIR recognition may have consequences for the cell's susceptibility to NK cell-mediated killing. In this respect, the three-domain KIR behave as TCRs as far as recognition of HLA molecules is concerned. However, the consequences of the recognition are quite opposite to each other: T cells recognize HLA via TCR and kill target cells, whereas NK cells recognize HLA via KIR3DL1 and spare them from killing. As three-domain KIR may recognize several HLA ligands in association with one or more pathogen-derived peptides, they essentially remain pattern-recognizing molecules, and TCRs recognize only a particular HLA in association with a well-defined, single foreign peptide and are antigen-specific.

CD94/NKG2C killer lectin-like receptor (KLR)-C (NKG2/CD94 family)

They are also known as the NKG2/CD94 family of receptors. The receptors of this family are type II, C-type, lectin-like integral membrane glycoproteins. As shown in Figure 4, they are expressed on the cell surface as heterodimers with CD94 (NKp43; KLR-D1), which is also a type II, C-type, lectin-like polypeptide. CD94 lacks a cytoplasmic tail and cannot transduce signals. However, it is essential for the cell surface expression of NKG2 receptors. Members of this family as well as other non-KIR human NKR are given in **Table 6**. There are four receptors in the family: A/B (KLR-C1), C (KLR-C2), E/H (KLR-C3), and F (KLR-C4). B and H represent splice variants of A and E genes, respectively [52, 54, 85]. The genes for these receptors are located on human chromosome 12p12.3–p13.2 in a region called NK gene complex (NGC). Of these receptors, CD94/NKG2A has inhibitory function, and it carries two ITIMs in its long cytoplasmic tail. It is expressed in a subset of human NK cells having the $CD56^{high}$ $CD16^{low}$ phenotype. It is also expressed, albeit at lower levels, on the $CD56^{low}$ subset of NK cells. NKG2C has a short cytoplasmic tail, associates noncovalently with a homodimer of DAP-12, and activates NK cells upon binding with its ligands. NKG2E is also considered an activating receptor. It has a charged amino acid (lysine) in its transmembrane region, but it does not associate with DAP-12. The NKG2A and NKG2C are expressed on overlapping subsets of $CD56^+$ NK cells [54]. NKG2F has a truncated extracellular domain comprising only 12 amino acids. It has a charged amino acid (lysine) in the transmembrane region and two ITIMs in its cytoplasmic tail. The protein is retained intracellularly. It does not form heterodimers with CD94 but can complex with DAP-12 [86]. Thus, it may sequester DAP-12 and regulate functional activities of other receptors that use DAP-12 as a signaling partner. Interestingly, DAP-12 has also been implicated in myelination and bone resorption (reviewed in ref. [87]).

The NKG2 receptors bind HLA-E: a nonclassical MHC class Ib molecule [88–90]. HLA-E is expressed in two isoforms

TABLE 6. Non-KIR NKRs

Receptor	Signaling partner	Distribution	Ligand (function)
1. NKp46	FcεR1γ, CD3ζ chain	NK	HA (+)
2. NKp44	DAP-12	Activated NK	HA (+)
3. NKp30	FcεR1γ, CD3ζ chain	NK	? (+)
4. FcγRIIIa (CD16)	FcεR1γ, CD3ζ chain	NK (s)	IgG, IgE (+)
5. FcγRIIc (CD32)	FcεR1γ, CD3ζ chain	NK (s)	IgG (+)
5. 2B4 (CD244)	SAP, EAT-2, SHIP-1	NK (s), T (s)	CD48 (+/–)
6. NKG2D	DAP-10	NK, CTL, Mac	SIP (+)
7. DNAM-1	Fyn	NK (s)	Nectin-2, PVR (+)
8. TACTILE (CD96)	Fyn	NK (s)	Nectin-2, PVR (+)
9. NKR-P1A	FcεR1γ	NK, NKT, CTL (s)	LLT-1 (+/–)
10. NKG2C/CD94	DAP-12	NK and CTL (s)	HLA-E (+)
11. CD94/NKG2A	SHP-1, 2	NK (s) CTL (s)	HLA-E (–)
12. ILT-2 (CD85j)	SHP-1, 2	NK (s) B, Mac, DC	HLA-G (–)
13. KLR-G1	SHP-1, 2	NK (s), CTL (s)	Cadherens (–)
14. SIGLEC-7	SHP-1, 2	NK	Sialic acid (–)
15. NKp80	?	NK	AICL (+)
16. FcRL6	SHP-2	NK	IgG complexes (–)
17. CD160 (BY55)	PI-3K	NK (s)	HLA-C. G (+)
18. CEACAM-1 (CD66a)	CD16?	NK	CEA and related proteins (+)
19. CD38	CD16	NK	CD31 (+)

AICL, Activation-induced C-type lectin expressed on monocyte/macrophages; CEA, carcinoembryionic antigen; CEACAM-1, CEA-related cell adhesion molecule; EAT-2, Ewing's sarcoma-activated transcript-2; FcRL6, FcR-like protein 6; SAP, signaling lymphocyte-activating molecule (SLAM)-associated protein; SIP, stress-induced proteins [MICA, MICB, UL16-binding protein (ULBP)]; PVR, Poliovirus receptor; DNAM-1, DNAX accessory molecule 1; TACTILE, T cell-activated increased late expression; SIGLEC-7, sialic acid-binding Ig-like lectin 7. The designations (+), (–), and (+/–) indicate that the function is activation, inhibition, and both, respectively. (s) indicates that expression is restricted to a subset of the cell population.

in humans, and both bind equally well to these receptors. HLA-E binds a peptide, VMAPRTVLL, which is present in the signal sequences of many classical MHC class I molecules (HLA-A, -B, -C), and a nonclassical MHC class I molecule HLA-G [89, 91, 92]. Without this peptide, HLA-E does not fold properly and therefore, is not expressed on the cell surface. Thus, NK cells have developed an elegant way to monitor the overall expression of MHC antigens on the surface of target cells by CD94/NKG2-mediated monitoring of HLA-E. Interestingly, human CMV (HCMV) encodes a glycoprotein UL40, which increases the expression of HLA-E on the surface of the virus-infected cells by providing this peptide and hence, increases their resistance to killing by NK cells [93]. HLA-E is normally expressed as well as released by placental trophoblasts, vascular endothelial cells, and peripheral blood cells (monocytes, DC, and T, B, and NK cells). Its expression is increased on human cells by certain cytokines, e.g., IFN-γ, TNF-α, and IL-1β [94]. The expression of HLA-E on human cells may protect them from NKG2A-bearing NK cells, and soluble HLA-E may interfere with this protection.

The KLR family NKRs have nonclassical, lectin-like domains. Therefore, they can bind nonsugar moieties on their ligands [95] (reviewed in ref. [96]). It has been demonstrated in vitro that NKG2A can bind efficiently to HLA-E produced in bacteria.

ILT (CD85) family

The family has also been given other names: leukocyte Ig-like receptor (LILR) and macrophage Ig-like receptors. It comprises 13 members. They vary in the number of Ig-like domains present in their extracellular regions and may be inhibitory or activating as in the case of KIR (reviewed in refs. [52, 54]). They are mostly expressed on monocytes, macrophages, DC, and certain subsets of B and T cells. One member of the family ILT-2 (LILRB1; CD85j) is also expressed on a subset of NK cells (**Fig. 5** and Table 6). The receptor has four Ig-like domains in its extracellular region and four ITIMs in its cytoplasmic tail. ILTs bind classical and nonclassical HLA molecules (e.g., HLA-G). ILT-2 preferentially binds HLA-G [97]. It interacts with the α3 domain of the MHC class I molecules and competes with CD8 for binding to the MHC ligand [98]. *ILT* genes are present on chromosome 19 close to the KIR gene cluster.

CD160 (BY55)

It is a GPI-anchored, Ig-like molecule expressed on the $CD56^{dim}$ subset of NK cells, γδ TCR-positive T cells, and a subset of $CD8^+$ T lymphocytes. It binds HLA-C, -G and other HLA molecules. NK cell stimulation via CD160 leads to secretion of a unique, proinflammatory profile of cytokines: IFN-γ, TNF-α, and IL-6. A soluble form of the molecule, shed from activated human NK cells, can inhibit functions of $CD160^+$ NK and T cells [99]. CD160-positive NK and CTL are usually implicated in inflammatory conditions.

Non-MHC-binding receptors

NK cells also express several types of receptors, which recognize different molecular structures (but not MHC antigens) present on the surface of target cells. These receptors are as below.

NKG2D receptor (KLR-K1; CD314)

Originally, this receptor was placed in the CD94/NKG2 family. However, it differs from members of this family in many

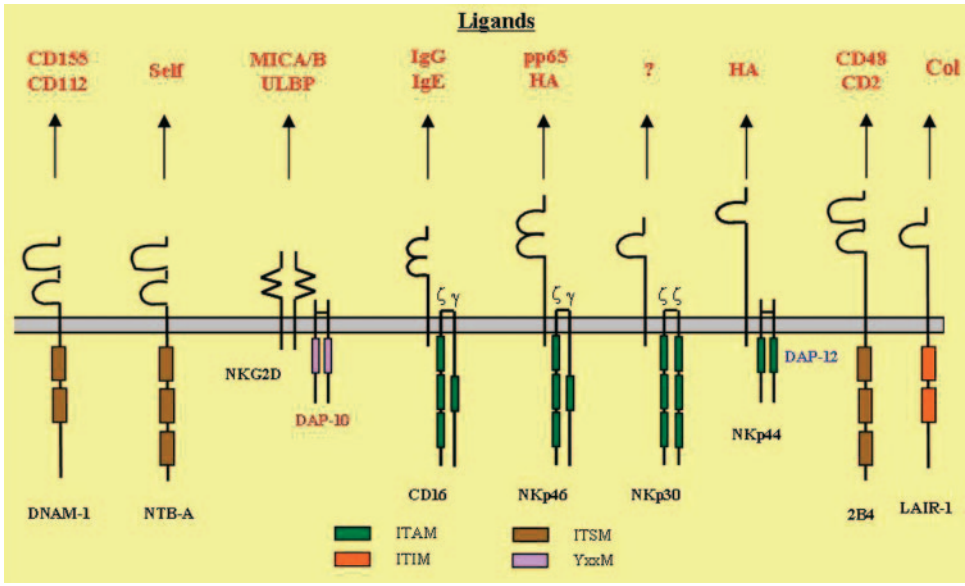

Fig. 5. Non-MHC-binding NKRs. The receptors, their ligands, and signaling partners are shown. ITSM, Immunoreceptor tyrosine-based switch motif; Col, collagens. The YxxM motif, when phosphorylated, recruits PI-3K; not drawn to the scale. The question mark (?) indicates that the ligand is unknown. NTB-A, NK-T-B (NK, T-B cell antigen) antigen; LAIR-1, leukocyte-associated Ig-like receptors.

respects. It does not form heterodimers with CD94 (see Fig. 5 and Table 6). It is expressed as homodimers, and each homodimer associates noncovalently with a homodimer of an adaptor protein DAP-10, which is a DAP-12-related protein but does not contain ITAM motifs in its cytoplasmic tails [100]. Instead, DAP-10 carries a YxxM motif, which upon phosphorylation of its tyrosine residues, can recruit the regulatory subunit p85 of PI-3K and growth-factor receptor-bound protein 2. This motif is also present in the cytoplasmic tail of the T cell costimulatory molecule CD28. However, there are important differences between CD28- and NKG2D-transduced signals. The engagement of NKG2D alone, but not of CD28, on NK as well as on T cells allows formation of an IS with target cells [101].

The NKG2D receptors do not recognize and bind HLA-E, as do all members of the CD94/NKG2 family. Instead, they bind MICA, MICB, and the HCMV ULBPs [102]. *MIC* genes are located on human chromosome 6q25 outside the MHC locus. Of the six distinct *MIC* genes, only *MICA* and *MICB* are transcribed. Structurally, MIC proteins resemble MHC class I heavy chain, and each has three (α1, α2, and α3) domains; however, they do not bind antigenic peptides and do not complex with β-2 microglobulin. The *MIC* genes are highly polymorphic. At least 54 *MICA* and 19 *MICB* alleles have been described. The MIC allotypes vary in their ability to bind NKG2D (reviewed in ref. [103]). For example, MICA*01 and 07 allotypes bind strongly, and MICA*016 binds NKG2D weakly. Some allotypes such as MICA*08 and *010 are defective and are not expressed on the cell surface. Thus, an individual's capacity to mediate NKG2D-mediated killing may depend on his/her inherited *MIC* genes. Under physiological conditions, the expression of MICA and MICB is restricted to the basolateral surface of intestinal epithelial cells and fibroblasts. However, they can be induced de novo on different host cells by stress, transformation, and viral infections. More specifically, DNA damage response triggered by stalled DNA replication, genotoxic drugs, irradiation, and hydroxyurea induces expression of MIC and other NKG2D ligands. The response is accompanied by activation of ataxia telangiectasia mutated (ATM) and ATM and Rad-3-related (ATR) kinases. The stimuli, which do not result in the induction of DNA damage response and activation of ATM and ATR (e.g., heat shock, hypoxia, inflammation, TNF-α, or IL-8), do not induce expression of NKG2D ligands [104–107]. MIC proteins may be cleaved via matrix metalloproteinases and shed from the cell surface as soluble proteins, which bind to and down-regulate the expression of NKG2D on NK cells. Tumor cells usually shed these proteins as an immune evasion mechanism [108, 109].

The ULBPs were first discovered by their ability to bind UL-16 of HCMV and appear to have ubiquitous expression at the mRNA level. Five distinct ULBPs (1–5) have been identified. Of these, ULBP3 and -4 do not bind UL16. Structurally, they resemble MIC proteins but lack α-3 domains and could be GPI-anchored (ULBP1, -2, and -3) or have transmembrane regions (ULBP4 and -5; reviewed in refs. [110, 111]). In the mouse, NKG2D recognizes H-60 (a minor histocompatibility antigen), the retinoic acid early inducible protein 1 (Rae-1), and a murine ULBP-like transcript 1 (reviewed in ref. [96]).

NKG2D is an activating NKR. In addition to NK cells, resting and activated human $CD8^+$ T lymphocytes express NKG2D [112]. Both of these cell types can efficiently kill NKG2D ligand-positive target cells. The $CD8^+$ T cells do so without their TCR coengagement [113]. Certain cytokines, e.g., IL-12 and IL-15, can increase expression of NKG2D on these cytotoxic cells, whereas cytokines such as TGF-β and IL-10 can decrease their expression. The cytokine-induced, increased expression of NKG2D on CTL greatly enhances their NK cell-like killing capabilities (reviewed in ref. [111]). NK cells may play an anti-inflammatory role by killing overtly activated macrophages, which start expressing ligands for NKG2D [17]. It is noteworthy that stimulation of macrophages via TLR induces NKG2DL in macrophages [114]. On the other hand, certain cytokines such as IL-1β and TNF-α have been shown to induce MIC and ULBP expression on oligodendrocytes and neurons. NKG2D-mediated killing of oligodendro-

cytes has been shown to play a role in the development of multiple sclerosis in humans [115].

Natural cytotoxicity receptors (NCRs)

Three NKRs, NKp46 (CD335), NKp30 (CD337), and NKp44, are called NCRs (Table 6 and Fig. 5). They trigger NK cell-mediated killing and secretion of IFN-γ upon their engagement. NKp46 and NKp30 are expressed on resting and activated NK cells, whereas NKp44 is expressed only on cytokine-activated NK cells [116–118]. NCRs belong to the Ig superfamily (IgSF). They have extracellular Ig domains and associate noncovalently with DAP-12 (NKp44), γ-ζ (NKp46), and ζ-ζ chains (NKp30). The ligands for the NCR mostly remain unknown. Two members of the group, NKp46 and NKp44, are known to bind the sialic acid-binding glycoproteins, e.g., HA and HA-neuraminidase, of the influenza and parainfluenza viruses, respectively [40]. α 2,6-Linked sialic acid moieties and the sugar-carrying residue Thr 225 near the membrane proximal region of the receptor play an important role in binding to the ligands [119]. The NCR ligands may also occur on normal hematopoietic cells. For example, NK cells specifically use NKp30 to kill immature DC, suggesting that these cells bear NKp30 ligands. NKp46-positive NK cells protect host from influenza viruses, as the viruses cause lethal infections in NKp46-deficient mice [120].

NKR-P1 (CD161; KLR-B1)

The receptors occur as s–s-bonded homodimers on the cell surface. They were first described in mice as NKR-P1C or NK1.1 antigen occurring on NK cells of C57BL/6 mice. In rodents, five distinct genes (*A*, *B*, *C*, *D*, and *F*) have been described. They encode activating receptors except for *B* and *D* genes, which encode inhibitory versions of the receptor. The humans have *A* gene. Its protein (CD161) is expressed on NK cells, NKT cells, and a fraction of $CD8^+$ T cells. The human CD161 binds LLT-1 and transmits activating signals via the γ-chain of the FcεRI. LLT-1 is usually expressed on monocytes and T, B, and NK cells. IL-2 can induce its expression on NK cells. The receptor differentially regulates NK and T cell functions: It increases TCR-mediated IFN-γ production in T cells but inhibits cytotoxicity and IFN-γ production in NK cells [121]. Another study has shown that the receptor cross-linking induces IFN-γ production but not cytotoxicity in human NK cells [122]. Thus, the receptor may perform dual functions. The occurrence of the receptor and its ligand on NK and T cells suggests that the receptor may interact with its ligand in cis.

SLAM-related receptors (SRRs)

The SLAM (CD150) is expressed in T cells and transmits its signals via an adaptor protein called SLAM-associated protein (SAP; or SH2D1A). The SRRs include 2B4 (CD244), NTB-A (Ly108), and CD2-like receptor on activated cytoxic cells (CRACC; CD139). These receptors are related to SLAM, as they all use similar signaling molecules, SAP, or related molecules (see Table 6). The genes for SRR are located on human chromosome1q22. They are expressed on NK cells, monocytes, basophils, γδ TCR-bearing T cells, and $CD8^+$ T cells of the effector/memory phenotype (Fig. 5). The receptors bear so-called ITSM (TIYxxV/I) in their cytoplasmic tails. These receptors transmit activating signals via SAP, which has a motif in its SH2 domain centered on Arg 78. This motif binds the SH3 domain of the src family kinase Fyn (reviewed in ref. [123]). SAP-Fyn-mediated signaling is important for TH2-type cytokine responses. Interestingly, SAP KO mice lack NKT cells [124]. A distal tyrosine-based motif (TVYxxV/I) in the cytoplasmic tail of the receptor can recruit SHIP, which can dephosphorylate phosphatidyl 3,4,5-triphosphate to phosphatidyl 3,4-biphosphate. Another SAP-like adaptor protein, EAT-2 or SH2D1B, can replace SAP and associates with SRRs. However, EAT-2 cannot recruit Fyn. In the absence of SAP, 2B4 may act as an inhibitory receptor. As a result of this ability of 2B4 to act as an inhibitory as well as an activating receptor, it is recognized as a receptor with dual functionality. Early in ontogeny when NK cells become cytolytic but still have not yet expressed KIR and NKG2A receptors, they do not express SAP. 2B4 acts as an inhibitory receptor and prevents autoaggression from these developing NK cells. It has been demonstrated that EAT-2 and SAP bind 2B4 in resting and activated NK cells, respectively. The ligand for 2B4 is CD48, which is widely expressed on human cells except plasmacytoid DC. Interestingly, 2B4 in mice mainly acts as an inhibitory receptor. 2B4 KO mice show increased cytotoxicity against CD48-expressing target cells. The mouse NK cells also express a EAT-2-related transducer, which like EAT-2, cannot recruit Fyn. The gene for SAP is located on the X chromosome in humans. It is noteworthy that genetic defects in SAP can cause X-linked lymphoproliferative disease and fatal EBV infections in humans [125, 126]. NTB-A, like 2B4, may bind SAP and EAT-2. The two adapters seem to control cytokine production and cytotoxicity in NK cells, respectively [127]. In resting NK cells, CRACC binds EAT-2 but dissociates from it upon activation and recruits PI-3K and PLC-γ. NK cells may interact with macrophages, CTL, and other cells via 2B4/CD48 interactions.

KLR-G1 or mast cell function-associated antigen

It is a type II lectin-like inhibitory receptor expressed as an s–s-bonded dimer on the surface of mast cells. It is also expressed on antigen-specific CTL of the effector/memory phenotype, a subset of $CD56^{dim}$ NK cells, and certain $CD4^+$ T cells in humans as well as in mice [128]. Its cross-linking inhibits cytokine secretion and cytotoxicity but not proliferation. It inhibits IgE-mediated activation of mast cells. The receptors bind ubiquitously expressed endothelial (E), neural, and retinal cadherins or junction proteins [129]. Interestingly, E-cadherins are lost in epithelial tumors undergoing metastasis.

FcRL

They make a growing family of molecules with homology to FcγRI. All FcRL are mainly expressed on B cells; however, one member of the family, FcRL6, is expressed on the surface of NK cells as well as on a subset of $CD8^+$ T cells of the effector-memory phenotypes [130]. Its novel cytoplasmic, cysteine-rich motif can recruit SHP-2 and inhibit cellular functions.

TABLE 7. Human NK Cell Coreceptors

Name	Ligand	Expression	Function
CD2 (LFA-2)	D58 (LFA-3) CD48 (weakly)	All NK	Costimulation, adhesion
LFA-1 (CD11a/CD18)	CD54 (ICAM-1–5)	All NK	Costimulation, adhesion Cytoskeleton rearrangement
CD8	MHC class I	Subset	Costimulation, adhesion
CD69	unknown	Activated NK	Costimulation
CD56 (N-CAM)	self	Subset	Homotypic adhesion
CD59	C8, C9	All NK	Adhesion, costimulation
CD57	unknown	Subset	Marker of senescence
CD28	B-7	Fetal NK	Costimulation
CD27	CD70	Subset	Costimulation
CD44	Hyaluronic acid	Activated NK	Costimulation, adhesion
VLA-4	Fibronectin, VCAM-1 MAdCAM-1	Subset	Adhesion, diapedesis
VLA-5	Fibronectin	Subset	Adhesion, diapedesis

N-CAM, Neural cell adhesion molecule; VCAM, vascular cell adhesion molecule; MAdCAM, mucosal adressin cell adhesion molecule.

NKp80 (KLR-F1)

It is a type II lectin-like molecule expressed as s–s-bonded homodimers on the surface of NK and $CD3^+CD56^+$ T cells. The cytoplasmic tail has two E/KxYxxL/T tyrosine-based motifs. It is expressed on NCR^{dull} and NCR^{bright} NK cells. The receptor binds AICL, which is a myeloid-specific activating receptor [131]. Its gene is located in the NGC close to that of CD69.

DNAM-1 (CD226)

The receptor belongs to the IgSF. It is expressed on the surface of NK, T, and a subset of B cells in physical association with LFA-1. Its gene is located on human chromosome 18q22.3 (Fig. 5). Its ligands include PVR (CD155), Nectin-2 (CD112), and nectin-like molecules, which are widely expressed on a variety of cells, e.g., endothelial, epithelial, and neuronal cells and fibroblasts [113]. The receptor is implicated in transendothelial migration, diapedesis, costimulation, and adhesion. A receptor closely related to DNAM-1 is CD96 (TACTILE). It bears 20% homology with DNAM-1 and also binds to nectins and nectin-like molecules.

Four Ig-like B7 homologues (4IgB7H or B7H)

These receptors belong to the B7 family and are expressed on NK and T cells. They may activate or inhibit the cell. One member of the family, B7-H1 [also called programmed death (PD) ligand-1], is expressed on NK cells. Its expression is increased by certain chemokines (e.g., CXCL-9, -10, and -11) on NK cells [132]. The receptor is also expressed and/or can be induced on several cell types in the body. The receptor interacts with its ligand PD-1 and causes premature activation of naïve T cells and inhibition/apoptosis of antigen-specific effector cells. It may be relevant to mention here that increased expression of PD-1 on HIV-specific T cells has been implicated in immunodeficiency in HIV-infected persons [133].

SIGLEC-7 [p75; adhesion receptor molecule-1; (AIRM-1); CD328]

SIGLECs are a family of sialic acid-binding Ig-like lectins, which belong to the superfamily of sialoadhesion proteins. Of these molecules, SIGLEC-7 (or AIRM-1) is expressed on the surface of human NK cells. Two other molecules, SIGLEC-9 (CD329) and SIGLEC-3 (CD33), are expressed relatively weakly on human NK cells. The SIGLECs transduce inhibitory signals upon binding with sialic acid moieties.

CEACAM-1 (CD66a)

It binds CEA and CEA-related antigens, which are expressed on tumor cells. The binding inhibits NK cell-mediated functions [134]. One of its ligands is PECAM-1 (CD31), which is expressed on vacscular endothelial cells as well as on NK cells. Cross-linking CD31 on NK cells activates LFA-1 [135].

LAIR

Two members of the family, LAIR-1 (CD305) and LAIR–2 (CD306), have been described [52, 54]. They are ubiquitously expressed on all leukocytes including NK cells. LAIR-1 has one Ig-like domain in its extracellular region and one ITIM in its cytoplasmic tail. The receptors bind collagens, which are abundantly expressed proteins in the body. Collagens are not present in blood, so leukocytes are only exposed to the LAIR ligands when they extravasate blood and enter tissues.

NK CELL CORECEPTORS

NK cells express several coreceptors, which bind to their cognate ligands on target cells and send costimulatory signals. These signals add to the overall strength of the activating signal. Adhesion molecules, which include integrins, selectins, and Ig-like molecules, usually act as coreceptors for NK cells (listed in **Table 7**). The distinction between NKRs and coreceptors is not very clear. Some molecules considered as coreceptors (e.g., LFA-1) may trigger NK cell-mediated lysis under appropriate conditions and hence, may qualify as activating receptors.

Integrins are heterodimers of α and β chains and may be of β1 and β2 types. LFA-1 is a β2 integrin (α_L:β2; CD11a/CD18), which is expressed by NK cells and other leukocytes. It is involved in essential early steps in NK cell-mediated killing [136]. It mediates contact and adhesion of NK cells with

the target cell as well as polarization of cytoskeleton (actin and microtubules) and granules in NK cells toward the target cell [44]. It is noteworthy that certain chemokines and cytokines (such as CCL-5, IL-2, and IL-15) as well as inside-out signaling from certain activating receptors (such as CD31) can induce conformational changes in LFA-1. These changes enhance affinity and avidity of the integrin for its ligands. Other β2 integrins expressed by NK cells include type III complement receptor (CR3; Mac-1; α_M:β2; CD11b/CD18) and CR4 (α_X:β2; CD11c/CD18). The β1 integrins expressed by NK cells include VLA-4 (α4β1; CD49d/CD29) and VLA-5 (α5β1; CD49e/CD29). They were named so because of their late expression in the course of T cell activation. Their ligands are indicated in Table 7. NK cells use these molecules to interact with vascular endothelial cells. These interactions are important for NK cell extravasation and also regulate angiogenesis. The costimulation of NK cells via integrins usually facilitates their cytotoxicity and cytokine secretion. However, coligation of α4:β1 has also been implicated in the inhibition of CD16-mediated killing of NK cells [137].

CD56 is an isoform of the N-CAM and is involved in homotypic cell adhesions. It is an IgSF member. The expression of CD56 on the surface of $CD8^+$ T lymphocytes coincides with their acquisition of cytolytic potential [138]. Twenty percent to 30% human (but not murine) NK cells express CD8 on their surface. This coreceptor stabilizes interaction of NK cells with target cells by binding with MHC [54]. CD69 is an early marker of NK cell activation. Its expression correlates with the cytotoxic potential of NK cells. CD25 represents the IL-2Rα chain. Its expression correlates with the proliferative capacity of NK cells in response to picomolar concentrations of this cytokine. CD27 is a member of the TNFR family. It binds transferin receptor (CD70) on target cells. CD44 binds hyaluronic acid on matrix proteins and facilitates LFA-1-mediated adhesion. CD59 is a GPI-anchored membrane glycoprotein, which physically interacts with NKp30 and NKp46 on the surface of NK cells. It is a complement regulatory protein and protects cells from death as a result of complement activation. It binds to the complement proteins C8a and C9. As a result of its physical association with the NCR, it transduces positive signals upon binding to its ligands and thus, acts as a coreceptor for NK cells.

Finally, resting fetal NK cells but not adult human NK cells express CD28, CD80 (B7.1), and CD86 (B7.2) as coreceptors.

DIVISION OF LABOR AMONG NKRs

It has been demonstrated that no activating NKR alone is able to kill insect (Drosophila) cells expressing its cognate ligands [44]. The engagement of LFA-1 by its ligands expressed on insect cells causes contact and adhesion as well as polarization of NK cell granules and cytoskeleton. Nevertheless, it does not result in degranulation of NK cells. On the other hand, the engagement of CD16 by human IgG expressed on insect cells causes contact and degranulation of NK cells but not polarization of their cytoskeleton. However, a simultaneous engagement of CD16 and LFA-1 results in contact, adhesion, polarization, and degranulation of NK cells resulting in secretion of cytokines and killing of the target cells. Thus, activating signals emanating from these two distinct receptors complement each other in NK cell-mediated killing of target cells. It has been demonstrated that cross-linking of CD2, 2B4, or NKG2D alone by specific mAb results in minor Ca^{++} fluxes in resting human NK cells [44, 137]. These data suggest that there exists a certain degree of division of labor among NK cell-activating receptors. It is noteworthy that different activating receptors and coreceptos may differ from each other in their signaling pathways. Two activating receptors with disparate signaling pathways may complement each other and trigger NK cell-mediated killing. On the other hand, a simultaneous engagement of two or more receptors with similar signaling pathways (e.g., use of ITAMs) may not result in triggering NK cell funtions. They may simply show functional redundancy and not complementarity. Therefore, a combination of at least two activating receptors with disparate signaling pathways must be coengaged by target cells to provoke NK cell-mediated killing. This implies that to be killed by a NK cell, it is compulsory for the target cell to express cognate ligands for at least two distinct activating NKRs. This provides a safety mechanism against autoaggression by NK cells. The reductionist approach of analyzing functions of activating NKRs in insect cells has challenged traditional concepts of NKRs and coreceptors. In the light of these observations, a reappraisal of the role of various NKRs in the process of NK cell-mediated killing is needed.

REGULATION OF NK CELL FUNCTIONS BY NKRs

NK cell-mediated killing of target cells is tightly controlled by inhibitory and activating receptors. This control ensures that NK cells do not kill normal, healthy cells of the body and cause tissue destruction (autoimmunity). As mentioned above, KIR and NKG2A are the main NKRs that inhibit NK cell-mediated killing. These receptors bind MHC class I antigens. Therefore, the body's healthy cells, which express normal levels of these self-antigens, are spared from NK cells. The existence of MHC class I antigen-specific inhibitory receptors was in fact predicted by the "missing-self hypothesis" proposed by Kärre's group in the mid-1980s [139]. These workers observed that NK cells preferentially killed tumor cells, which lacked or have reduced expression of MHC class I antigens. They also observed that MHC-positive cells were resistant to killing by NK cells (reviewed in ref. [139]). Their hypothesis was based on these observations. It posited that NK cells sense the expression of MHC class I antigens on the surface of target cells and kill those cells that lack these antigens. The discovery of MHC class I-binding inhibitory receptors (e.g., KIR and LY49 on human and mouse NK cells, respectively) proved validity of their hypothesis. However, NK cells have also been found to express a multitude of activating receptors and coreceptors, which bind to various MHC and non-MHC ligands expressed on the surface of target cells. The discovery of these receptors was a surprise, as their existence was not predicted by the missing-self hypothesis.

The current model of NK cell function is based on the engagement of its inhibitory and activating receptors by their cognate ligands expressed on the surface of target cells. When an NK cell comes in contact with a target cell, inhibitory and activating receptors may bind with their cognate ligands expressed on the surface of the target cell. The balance between inhibitory and activating stimuli received by the NK cell determines whether it will kill or spare the target cell (Fig. 2). Under physiological conditions, inhibitory signals usually remain dominant over activating ones. If for some reason, MHC class I expression is reduced on body cells, it may turn the balance of NK cell-received signals in favor of activating ones (as a result of loss of MHC-mediated inhibition of NK cells) and may make these cells susceptible to NK cell-mediated killing. Viral infections and malignancy may cause a reduction in the expression of MHC class I antigens on the surface of infected cells. That may explain, at least in part, why NK cells can kill virus-infected and cancer cells. However, it may be noted that a MHC-deficient cell is only killed if it expresses ligands for one or more activating NKRs. Furthermore, down-regulation of MHC expression by a target cell is not a prerequisite: It may become killed despite expressing normal levels of MHC antigens if it increases the expression of ligands for one or more activating receptors. Consequently, if target cells express NKG2D ligands de novo, the engagement of KIR may not be able to inhibit NK cell-mediated killing via NKG2D. If target cells express de novo NKG2D ligands and/or increase the expression of ligands for other NK cell-activating receptors as well as down-regulate the expression of MHC antigens, they would become super-susceptible to NK cell-mediated killing. The ligands for the activating NKG2D receptor may be induced on the body cells by viral infections, malignancy, or other stimuli causing genotoxic stress. Thus, NK cells sense not only the "missing or altered self" but also "induced self" to detect hazardous cells and kill them. It is noteworthy that different NK cell clones may vary in their ability to kill a given target cell. This ability depends on the repertoire of its activating and inhibitory receptors as well as its repertoire of signaling and effector molecules (perforin, FasL, etc).

EXPRESSION OF NKRs

The expression of the main NKR genes, KIR, is controlled at the transcriptional level. Once expressed, the expression remains fairly stable. KIR genes are expressed clonally on overlapping subsets of NK cells in a cumulative and stochastic manner, independently of each other. A developing NK cell keeps on expressing its KIR genes at random one after another until it has acquired an overall level of inhibition that is just sufficient to make the cell tolerant to autologous cells. An individual NK cell does not express all KIR genes present in its genome. It expresses only a subset of these genes. This mode of expression makes NK cells of an individual heterogeneous and variegated with respect to the expression of KIR (reviewed in ref. [140]). All clonally expressed *KIR* genes have more or less similar upstream regulatory regions (reviewed in ref. [141]). These regions are characterized by the presence of at least two promoters: a proximal (5′ to ATG codon) and a distal (located 891–1347 bases upstream of the ATG) promoter. The proximal promoter is composite and consists of overlapping and bidirectional promoters, which work in opposite directions. It can produce sense and antisense transcripts. The sense transcripts from the promoter result in splicing and expression of the KIR protein. The antisense transcripts from the proximal promoter join sense transcripts from the distal promoter, make dsRNA, and silence transcription through RNase III-type nucleases (which cleave dsRNA into small, uniform, 21 bp segments) and RNA-induced silencing kinase (reviewed in ref. [142]). The ratio between forward and reverse transcriptional activities of the proximal promoter of a KIR gene determines whether the gene is expressed in an NK cell. The promoter activities involve cell-specific and nonspecific transcription factors. The bidirectional proximal promoters act as probabilistic binary switches for turning on or off transcription of individual KIR genes (reviewed in refs. [141, 143, 144]). The direction of the transcription, once selected, remains fixed for the life of the cell. The presence of reverse transcripts for a given KIR gene from its bidirectional promoter represents lack of expression of the gene. The KIR genes, not expressed in NK cells, are maintained silent, mainly by DNA methylation of their promoter regions. The transcription start sites in KIR genes are surrounded by CpG islands, which are always methylated in nonexpressed genes. Consequently, treatment of NK cells with demethylating agents, e.g., 5-aza-2 deoxycytidine, causes expression of all silenced KIR genes. It was demonstrated that single base-pair mutations in the acute myeloid leukemia-binding sites in the promoter regions of two *KIR2DL5* alleles (*KIR2DL5.2* and *KIR2DL5.4*) result in their transcriptional silence and nonexpression as a protein [145]. It is noteworthy that the KIR2DL4 promoter is quite different from those present in other clonally expressed KIR genes and is not controlled by probabilistic transcriptional switches. This explains why this KIR gene is expressed ubiquitously.

A consequence of the stochastic expression of KIR genes is that each NK cell of an individual has a unique repertoire of expressed KIR. On the average, three to four *KIR* genes (inhibitory and activating ones) are expressed in an individual NK cell [146–148]. At least one of these receptors binds a self-MHC class I ligand and induces tolerance to self. The KIR genotype of an individual determines the repertoire of KIR expressed on his/her NK cells. The HLA genotype of the person affects this repertoire in a subtle way. The percent of expression of a KIR on NK cells of an individual is slightly increased if he/she also expresses an HLA ligand for that KIR [149]. A higher number of copies of an individual KIR allele also enhance its frequency of expression on NK cells. Furthermore, the number of other inhibitory KIR-HLA ligand pairs expressed in an individual also affects the expression of a given KIR on his/her NK cells; the higher the number, the lower the frequency. Therefore, it is not surprising that KIR haplotype identical sibling pairs with different MHC class I haplotypes have significant differences in the frequencies of expression of different KIR genes on their peripheral blood NK cells [150].

KIR and NKG2/CD94 receptors are usually expressed on mutually exclusive subsets of NK cells and complement each other (Table 2). In the course of NK cell development and differentiation, NKG2/CD94 receptors are expressed earlier.

Later in development, these receptors are replaced by KIR. However, a small proportion of NK cells continues to express NKG2/CD94 receptors and does not express KIR. In blood, $CD56^{high}CD16^{low}$ NK cells usually express NKG2/CD94 receptors.

Although an individual NK cell may express three to four KIR genes, rarely does more than one of these receptors bind to a self-MHC antigen. Consequently, each individual NK cell can sense and respond to changes in individual MHC antigens on autologous cells. It would not have to wait until there is a global decrease in the expression of all MHC antigens. A distinct advantage of the clonal and variegated pattern of expression of KIR on NK cells is that different NK cells can sense different MHC class I antigens on target cells. NK cells in an individual may express certain KIR, which may not bind to any HLA antigen expressed by him/her. The individual may not have inherited HLA ligand genes for the receptor. For example, an individual may have a *KIR2DL1* gene but may lack group II HLA-C genes, which encode its ligands. This may happen, as KIR and MHC class I genes are located on two separate chromosomes (12 and 6, respectively) and are assorted independently of each other.

The expression of KIR genes on NK cells usually remains stable and is least affected by cytokines. IL-21 plays a role in the induction of these receptors on developing NK cells from $CD34^+$ progenitor cells in in vitro cultures [151]. The cytokine, however, does not affect KIR gene expression in mature NK cells. In contrast to KIR, cytokines may regulate expression of other NKRs. For example, IL-15, IL-10, and TGF-β1 were shown to induce expression of CD94/NK2GA on developing NK cells as well as on the TCR-stimulated $CD8^+$ T cells. TGF-β1 also reduces expression of NKp30 and NKG2D on NK cells. IL-21 increases the expression of NCR and 2B4 on NK cells. The cytokine, however, decreases expression of NKG2D on NK cells as well as on CTL. Glucocorticoids also decrease NCR expression on NK cells [152–156]. These studies show that changes in cytokine production, which usually accompany viral infections and malignancy, may cause changes in the expression of different receptors on NK cells as well as on T cells. An altered expression of NKR has important implications for the functional activity of NK cells; it may lead to the emergence of autoimmune NK cell clones if inhibitory receptors on NK cells are reduced, and/or activating receptors are overexpressed. NK cells may also become immunodeficient if inhibitory receptors to MHC antigens are overexpressed on them. It has been demonstrated in the mouse model in vitro and in vivo that blocking NK cell inhibitory receptors by small molecular weight inhibitors or by receptor-specific antibodies increases NK cell activity against tumors and results in their regression [157]. The autoimmune cells may kill normal, autologous cells, whereas the immunodeficient ones may not be able to kill otherwise susceptible malignant or virus-infected cells. A dysregulated in vivo expression of KIR genes has been documented to cause immune deficiency in humans [120]. The authors described the case of a person who expressed KIR2DL1 on all of his NK cells. He was immunodeficient and suffered from repeated viral and bacterial infections.

ROLE OF INHIBITORY RECEPTORS IN NK CELL "EDUCATION"

It was proposed that each NK cell in an individual expresses at least one inhibitory receptor (KIR or NKG2/CD94) capable of binding to a self-MHC antigen. This ensures that NK cells do not kill normal, autologous cells. However, it has been demonstrated that some NK cells may not express any inhibitory receptors (KIR or NKG2/CD94) specific to a self-MHC antigen. These cells do not kill autologous target cells and are hyporesponsive [158]. According to one school of thought, these cells have not been "licensed" to kill and therefore, are tolerant of autologous target cells. According to the "licensing" hypothesis, NK cells must express inhibitory receptors specific for self-MHC class I antigens for acquiring killing capacity [159, 160]. This hypothesis implies that these NK cells are somehow immature. Another group of researchers thinks that these NK cells are fully mature and capable of killing but are "disarmed" to kill autologous cells. They argue that as a result of persistent activation of these cells, their signaling pathways become attenuated [161]. The disarmed NK cells express all of the phenotypic markers of fully differentiated, mature NK cells. Upon activation with pharmacological activators, e.g., ionomycin, they can release cytokines such as mature NK cells. In conformity with the "disarming" hypothesis, NK cells from Rae-1 transgenic mice are also hyporesponsive [162]. Furthermore, NK cells are chronically activated in transporter associated with antigen processing 2-deficient humans but are hyporesponsive and tolerant to self. This self-tolerance could be ascribed at least partially to their reduced expression of NKG2D, ζ-chain, and NKp46 [10].

KIR HAPLOTYPES

As mentioned above, all *KIR* genes are tightly packed in a head-to-tail manner in the LRC region (reviewed in refs. [163, 164]). Each KIR gene is ~2.0 kb apart. The order of the genes in the LRC region has been deduced from sequencing of the KIR haplotypes as well from segregation analyses. KIR haplotypes vary in humans with respect to the number of activating and inhibitory genes as well as to their allelic forms. Because of these variations, a large number of KIR haplotypes have been identified. These haplotypes may be classified into two broad types: A and B. The type A haplotypes usually contain five inhibitory KIR genes. They also contain one S or activating KIR gene (*KIR2DS4*). This activating KIR, however, is frequently mutated and encodes a nonfunctional receptor as a result of the presence of a 22-bp deletion in exon 5 of the gene. About 80% of the Caucasians have this deletion [165]. Therefore, type A haplotypes usually do not express a functional, activating KIR. They have an inherent tendency to strongly inhibit NK cells. Type B haplotypes are more diverse and may contain more (up to 14) KIR genes, which may include as many as five activating *KIR* (*KIR2DS1*,- *2*, and *-3*, *KIR2DS5*, and *KIR3DS1* but not *KIR2DS4*). It is noteworthy that all human KIR haplotypes contain *KIR2DL4*, which acts as an activating receptor despite having a long cytoplasmic tail. **Figure 6**

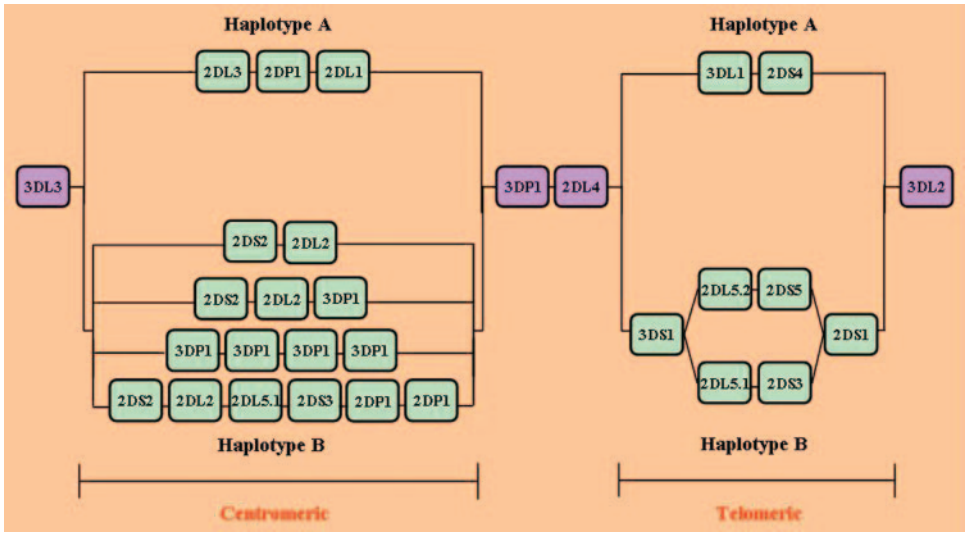

Fig. 6. Schematic representation of KIR haplotypes in humans. A haplotypes contain five inhibitory *KIR* and only one activating *KIR* (*2DS4*), which is frequently mutated. B haplotypes differ from A in having many activating *KIR* genes. The framework genes (*3DL3*, *3DP1*, *2DL4*, and *3DL2*; in violet color) are present in each haplotype. The figure shows KIR genes present in the centromeric and telomeric halves of the frequently found A and B haplotypes above and below the framework genes, respectively. Each box in the figure represents a *KIR* gene. *3DP1* is a pseudogene.

shows the prevalent KIR gene arrangements present in the most common A and B types of KIR haplotypes. Four *KIR* genes *KIR3DL3*, *KIR3DP1*, *KIR2D4*, and *KIR3DL2* are present in all KIR haplotypes. Of these, *KIR3DP1* is a pseudogene. These genes have been termed the "framework genes" [163, 166]. The frequencies of the two haplotypes vary significantly in different human populations. For example, ~60% Japanese, 30% Caucasians, and 2% Australian Aborigines are homozygous for A haplotypes [166, 167]. It has been demonstrated that overall, 12% humans have predominantly inhibitory KIR genotypes (more inhibitory receptors than activating ones), and 36% have predominantly activating ones (more activating than inhibitory receptors). The rest (52%) have neutral KIR genotypes (equal number of activating and inhibitory KIR genotypes). Furthermore, only less than 1% of unrelated humans has the same KIR genes [168]. In a small percentage of people, KIR may be truncated or unusually elongated. These inviduals may lack or may have duplicated copies of certain KIR genes. The KIR genotype of an individual greatly affects his/her NK cell responses to viral infections.

KIR-HLA EPISTATIC INTERACTIONS AND RESISTANCE TO VIRAL INFECTIONS

The KIR bind specific HLA antigens and regulate NK cells functions. The two gene families are located on two different chromosomes in humans. Therefore, they segregate independently of each other in meiosis and may result in unique *KIR/HLA* genotype combinations in humans. These unique *KIR/HLA* combinations translate into unique interactions and control over NK cell functions in humans. The degree of inhibition mediated by interactions between coinherited KIR and MHC class I gene products in an individual determines the activation threshold for his/her NK cells. The differences in the inhibition of NK cells result from different affinities of different KIR for their cognate MHC ligands. Furthermore, individuals may vary in the number of KIR/MHC inhibitory pairs as well as in the number of inherited, activating KIR genes [163, 168]. Apart from playing a direct role in killing virus-infected cells, NK cells effectively regulate several aspects of virus-specific, adaptive immune responses. Moreover, KIR (especially the inhibitory ones) are also expressed on virus-specific $CD8^+$ T cells, and NKR/MHC interactions have been documented to control the effector function of these cells. For this reason, the effects of the KIR/MHC combinations may go beyond NK cells and determine the overall immune efficiency of the individual. For example, if a person X expresses KIR and MHC antigens that interact strongly with each other, he/she would be relatively immunodeficient as compared with person Y, whose KIR and MHC antigens interact relatively weakly with each other. From the point of view of a viral infection, the X may be at a relative disadvantage to mount an effective antiviral immune response and clear the infection as compared with the Y. However, it has also been argued that X individuals may be better than Y individuals in dealing with viral infections as a result of enhanced licensing of their NK cells for killing by high-affinity KIR [75]. This argument cannot explain the experimental findings that the individuals who are homozygous for group I *HLA-C* and *KIR2DL3* can more efficiently clear needle-prick hepatitis C virus (HCV) infections as compared with the individuals who are homozygous for *KIR2DL1* and group II *HLA-C* [79]. As mentioned above, KIR2DL1/group II HLA-C interactions are stronger and mediate tighter inhibition of NK cells as compared with that mediated by the KIR2DL3/group I HLA-C. The implications of coinherited KIR and HLA genotypes are not restricted to resistance to viral infections (reviewed in ref. [51]). The individuals with relatively weakly inhibited NK cells would not only better control viral infections and other intracellular pathogens, but they would also be in a better position to control malignancy. On the other hand, they may be more susceptible to the development of various autoimmune diseases. Indeed, persons suffering from type I diabetes have been reported to carry a lower frequency of inhibitory *KIR/HLA* gene pairs, a higher frequency of activating *KIR* genes, and a higher frequency of KIR2DS2/ligand gene combinations [169, 170]. It is likely that a genetic imbalance between KIR and their ligands may have resulted in increased NK and CTL activities toward pancreatic antigens in these patients. According to this paradigm, the persons susceptible

to developing autoimmune diseases may control viral infections more efficiently. Consistent with this theme, it has been reported that HIV infection progresses more slowly toward AIDS in Zambian individuals suffering from the genetically determined, autoimmune spondylarthritis as compared with the spondylarthritis-free, HIV-infected persons [171]. The frequent presence of HLA-B27 in these patients may be a contributing factor in slowing the progression to AIDS in these patients.

KIR-HLA INTERACTIONS IN RESISTANCE TO HIV INFECTION AND PROGRESSION TO AIDS

Role of Bw4-binding KIR

Several genetic and functional studies have associated certain HLA allotypes with a rapid development of AIDS in HIV-infected persons. For example, the *HLA-B35 Px* allele has been associated with rapid progression toward AIDS, whereas *HLA-B57* and *HLA-B27* alleles have been associated with delayed progression. The HIV-infected persons carrying the protective alleles have slower decline of $CD4^+$ T cell counts, lower viral loads in their blood (viremia), and longer AIDS-free survival. The protective HLA alleles are also over-represented in HIV-infected persons, who can control HIV replication and do not develop AIDS for long periods of time (so-called natural controllers or long-term nonprogressors). The HLA-conferred protection from AIDS has been explained on the basis of the ability of these HLA allotypes to present immunodominant viral epitopes and induce broadly reactive polyclonal antiviral CTL responses [172–175]. It is noteworthy that these protective allotypes are seropositive for the Bw4 determinant. Furthermore, Bw4/Bw4 individuals have been shown to be relatively resistant to the development of AIDS as compared with Bw6/Bw6 individuals [75, 176]. As only Bw4-serospecific HLA allotypes have been shown to bind three-domain KIR, the question arises whether KIR/HLA-Bw4 interactions and NK cells could be involved in the Bw4-mediated resistance to the development of AIDS. Only a few studies have addressed this question and investigated the impact of KIR polymorphism as well as other *HLA/KIR* gene coinheritance on resistance to the development of AIDS. In this regard, Martin et al. [77] conducted investigations in a cohort of more than 1000 North American HIV-infected persons. They reported that an activating allele of the *KIR3DL1* gene, *KIR3DS1*, was associated with delayed progression to AIDS in humans coinheriting the *HLA-Bw4-I* alleles. In the absence of *KIR3DS1*, *HLA-Bw4-I* alleles were not associated with the protection. More surprisingly, *KIR3DS1* alone without its HLA ligand was associated with a relatively rapid progression of the disease. In a subsequent study, the group showed that coinherited *KIR3DS1* and *HLA-Bw4-I* alleles protected HIV-infected persons from viral burden in early stages of the infection and from opportunistic infections in late stages. Their coinheritance, however, afforded no protection from HIV-related malignancies [177]. As mentioned above, KIR3DL1 receptors bind the HLA-Bw4-I allotypes with higher affinity [70, 71]. Therefore, it can be argued that a high-affinity interaction between KIR3DS1 and HLA-Bw4-I allotypes may have resulted in more effective immune responses (through enhanced activation of NK cells and/or of T cells, were KIR3DS1 expressed on CTL in these patients). The problem in this interpretation is that in in vitro studies, although KIR3DL1 allotypes have been shown to bind HLA-Bw4 antigens in a peptide-dependent manner, KIR3DS1 does not bind any Bw4 allotype, even when complexed with the peptides that allow binding of KIR3DL1 allotypes [76, 78]. Alternately, a protein of viral or cellular origin, expressed de novo on the surface of HIV-infected cells, may have acted as a ligand for KIR3DS1. It is also possible that another unknown genetic determinant, which may be in linkage disequilibrium with *KIR3DS1*, may have been responsible for this protection. Furthermore, reasons behind the reported association of *KIR3DS1* with rapid progression of AIDS in the absence of its HLA ligand remain enigmatic. Another group of researchers could not corroborate these results in an ethnically different cohort of HIV-infected persons [178, 179]. They found no protective effect of coinherited *KIR3DS1* and *HLA-Bw4-I* genes on AIDS progression. Instead, they found that inheritance of two linked KIR genes (*KIR2DL2* and *KIR2DS2*; see Fig. 6) posed a greater risk of developing AIDS if their ligands were also present, and the presence of the *KIR2DL3* gene had a moderating effect on this risk. They also found that coinheritence of the genes for Bw6 and group II HLA-C allotypes was associated with rapid progression toward AIDS. The authors concluded that HLA and KIR affect HIV progression independently as well as synergistically. In another study on 88 HIV-infected individuals, it was shown that KIR3DL1 had a protective effect on the development of AIDS when coinherited with HLA-B*57 (an allotype with Ile at p80 [180]). An important drawback of these studies is that they did not characterize the *KIR3DS1* allele in the patients and control subjects. It is noteworthy that a mutant, nonfunctional form of *KIR3DS1* (**049N*) also exists [181, 182]. However, this mutant allele is rare. Martin et al. [181] reported later that their disease association data were not affected when they adjusted them for the presence of this allele. It may be relevant to mention here that KIR3DS1-expressing NK cells as well as T cells expand in viremic, HIV-infected persons [182]. These data suggest strongly that KIR3DS1 expression is relevant to this infection. Further studies are needed to pinpoint its exact role.

Role of KIR3DL1 alleles

It is noteworthy that *KIR3DL1* has 59 alleles, 12 of which encode the activating allotypes (KIR3DS1), and 47 encode inhibitory (KIR3DL1) allotypes. Depending on their level of expression on the cell surface, Martin et al. [75] have divided the inhibitory allotypes into high expressors (*KIR3DL1*001*, **002*, **008*, **015*, **009*), low expressors (*KIR3DL1*005*, **007*), and no expressor (*KIR3DL1*004*). The last one is retained within cells as a result of its truncated, cytoplasmic tail. They presumed that the affinities of these allotypes for their MHC ligands correlated with their expression levels. They further demonstrated that highly expressing *KIR3DL1* alleles were protective when coinherited with *HLA-Bw4-I* alleles as compared with the low-expressing allotypes [75]. This study also showed relative protection afforded by low-expressing *KIR3DL1* alleles when coinherited with *HLA-Bw4-T* as

compared with the individuals homozygous for *Bw6* alleles. More surprisingly, *KIR3DL1*004* also provided protection, despite the fact that this allotype is not expressed on the cell surface. This shows that even an intracellularly retained KIR3DL1 allotype may have functional significance. These results from Martin et al. [75] suggest that the presence of a pair of inhibitory KIR3DL1 and its HLA ligand in HIV-infected persons affords protection from AIDS progression. Furthermore, the strength of the inhibition between the receptor-ligand pair correlates positively with the degree of protection. These results are not congruent with the paradigm that the KIR/HLA combinations that favored NK cell activation provided protection from viral infections and from the development of AIDS in HIV-infected individuals. The authors have argued that if NK cells were more strongly inhibited by KIR3DL1 receptor/ligand pairs, they would be more cytotoxic once they were relieved of their inhibition. The argument derives from the hypothesis that NK cells continue to express inhibitory KIR one after the other until they acquire sufficient inhibition to become self-tolerant. Thus, more tightly inhibited NK cells would be inherently more cytotoxic once their KIR/HLA-mediated inhibition is lost. The inhibition may be lost if the expression of HLA-Bw4 decreases on the surface of the virus-infected cells. Alternately, HLA-Bw4 may present a virus-derived peptide that may make it unrecognizable by the KIR3DL1 receptor. However, this argument cannot explain why coinheritence of *KIR/HLA* gene pairs, which inhibit NK cells rather loosely, or the inheritance of KIR without the genes for their HLA ligands protects humans from HIV and HCV infections [79, 183]. The authors have put forward another argument to explain their findings: In the presence of tightly inhibited NK cells, only a virus-specific, immune response is generated, avoiding a nonspecific, overall activation of the immune system. This response is more effective in suppressing HIV replication. Weakly inhibited NK cells may lead to a generalized activation of the immune response, which may cause immune-mediated pathology. However, there is no experimental evidence to support this argument. On the contrary, enhanced NK cell activation has been shown to induce better and stronger antigen-specific immune responses [184]. Furthermore, it does not explain results from several other studies in which relatively weakly inhibited NK cells (as a result of weak-affinity KIR/HLA interactions) have been shown to provide protection from viral infections as well as from tumors [51, 79, 178]. Even in the case of HIV infection, it has been reported that persons having weakly inhibited NK cells have reduced risk of contracting the infection. The study, conducted in African female sex workers, has shown that the inheritance of inhibitory *KIR* genes was protective from contracting HIV infection when the genes for their cognate MHC ligands were not coinherited. More specifically, *KIR2DL2/3* heterozygotes without group I HLA-C and KIR3DL1 homozygotes without HLA-Bw4 were relatively protected [183]. Furthermore, it has also been reported that the persons with KIR genotypes having more activating KIR genes were also relatively protected [183, 185]. In the case of i.v. drug users, stronger NK cell activities as well as a predominantly activating KIR repertoire (high KIR3DS1/KIR3DL1, NKG2C/NKG2A ratios, low expression of KIR3DL1, coinheritance of weakly inhibiting KIR/MHC pairs, i.e., KIR2DL3/HLA-C of group I) also protect from contracting HIV infection [185, 186]. Taken together, these studies suggest that weakly inhibited NK cells not only may slow progression of HIV infection toward AIDS but also may protect from contracting HIV infection. It is noteworthy that a hierarchy of KIR3DL1 allotypes for HLA-Bw4 binding has been described [149] that differs significantly from the one used by Martin et al. [75].

Concerning the impact of KIR3DS1 and its HLA ligands, Barbour et al. [187] have shown that the two genes affect AIDS progression independently from each other. The researchers analyzed viral load, $CD4^+$ T cell counts, and *KIR3DS1* and *HLA-Bw4* genotypes of a cohort of 255 treatment-naïve, HIV-infected persons during the first 2 years of infection. They found that the *KIR3DS1* and *HLA-Bw4-I* genes had distinct but independent effects on $CD4^+$ T cell counts and viral loads, respectively. They noted that *KIR3DS1*-positive, HIV-infected persons maintained $CD4^+$ T cells counts at higher levels as compared with the *KIR3DS1*-negative persons, irrespective of coinheritence of any *Bw4-I* alleles. The persons possessing *HLA-Bw4-I* alleles maintained lower viral loads all along the 2 years of the study period, irrespective of their *KIR3DS1* status. In the persons having the receptor and the ligand genes, the effects on $CD4^+$ T cell counts and viral load were simply additive and not synergistic. This study suggests a direct relationship between KIR3DS1 expression and $CD4^+$ T cell counts in HIV-infected persons. It may be interesting to investigate potential interactions between KIR3DS1-positive NK cells and $CD4^+$ T cells in humans. More recently, a direct role for an activating KIR, KIR3DS1, has been demonstrated in controlling HIV replication. In in vitro studies, KIR3DS1-positive NK cells inhibited HIV replication in HLA-Bw4-I-positive cell cultures in a contact and dose-dependent manner. The inhibition was significantly more as compared with KIR3DS1-negative NK cells [188]. Collectively, these studies do suggest a role of activated NK cells in controlling HIV infection. The caveat is that uncontrolled activation may contribute to immunopathogenesis.

It is noteworthy that HIV infections in humans have arisen relatively recently. The pathogen and its host have not had sufficient time to coevolve and eliminate deleterious genes from each other. Nevertheless, KIR and MHC antigens have evolved in humans under pressure from infectious agents, malignancy, and autoimmunity over millenia. Therefore, the impact of KIR genes, especially in combination with coinherited HLA genes on the susceptibility to HIV infection and development of AIDS in human populations, should be forthcoming. The studies conducted so far have yielded discordant results. The reasons for these discordant results may include variations in the pathogenicity of HIV viruses, treatment regimens, sample sizes, variable frequency of different genes in human populations, improper statistical models, etc. A part of the problem in formulating a uniform hypothesis regarding the impact of HLA/KIR interactions on the AIDS pathogenesis is the heterogeneous nature of the KIR with respect to their dependence on MHC-bound peptides. The two-domain KIR bind HLA-C and are affected by certain amino-acid side-chains at p7 and p8 of the MHC-bound nonamer peptides [83]. The three-domain KIR bind HLA-A and -B and are relatively

more discriminating between the peptides bound to their MHC ligand Bw4. Their recognition may have implications for antiviral CTL responses. For example, if an individual has HLA-Bw4 alleles, he/she will be expressing KIR3DL1 on a subset of his/her NK cells, which will recognize HLA-Bw4 complexed with endogenous self-peptides and will be tolerant to them. If the individual becomes infected, his/her cells may bind a foreign (antigenic) peptide to its HLA. If the new peptide-bound HLA is recognized by KIR3DL1, the cell still will be protected from NK cells but may be killed by antiviral CTL. If the new HLA-peptide complex is not recognized by KIR3DL1, the cell will no longer be protected from KIR3DL1-positive NK cells. The HLA-peptide complex may, however, be recognized by the CTL, and the infected cell will also be killed by the CTL. Thus, NK cells and CTL will eliminate the infected cell. Thus, the person coinheriting *KIR3DL1* and *HLA-Bw4* may be better equipped to eliminate HIV-infected cells compared with the person who is homozygous for *HLA-Bw6*. It is noteworthy that KIR and TCR bind their cognate MHC ligands with much different kinetics and thermodynamic properties [189, 190]. Therefore, if CTL and the KIR3DL1-positive NK cell recognize and bind the same Bw4-peptide complex, CTL may preclude the NK cell binding to the complex.

No KIR has been described that could bind HLA-Bw6 allotypes. Individuals bearing this HLA may be killed only by antiviral CTL (in the context of HLA-Bw6). It may explain why HLA-Bw4 and KIR3DL1 have synergistic effects in slowing down the progression of HIV toward AIDS (**Fig. 7**). As *HLA-Bw4* homozygous persons are not likely to lack *KIR3DL1*, this may also explain why *HLA-Bw4* homozygous individuals are relatively resistant to the development of AIDS as compared with *HLA-Bw6* homozygous ones [75, 176]. Furthermore, more protection may be afforded by high-affinity KIR3DL1 allotypes, as they may be more sensitive to the peptide requirements and therefore, may be less likely to recognize the HLA ligand if bound to a foreign peptide. However, at this point, it remains a speculation and needs to be proved by experimental data.

KIR3DS1 may be protective, as in normal uninfected persons, this receptor does not recognize autologous cells; otherwise, host NK cells may kill these autologous cells and would cause autoimmunity. In HIV-infected persons, a viral peptide may bind HLA-Bw4 and make it recognizable by the KIR3DS1. Under these conditions, KIR3DS1-positive NK cells will cause killing of the infected cell. Although KIR3DS1 and KIR3DL1 allotypes have more than 95% sequence identity at the amino-acid level in their ligand-binding, extracellular regions, they may still differ from each other in their three-dimentional structures. That is why a mAb DX9 binds KIR3DL1 but not KIR3DS1 [191]. Thus, it may not be surprising if KIR3DS1 and KIR3DL1 bind HLA-Bw4/peptide complexes.

One should not overlook the fact that KIR are also expressed on T cells, especially on the memory/effector phenotype. This is an area that needs to be addressed in HIV immunology. Furthermore, one should also keep in mind that Nef may be attenuating the protective effects of KIR3DL1/Bw4 interactions by down-regulating the expression of these MHC antigens.

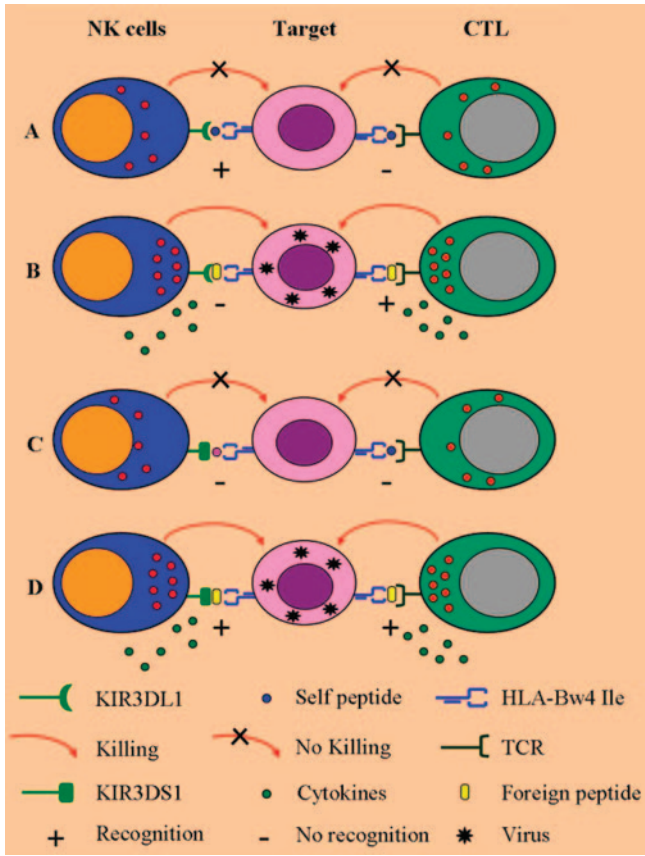

Fig. 7. NK cells with three-domain KIR behave like CTL in killing HIV-infected cells. (A) KIR3DL1 on NK cells recognize HLA-Bw4 with a self-peptide and are inhibited from killing normal, autologous cells. CTL do not recognize the self-peptide bound to the HLA and also do not kill the cell. (B) The virus-infected cell presents a virus-derived foreign peptide bound to HLA-Bw4. KIR3DL1 on the NK cell may not recognize the HLA with foreign peptide and is not inhibited from killing. The CTL may recognize the viral peptide bound to HLA-Bw4 and kill the infected cells. So, HIV-infected cells are more likely to be killed (from CTL as well as from NK cells). (C) KIR3DS1 expressed on NK cells does not bind to HLA-Bw4-I complexed with a self-peptide. CTL also do not recognize self-endogenous peptides and therefore, would not kill the healthy autologous cells. (D) A virus-infected cell presents a virus-derived foreign peptide bound to HLA-Bw4. It may be recognized by KIR3DS1 and be killed by KIR3DS1-positive NK cells. The CTL may also recognize the foreign peptide bound to HLA-Bw4 and therefore, will also kill the infected cell. Thus virus-infected, HLA-Bw4-positive cells are more likely to be killed (by virus-specific CTL and NK cells).

Role of HLA-G-binding KIR

As stated above, nonclassical MHC class I antigens, HLA-G and -E, also act as ligands for some NKRs. HLA-G binds KIR2DL4, ILT-2, and possibly KIR2DL5. The gene for HLA-G shows limited polymorphism. It was demonstrated that an *HLA-G* allele, *HLA-G*0105N*, which codes for a nonfunctional mutant HLA-G, provides protection from HIV infection in East African female sex workers. On the other hand, inheritance of functionally active HLA-G alleles increased the risk of the infection in these workers [192]. It is noteworthy that HLA-G is expressed in the mucosal tissues of the female genital tract and may play a role in regulating local antiviral immunity. The authors interpreted the data by concluding that the mutant

antigen protected women, as it could not inhibit their NK cells in the reproductive tract of the HIV-exposed women. Of the NKRs, which could bind HLA-G, KIR2DL4 is expressed on all $CD56^+$ NK cells present in tissues in humans. However, as mentioned above in the KIR section, this receptor is not an authentic inhibitory KIR: It induces secretion of IFN-γ but does not trigger cytotoxicity upon binding with its ligand [60, 63, 64]. Two other receptors, ILT-2 and possibly KIR2DL5, may also bind and inhibit NK cell functions. However, they are only expressed on a minor subset of NK cells. Notably, monocytes, macrophages, and DC abundantly express different ILT, including ILT-2, which preferentially bind and are inhibited by HLA-G [97]. Thus, in the persons having mutant HLA-G, activated monocytes, macrophages, and DC may play a greater role than NK cells. It has also been reported that the African women having a mutant *HLA-E* allele (*HLA-E*0103-G* or *HLA-E^G*) have four times less risk of contracting HIV infection as compared with the women with wild-type *HLA-E* [193]. It is noteworthy that the mutant *HLA-E* allele is expressed at lower levels and has decreased affinity for CD94/NKG2A receptors. It has also been shown that the sex worker women with HLA-E and HLA-G mutant genotypes (homozygous for *HLA-E^G* as well as heterozygous for HLA-*G*0105N*) had more than 12-fold decreased risk of contracting HIV infection [193]. These results implied a synergistic interaction between mutant HLA-E and HLA-G alleles in affording protection from HIV infection. These studies also suggest that blocking HLA-G and HLA-E interactions with NKR may enhance innate resistance to HIV.

Taken together, it can be postulated that coinheritance of genes for any NKR/ligand pair that weakens NK cell inhibition in the body and decreases activation threshold of NK and T cells is likely to provide protection from HIV and other viral infections. In this respect, the three-domain KIR behave as TCRs in recognizing MHC-bound peptides. If they fail to recognize the MHC-bound foreign peptide, they release the NK cell from inhibition. The NK cell will kill the virus-infected cell and so will do the virus-specific CTL. These two effector cells will be more effective in controlling HIV infection and delaying onset of AIDS in the persons who coinherit *KIR3DL1/HLA-Bw4* genes. If the three-domain KIR also recognize the foreign peptide, then the NK cell will be inhibited from killing the target cell. These results have implication for HIV vaccine strategies. Viral peptides, which are recognized by CTL, but not by KIR3DL1, may serve as better immunogens. They may arouse NK and CTL responses for killing the infected cells.

PERSPECTIVE AND FUTURE DIRECTIONS

Advances made in understanding NK cell immunobiology have enabled us to appreciate the role of NK cells in host resistance to these infections. The functional activities of these cells are controlled by a multitude of inhibiting and activating receptors and coreceptors. Of these receptors, the genes encoding KIR and their HLA ligands are highly polymorphic. Therefore, it is not surprising that coinheritance of these genes and their epistatic interactions is important in determining the status of NK cell activation, overall immune reactivity, and innate genetic resistance of humans to viral infections, malignancy, and autoimmune diseases. Researchers have just begun unraveling the role of these interactions in these diseases.

In the case of HIV infection and AIDS, few studies conducted so far highlight the significance of *HLA* and *KIR* genes as well as of their interactions in determining our innate susceptibility to the infection and its progression to AIDS. Many of these studies lacked adequate sample sizes and did not take into account allelic variations. For example, all major KIR genes have allelic variants that encode nonfunctional receptors. Furthermore, different allotypes of a given KIR differ widely in their affinities for MHC ligands. Future studies should take into account the impact of these variants on the susceptibility/resistance of humans to the infection as well as on the rate of progression toward AIDS. The results could have a profound impact on our understanding of the role of NK cells in controlling HIV infection. In view of the large number of KIR genes, their alleles, differences in gene doses, and lack of knowledge about the ligands for activating KIR, the task of investigating the impact of KIR/HLA interactions on HIV infection is challenging but worth undertaking. The results would have enormous implications for the immunotherapy, prognosis, and vaccination of HIV infections. Such studies would require larger sample sizes, accurate clinical data, and proper stratification of study participants with respect to their ethnic backgrounds. Furthermore, the researchers must use models that include all KIR alleles and their ligand genes as well as all other known genetic determinants that affect host resistance to HIV infection.

In determining the role of genes for NKRs and those of their ligands, researchers have ignored the potential involvement of the ligands for KLR-D (NKG2D) receptors, i.e., MICA and MICB proteins, which exist in more than 50 and 20 allotypes, respectively. These allotypes vary in their affinity for NKG2D. The nature of the allotype(s) carried by an individual could greatly affect his/her NK cell ability to kill target cells. They have been shown to play a role in autoimmune diseases such as celiac disease, diabetes, etc.

Finally, research in this field is seriously hampered by lack of appropriate mAb to identify individual NKR genes and their allelic variants. Development of such reagents should be a priority. This would allow determining the level of expression of the genes at the protein level and supplement genetic data with more relevant protein data. Furthermore, these antibodies could serve as important tools for manipulating receptor/ligand interactions for therapeutic purposes.

NOTE ADDED IN PROOF

A recent publication showed significantly enhanced frequency of *KIR3DS1* homozygotes but not of the combination of the *KIR3D* gene with *HLA-Bw4* or *HLA-Bw4-I* genes in HIV-exposed uninfected individuals compared to HIV-infected ones [194].

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1.2.3.2 Article 3:

1.2.3.2.1 Antiviral NK cell responses in HIV infection: II. Viral strategies for evasion and lessons for immunotherapy and vaccination

Alexandre Iannello, Olfa Debbech, Suzanne Samarani, and Ali Ahmad. 2008. Journal of Leukocyte Biology, 84 (1): 27-49.

RÉSUMÉ

Les cellules NK sont présentes dans la circulation, dans les ganglions lymphatiques, le foie et la rate. Elle peut atteindre toute place où il existe une infection virale et exécute une réponse anti inflammatoire. Après l'infection, le virus induit la production des cytokines, comme l'INF de type I, l'IL-12, l'IL-15, l'IL-18 etc… qui sont responsable de l'activation des cellules NK, et qui a leur tour induit une réponse immunitaire adaptative contre les pathogènes intracellulaire. Les cellules NK peuvent tuer les cellules infectées directement ou indirectement par ADCC. Les fonctions cytolytiques et sécrétoires deviennent compromises, durant la progression de la maladie. Alors, il y a une déplétion du nombre des cellules NK et expansion des cellules NK non fonctionnels. Alors dans cette revue, on discute les stratégies développer par le virus pour échapper à la réponse médié par les cellules NK, la dérégulations des cytokines qui induisait l'activation des cellules NK, et d'autre aspect qui touche à l'apoptose, et l'effet de la thérapie anti virale. Finalement on discute des nouvelles approches qui impliquent les cellules NK comme une immunothérapie contre le VIH et la vaccination.

Contribution

J’ai contribué dans la rédaction de cet article, de donner mes idées, à la recherche des nouvelles idées et informations et finalement à chercher des nouvelles références.

Antiviral NK cell responses in HIV infection: II. viral strategies for evasion and lessons for immunotherapy and vaccination

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Abstract: As is the case in other viral infections, humans respond to HIV infection by activating their NK cells. However, the virus uses several strategies to neutralize and evade the host's NK cell responses. Consequently, it is not surprising that NK cell functions become compromised in HIV-infected individuals in early stages of the infection. The compromised NK cell functions also adversely affect several aspects of the host's antiviral adaptive immune responses. Researchers have made significant progress in understanding how HIV counters NK cell responses of the host. This knowledge has opened new avenues for immunotherapy and vaccination against this infection. In the first part of this review article, we gave an overview of our current knowledge of NK cell biology and discussed how the genes encoding NK cell receptors and their ligands determine innate genetic resistance/susceptibilty of humans against HIV infections and AIDS. In this second part, we discuss NK cell responses, viral strategies to counter these responses, and finally, their implications for anti-HIV immunotherapy and vaccination. *J. Leukoc. Biol.* 84: 27–49; 2008.

Key Words: *ADCC · AIDS · CD94/NKG2 · chemokines · cytokines · HIV-1 · HLA · KIR · KIR haplotypes · MHC class I · MICA · MICB · NK cell receptors · NKG2D · ULBP*

INTRODUCTION

This is second part of a review article on NK cell responses in HIV infections. The first part gives an overview of our current knowledge about NK cell immunobiology, receptors, and their ligands. The part also describes how polymorphism in the genes encoding killer-cell Ig-like receptor (KIR) and their HLA ligands determines innate genetic resistance/susceptibility to HIV infection and development of AIDS. This second part of the article deals with functional defects that occur in NK cells in the course of HIV infection, viral strategies to counter host's NK cell responses, and their implications for anti-HIV immunotherapy and vaccination. We recommend that this article be read in conjunction with its first part.

NK CELL ACTIVATION IN HIV INFECTION

As mentioned earlier, NK cells are present in the circulation, bone marrow, lymph nodes, spleen, lung, liver, omentum, etc., and can reach almost any place in the body where a viral infection occurs and induces an inflammatory response. Viral infections generally activate NK cells, especially in early stages of the infection. Studies from animal models as well as in individuals in acute stages of the infection suggest that HIV is no exception to this rule. The infection also causes activation and expansion of NK cells. NK cell activation and expansion have been observed in humans in primary HIV infections and precede the appearance of virus-specific CTL responses. The expansion usually occurs in the highly cytotoxic $CD56^{dim}CD16^{+}$ subset of NK cells [1]. Increased NK cell activities were also observed in monkeys after experimental infection with SIV [2]. This initial NK cell expansion and activation probably result from direct and indirect effects of the infection. Virus-induced cytokines, e.g., type I IFN, IL-12, IL-15, IL-18, etc., are usually responsible for early NK cell activation and expansion. Viral proteins and nucleic acids may bind to TLRs and/or other receptors on a variety of host cells including NK cells themselves, resulting in their activation. As mentioned in the first part of this review, to become functionally competent, TLRs expressed on NK cells seem to require help from accessory cells [3, 4]. Thus, it is not surprising that a uridine-rich ssRNA derived from HIV-1 long-terminal repeat has been shown to activate NK cells but requires the presence and activation of plasmacytoid DC or $CD14^{+}$ monocytes [5]. Activated NK cells activate DC, secrete IFN-γ, and act as adjuvants by killing virus-infected cells and by causing release of intracellular proteins from the killed cells. NK cell activation has been shown to be important in inducing an effective adaptive immune response against intracellular pathogens in several animal models. In the context of HIV infection, NK cells may control the infection, not only by killing virus-infected cells directly as well as indirectly by antibody-dependent, cell-mediated cytotoxicity (ADCC), but also, they serve as an important source of β-chemokines (MIP-1α, MIP-1β, and RANTES) and undefined soluble factors, which can sup-

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press replication of M- and T-tropic HIV viruses [6–8]. NK cell-secreted cytokines, especially IFNs, may induce the antiviral state in host cells and cure HIV-infected cells via noncytolytic mechanisms.

Many studies have shown that NK cells play an important role in controlling HIV replication. The presence of NK cells suppresses HIV replication in cell cultures [9–11]. It has been demonstrated that i.v. drug users, who are at high risk of contracting HIV infection, resist infection, as long as they have elevated NK cell activities. In this regard, researchers have shown that these uninfected but highly exposed drug users have NK cells, which produce more chemokines and cytokines in vitro with or without simulation and whose KIR repertoire is predominantly of an activating type. They have high ratios of $KIR3DS1^+/KIR3DL1^+$ and NK cell group 2C+ ($NKG2C^+$)/$NKG2A^+$ NK cells and coinherit the weakly inhibiting *KIR-MHC* gene pair (*KIR2DL3/HLA-C* of group I). They also have low expression of KIR3DL1 and an increased expression of CD107a and CD69 on their NK cells [12, 13]. Apart from protecting from HIV infection, high NK cell activities also delay progression of the infection toward AIDS [14, 15]. It has been demonstrated that decreases in NK cell cytotoxicity as well as in NK cell counts in the circulation of the infected persons were associated with their rapid $CD4^+$ T cell depletions and rapid progression toward AIDS [14, 16]. However, the infected persons who are able to maintain their NK cell functions remain healthy, despite having decreased $CD4^+$ T cell counts [15]. Animal models of HIV infection also support a role of NK cells in controlling this infection. It has been shown that our closest relatives, chimpanzees (Pan troglydytes), can be infected with HIV-1 and SIV_{cpz}. The viruses replicate in this species but cause no AIDS-like disease. It is noteworthy that NK cells are more abundant in this species than in humans; they remain fully functional throughout the course of infection and unlike humans, can up-regulate certain natural cytotoxicity receptors (NCR; NKp30) in response to the infection. As mentioned above, this receptor plays a role in NK cell–dendritic cell (DC) interactions. Higher NK cell responses in chimpanzees are thought to be a factor in their resistance to progression to an AIDS-like disease [17, 18].

Several workers have investigated NK cell responses in HIV-infected humans. An exhaustive list of these studies, along with their major findings, is given in **Table 1**. It is quite evident from this table that NK cell functions (cytolytic and secretory) become compromised in HIV-infected persons; depletion of functional NK cell subsets and expansion of nonfunctional NK cells occurs; the infection causes changes in the expression of NKRs and their ligands; HAART tends to normalize changes in the number and functional capabilities of NK cells, but they never become normal. Few studies have been undertaken to translate our current knowledge into ways and means to invigorate NK cells and develop novel, anti-HIV vaccines.

ADCC

NK cells not only can kill virus-infected cells alone, they can also do so in combination with antibodies for which the antigen is expressed on the surface of the infected cells. The process is called ADCC. The antibodies bind through their variable antigen-binding sites to the viral antigen on the surface of the virus-infected cells and through their so-called crystallizable fragments (Fc) to CD16 on NK cells [63, 64]. The antibodies cross-link CD16 on NK cells and consequently, trigger their cytolytic functions. This results in killing of the virus-infected cells and secretion of cytokines and chemokines from NK cells (**Fig. 1**). The ADCC is a classical example of cooperation between innate and adaptive immune responses in protecting host from viral infections and malignancies. CD16 is a type I Ig-like integral membrane glycoprotein, which is expressed on the surface of NK cells, monocyte-macrophages, Langerhan's cells, DC, etc. It is a low-affinity type III receptor for the Fc part of IgG (FcγRIII; CD16). It binds aggregated but not monomeric human IgG1 and IgG3. The aggregated Ig are present in immune complexes. NK cells express the CD16A or FcγRIIIA form of the receptor. This form associates noncovalently via its transmembrane region with signaling adaptors (γ and/or ζ chains) and can transmit signals intracellularly. The receptor plays a predominant role in NK cell-mediated ADCC. Therefore, it is also commonly referred to as the "ADCC receptor." Another form of the receptor (CD16B or FcγRIIIB) is anchored in the plasma membrane via GPI and cannot transmit intracellular signals. This form acts as a sink for antigen/antibody complexes and is expressed on neutrophils and eosinophils [64]. The level of expression of CD16 on the surface of NK cells correlates with their functional ADCC activity. CD16 interacts physically with CD38 on the surface of NK cells. CD38 is a surface glycoprotein with ADP ribosyl cyclase/cyclic ADP-ribose hydrolase activities. It regulates cytoplasmic calcium and also acts as a receptor modulating cell–cell interactions. It binds CD31 (PECAM-1), which is a transmembrane Ig-like glycoprotein expressed on human vascular endothelial cells, and plays a role in angiogenesis, cell adhesion, and diapedesis. When cross-linked, CD38 transmits activating signals to NK cells via CD16 [65]. Interestingly, cells may shed CD16 upon activation, and cleaved sCD16 interferes with the ADCC process. Increased concentrations of sCD16 have been reported in the sera of HIV-infected persons, which correlate with disease progression. Interestingly, sCD16 seems to be shed from non-NK cells in these patients [66].

In addition to CD16, NK cells express an activating version of the FcγRIIC (CD32C), which also takes part in ADCC [67]. However, only 40–45% humans express this receptor on their NK cells. It is noteworthy that CD32 is encoded by three diferent genes: *CD32A*, *-B*, and *-C*. CD32A is an activating receptor expressed on neutrophils, monocytes, and DC. CD32B is an inhibitory receptor expressed on DC, monocytes, neutrophils, and B cells. An allelic variant of CD32A expresses arginine at position 131 (R131) instead of histidine (H131). The R131 variant responds vigorously to IgG and has been implicated in the development of systemic lupus erythrematosus (for details, see ref. [67]).

The major FcR involved in ADCC may be mutated and nonfunctional in some individuals. This happens as a result of a deletion of a single base (adenine) in exon 4 at nucleotide 550, resulting in a premature stop codon and truncated protein [68, 69]. Another mutation has been described that results in

polymorphism at position 158 in the amino-acid sequence. The amino acid at this position could be valine (V) or phenylalanine (F). The V allotype has higher affinity with IgG than the F one. The individuals with the *V/V* genotype are more efficient in mediating ADCC [70]. Few studies have investigated the impact of these mutations on the clinical course of HIV infection. In this regard, one group of researchers has demonstrated that HIV-infected persons bearing the FcγRII RR genotype progress more rapidly toward AIDS than those bearing HH or HR genotypes [71].

The ADCC-mediated destruction of tumor cells as well as of virus-infected cells can be readily demonstrated in vitro in the presence of NK cells and tumor or virus-specific antibodies of the appropriate IgG isotypes. The process also occurs in vivo. Although macrophages and neutrophils can also mediate ADCC, NK cells are the main cell type that mediates this process. Their depletion, therefore, abrogates the ADCC-mediating ability of PBMC [64].

ADCC IN HIV INFECTION

NK cells may eliminate HIV-infected cells in combination with HIV-specific antibodies via ADCC. The destruction of HIV-infected cells can be readily demonstrated in in vitro ADCC reactions in which HIV-infected cells are incubated with NK cells in the presence of HIV-specific antibodies. The phenomenon has been demonstrated to occur in vivo in these infections [26]. The antibodies specific to the viral envelope protein gp120/41 have been shown to mediate ADCC against the virus-infected cells. A prerequisite of the ADCC against HIV-infected cells is that the virus must be replicating in the cells, and the viral envelope proteins must be expressed on the surface of these cells. Anti-HIV, ADCC-mediating antibodies have been demonstrated in the sera of HIV-infected persons in several studies [9, 33, 63].

Although HIV-specific ADCC eliminates HIV-infected cells, it also has the potential to contribute to AIDS pathogenesis (Fig. 1). In in vitro experiments, uninfected $CD4^+$ T cells may bind exogenous recombinant gp120 and be killed by NK cells in the presence of gp120-specific antibodies of the IgG isotype [72, 73]. Furthermore, anti-gp120 antibodies may complex with the virus and facilitate uptake of the virus by monocytes. They may also cause NK cell activation and hence, excessive production of chemokines and cytokines. In fact, a group has demonstrated a correlation between the presence of HIV-specific ADCC antibodies and the development of AIDS [74]. However, these studies have not been corroborated. On the other hand, several researchers have demonstrated that these antibodies correlate with better clinical condition and better prognosis in HIV-infected children and adults [63, 75–77]. The protective nature of anti-HIV ADCC antibodies could also be demonstrated in in vitro experiments in which HIV-specific antibodies or NK cells alone are not able to inhibit replication of primary isolates of HIV-1 in human PBMC. However, they do so efficiently via ADCC when added together to these cultures [9]. Studies in animal models of HIV infection have also shown a protective effect of ADCC against disease progression [78]. Many researchers regard anti-HIV ADCC as a reliable correlate of immune protection from HIV infection [17, 63]. However, it remains to be tested in HIV vaccination studies. It has been demonstrated that vaccines may elicit ADCC antibodies, which could inhibit replication of clinical strains of HIV in the presence of NK cells [9].

Although anti-HIV ADCC antibodies can be demonstrated in HIV-infected individuals, even in late stages of the infection, the full host beneficial potential of this ADCC cannot be realized in vivo, as NK cell functions become compromised in a majority of these individuals [14, 27, 79, 80]. The decreased ADCC effector function of NK cells in HIV-infected persons could be a result of several reasons: decreased number of $CD16^+$ NK cells, decreased expression of the signaling partner ζ chain in NK cells, and overall decreased cytolytic capacity of NK cells (see Table 1). It is noteworthy that the engagement of CD16 alone cannot mediate killing of the target cells. For this purpose, it needs simultaneous engagement of LFA-1 or 2B4 (reviewed in ref. [81]). The receptor activities may be neutralized by increased concentrations of ICAMs and sCD16 in the circulation of HIV-infected patients [66, 82]. Interestingly, these concentrations increase with disease progression and serve as prognostic markers. Increased expression of HLA-C and -E on the surface of HIV-infected T cell blasts also interferes with their killing by autologous NK cells via ADCC. The blockage of interactions between KIR and HLA-C and between NKG2A and HLA-E with specific antibodies enhances this immune effector mechanism against this virus [83].

Attempts to control HIV replication in HIV-infected patients via passive immunotherapy (infusion of anti-HIV antibodies or i.v. Igs) have not yielded desired results. Passively infused i.v. Igs are known to have immunosuppressive effects (reviewed in ref. [84]). The infused antibodies form multimeric IgG complexes on DC. Such DC are killed by NK cells via ADCC or become defective for their ability to activate NK cells and to prime T cells. They decrease the expression of NKp30 and KIR on interacting NK cells [85]. Therefore, they may aggravate the defects, which already exist in an NK cell compartment in HIV-infected patients. HIV-1 has developed myriad strategies to evade a neutralizing antibody response of the host, e.g., mutation of epitopes, masking of epitopes by glycosylation and trimerization of gp120/41 spikes, shedding of envelope proteins, etc. (reviewed in ref. [86]). Nevertheless, the infusion of a combination of HIV-specific neutralizing antibodies does provide protection from infection in the animal models. However, it has been demonstrated that the antibodies require binding to FcγR for full efficacy [87, 88]. These results highlight a beneficial role of ADCC for the host. FcγR can also mediate uptake of antibody-coated viruses by monocytes and macrophages. The potentials and limitations of the i.v. use of neutralizing antibodies in HIV-infected patients have been demonstrated by the results of a small trial, in which a combination of HIV-neutralizing antibodies was infused into HIV-infected persons. Their HAART treatment was stopped 1 day after the infusion, and HIV rebound was measured [89, 90]. The virus rebound was delayed in acutely infected persons. However, this delay in the virus rebound was seen only in two of the chronically infected persons. Escape mutants also appeared for one of the three antibodies in the rebound viruses.

TABLE 1. List of Published Research Papers and Their Major Findings on NK Cells in HIV-Infected Persons

Year	Major Findings	Reference
1986	Defective trigger and release of NKCF; partial restoration by IL-2.	[19]
1986	Decreased NK cell activity as a result of decreased IL-2 production; *in vitro* IL-2 restores this activity.	[20]
1986	IL-2 increases NK cell-mediated killing of HIV-1-infected cells; HIV-1 infects NK cells *in vitro*.	[21]
1987	Defective natural cytotoxicity, normal ADCC.	[22]
1987	Defective NK cell lysis in patients with AIDS and AIDS-related complex; increased number of $CD8^+CD57^+$ and $CD16^+CD57^+$ cells.	[23]
1988	Normal actin polarization but defective tubulin rearrangement in NK cells from AIDS patients.	[24]
1990	Defective inducibility of LAK activity in blood lymphocytes.	[25]
1990	Defective ADCC effector functions of NK cells.	[26]
1990	CD16+ NK cells decrease in all stages of infection; specific depletion of $CD3^-CD8^+CD16^+$ cells.	[27]
1992	NK cell activation and killing decreased, not restored by long-term in vitro culture with IL-2; defect at clonal level.	[28]
1994	$CD56^+$ and KIR^+ NK cells decrease in AIDS patients; NK cell activity decreases, and $EB6^+$ $GL183^+$ (double-positive) NK and T cells appear in all HIV-infected persons.	[29]
1994	Impaired NK and LAK activity; no effect of AZT therapy.	[30]
1994	NK cell activity decreased with disease progression starting in CDC stage IVC2; Defect not corrected by in vitro culture with IL-2.	[31]
1994	Decreased $CD16^+CD56^+$ NK cells in primary and chronic HIV infections in children.	[32]
1994	Defective ADCC effector function of NK cells from HIV-infected persons; IL-2 and IFN-γ restore partially.	[33]
1995	Decreased NK cell activity early in infection; CD16+ decreased; $CD16^+CD56^+$ percentage normal or elevated, reduced response to IFN-α.	[34]
1995	$CD16^+CD56^+$ subset decreases; expansion of less-cytotoxic $CD16^{dim}CD56^-$ cells.	[35]
1995	$CD8^+CD16^+CD56^+$ NK cells depleted.	[36]
1996	Defective NK cell-mediated killing of gp120/41-expressing target cells.	[37]
1997	$CD16^+$, $CD56^+$ and $CD16^+CD56^+$ NK cell percentage and concentration of $CD16^+CD56^+$ NK cells decrease in correlation with disease progression.	[38]
1997	AIDS patients have lower NK cell activity than HIV-infected asymptomatic and seronegative controls; at least in part as a result of reduced IL-2 production.	[39]
1997	KIR^+ $CD3^+$ $CD8^+$ cells increase, masking KIR-increased cytotoxicity against HIV-infected cells.	[40]
1999	Increased percentage of $CD94^+CD8^+CD56^+$ cells; no change in KIR, IL-10 implicated.	[41]
1999	KIR increased transiently on T but not on NK cells; CD94 increases on both cell types.	[42]
1999	Poor response to IFN-α and low LAK activity associated with poor prognosis and death.	[43]
1999	Decreased expression of ζ chain in NK cells; defective, CD16-mediated lysis.	[44]
2001	NK activity decreased with decrease in VL; decreased iKIR on $CD56^+$ but increased on $CD8^+$ cells; CD57 and CD94 expression increased.	[16]
2001	Decreased NK cell numbers with decrease in $CD4^+$ T cell counts; decreased $CD16^+CD56^+$ NK cells; expansion of $CD8^+CD38^+DR^+$ lymphocytes associated with low cytotoxicity.	[14]
2001	HAART normalizes expression of iKIR and NKG2A but not of ILT-2 on CTL; blocking of ILT-2 increases CTL activity.	[45]
2002	$CD56^+$ cells coexpressing CD16, CD161, or CD94 become depleted; CD56– cells coexpressing CD16, CD161, or CD94 expand; defective differentiation.	[46]
2002	Defective modulation of CD69 and CD16 expression on NK cells.	[47]
2002	Increased NK cell activity that decreases with HAART; more NK cells expressing CD158a and CD94 appear; IL-10 implicated.	[48]
2003	Decreased production of chemokines and viral suppression by NK cells from viremic patients.	[11]
2003	Decreased expression of NCR on fresh and in vitro-activated NK cells.	[49]
2003	Decrease in $CD16–CD56^{bright}$ subset in patients undergoing HAART; IL-12 plus IL-15 or HGH partially restore NK cell number and IFN-γ production.	[50]
2003	Expansion of $CD56^-CD16^+$ dysfunctional NK subset; decreased expression of actNKR but not of NKG2D; stable or increased KIR expression; impaired NK cell cytotoxicity; HAART normalizes NKR expression.	[51]
2004	Aberrant NK cell activation; NCR expression decreased; no NKp44 expression; decreased cytotoxicity in viremic patients.	[52]
2004	Increased CCR5 and iKIR expression on NK in viremia; became normal after HAART; direct correlation of iKIR expression with viral load.	[7]
2004	iKIR expression increases on NK in viremic patients; $CD56^+CD94^+$ and $CD56^+CD161^+$ NK decreased; NK cell number decreased; overall NK activity and CD107a expression increased; NK produce more TNF-α and IFN-γ in viremic patients.	[1]
2005	Expansion of $NKG2C^+$ and loss of $NKG2A^+$ NK; no effect of HAART.	[53]
2005	Expansion of dysfunctional $CD56^-CD16^+$ subset in viremia; iKIR expression increased on NK; major activating NKR decreased but not NKG2D; NK less cytotoxic, produce less chemokines and cytokines.	[54]
2005	Decreased $CD94^+$ NK cell numbers in viremic patients and advanced disease.	[55]
2005	$2B4^+$ cells transiently decrease early in infection; decrease is related to VL and sTNFRII; later expression increases on NK and T cells and normalizes with HAART.	[56]
2005	Increased NK cell numbers with early depletion of the CD56highCD16$^-$ subset and expansion of the CD56lowCD16$^+$ subset in acute infection; the depletion of the $CD56^{low}CD16^+$ subset continues with a parallel increase in the anergic $CD56^-CD16^+$ subset.	[57]
2006	Decreased perforin and increased SHIP expression in NK cells in chronic infection.	[58]
2007	Expression of inhibitory FcRL6 on all lymphocytes (NK, $CD4^+$, $CD8^+$ effector memory cells).	[59]

TABLE 1. (Continued)

Year	Major Findings	Reference
2007	NK cell activation and expansion precede CTL response in acute infection; NK cell activity decreases with HAART; NK and CTL respond with similar kinetics upon antigen exposure or upon discontinuation of therapy.	[60]
2007	CD56dim NK cell subset expanded in primary infection; partial normalization after 6 months HAART; $CD27^+CD70^+$ expression increased in chronic infection.	[61]
2007	$KIR3DS1^+$ NK and T cells increase in number in viremic HIV-infected persons.	[62]

actNKR, Activating NK cell receptors (NKR); FcRL6, FcR-like protein 6; HGH, human growth hormone; iKIR, inhibitory KIR; LAK, lymphokine-activated killer cell activity; NKCF, NK cell cytotoxic factor, HAART, highly active antiviral therapy; sTNFRII, soluble type II TNFR; VL, viral load; ILT-2, Ig-like transcript 2. EB6 and GL183 are mouse MAb (from Immunotech/Coulter, France) that bind to KIR2DL1 (p58.1) and KIR2DL2/3 (p58.2), respectively.

As mentioned above, the $CD16^+$ NK cell subset is mainly involved in mediating ADCC. NK cell therapy with or without anti-gp120/41 antibodies may be more effective in restoring ADCC and controlling HIV replication in HIV-infected patients. Finally, several cytokines are known to increase ADCC against HIV-infected cells (reviewed in ref. [80]).

HIV STRATEGIES TO EVADE NK CELL RESPONSES OF THE HOST

Viruses generally use multiple strategies to counter NK cell responses of the host. HIV is no exception. The strategies aimed at evading antiviral effects of the host's NK cell responses are listed in **Table 2** and are discussed below.

Changing the expression of MHC and non-MHC ligands for NKRs

The down-regulation of MHC class I antigens on the surface of infected cells is a common strategy used by a variety of viruses to evade antiviral CTL responses of the host, as CTL recognize viral peptides in association with these antigens (reviewed in refs. [91–93]). A global decrease in the expression of MHC antigens, however, makes virus-infected cells susceptible to NK cell-mediated killing. Therefore, viruses have developed various strategies to evade this NK cell-mediated killing. Two HIV proteins have been shown to affect expression of MHC class I antigens: Tat represses promoters of the MHC class I and the β-2 microglobulin genes, and viral protein U (Vpu) interferes with an early step in the biosynthesis of MHC antigens [94, 95]. However, a global down-regulation of MHC class I antigens has rarely been observed in HIV-infected cells. Instead, several studies have documented that HIV differentially down-regulates the expression of MHC class I antigens on the surface of the infected cells. The viral protein Nef recognizes certain motifs present in the cytoplasmic tails of MHC class I antigens and causes their degradation. These motifs are present mostly in the cytoplasmic tails of HLA-A and -B but not of HLA-C and HLA-E antigens. Consequently, the expression of HLA-A and -B but not of HLA-C and -E is decreased on the surface of HIV-infected cells [96–98]. It is noteworthy that HLA-A and -B predominantly present viral peptides to CTL. Of these, only Bw4-serospecific HLA-A and HLA-B allotypes act as ligands for KIR3DL1. On the other hand, HLA-C and HLA-E present relatively fewer HIV-derived. immunodominant peptides to CTL. Nevertheless, all known HLA-C and HLA-E allotypes act as ligands for KIR and CD94/NKG2, respectively. From the perspective of NK cell functions, HLA-C and HLA-E are more important. The maintenance of these HLA molecules on the surface of HIV-in-

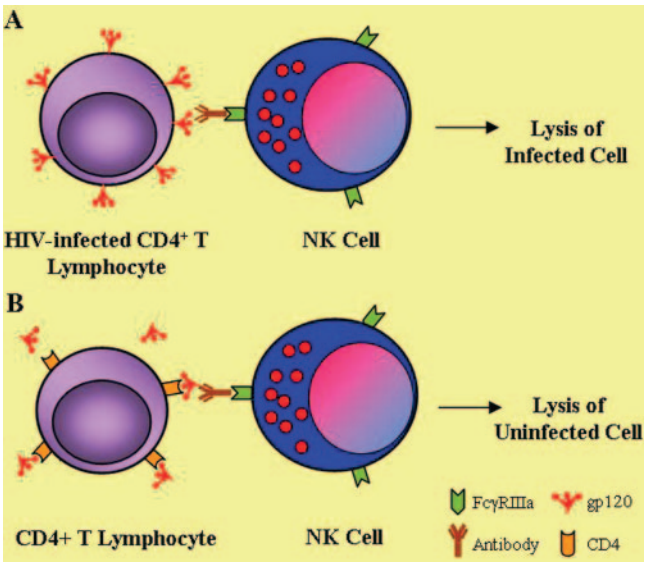

Fig. 1. Schematic representation of ADCC phenomenon. (A) NK cells kill HIV-infected $CD4^+$ T cells expressing gp120 via gp120-specific antibodies. The antibodies on one hand bind to gp120 and on the other hand, to CD16 on NK cells via their Fc regions. By killing HIV-infected cells, ADCC may help control the infection. (B) NK cells may also kill uninfected, gp120-bound $CD4^+$ T cells. The process may not discriminate between HIV-infected and gp120-bound, uninfected $CD4^+$ T cells.

TABLE 2. How HIV Evades Host's NK Cell Response

It does so by:

1. Differential down-regulation of MHC class I antigens via the negative factor (Nef) protein.
2. Inducing and stabilizing the expression of HLA-E and -G in the infected cells.
3. Directly infecting and eliminating NK cells.
4. Blocking NK cell-mediated killing.
5. Promoting apoptosis of NK cells.
6. Dysregulating the expression of NKRs.
7. Dysregulating the production of NK cell-activating cytokine.
8. Down-regulating the expression of adaptor molecules involved in the signaling cascade of NK cell activation.
9. Modulating NK cell differentiation and maturation program.
10. Interfering with NK cell interactions with DC and other immunocytes.

fected cells protects them from NK cell-mediated lysis. A decreased expression of HLA-A and -B makes them invisible to most of the virus-specific CTL. However, it comes with some price. The infected cells become susceptible to killing by the NK cells expressing HLA-Bw4-specific KIR. By a differential modulation of HLA antigens on the surface of infected cells, virus evades most if not all CTL and NK cell-mediated killing. Indeed. autologous NK cells do not kill HIV-infected T cell blasts despite decreased HLA-A and -B antigens on their surface [99].

In addition to classical MHC class I antigens, HIV modulates expression of nonclassical MHC antigens. The infection increases the expression of HLA-E on the surface of $CD4^+$ T cells in in vitro experiments [55]. At least one potential mechanism of this increase is a peptide from the viral protein p24 (residues 14–22), which can bind and stabilize HLA-E on the surface of HIV-infected cells [100]. In line with these results, increased expression of HLA-E has been reported on the surface of $CD4^+$ T cells in HIV-infected persons. The increase was more pronounced in advanced stages of the infection and correlated with peaks in viremia [55].

Conflicting results have been reported concerning the effect of HIV infection on the expression of HLA-G. The infection was reported to cause down-regulation of HLA-G on the surface of HIV-infected cells in a Vpu-dependent manner [96, 101]. However, the molecule is expressed on monocytes and T lymphocytes in HIV-infected persons, probably as a result of HAART and increased concentrations of IL-10 in the circulation [102, 103]. HLA-G is normally expressed on certain immune-privileged sites, e.g., cornea, and on invading cytotrophoblasts in pregnancy and is believed to protect a developing fetus from the mother's immune responses (reviewed in ref. [104]). Increased expressions of HLA-E and -G on the surface of HIV-infected cells would increase their resistance to NK cell-mediated killing as well as to macrophage activation.

Although exact ligands for NCR are not known, Vieillard et al. [105] have reported increased expression of NKp44 binding to HIV-infected $CD4^+$ T cells. They have shown that a peptide (SWSNKS) derived from the transmembrane unit (gp41) of a viral envelope protein induces the unknown NKp44 ligand on $CD4^+$ T cells. It also suggests that the unknown ligand for this receptor could be a peptide-binding MHC or MHC-like molecule. The induction of NKp44 ligand on the HIV-infected cells may promote their killing by cytokine-activated NK cells. The increased expression of NKp44 ligands on the surface of HIV-infected cells has been corroborated by Ward et al. [83], who also reported increased expression of MHC class I chain-related protein A (MICA), MICB, and the human CMV (HCMV) glycoprotein UL16-binding protein 1 (ULBP-1), 2, and 3 on T cell blasts infected with HIV. They also reported a decrease in the expression of CD48 and NK-T-B antigen, and no change was observed in the expression of NKp30 and NKp46 ligands. As mentioned earlier, MICA, MICB, and ULBPs serve as ligands for NKG2D, which is an activating receptor expressed on all NK and $CD8^+$ T cells in humans. It is not in the interest of a virus to induce expression of these ligands on the surface of infected cells, as the virus-infected cells would be killed by NK cells as well as by $CD8^+$ T cells via NKG2D. Therefore, viruses have developed different strategies to evade this NKG2D-mediated killing. For example, HCMV encodes a glycoprotein UL-16, which can bind ULBPs intracellularly and prevent them from reaching cell surface and interacting with NKG2D [106]. Another protein from this virus, UL142, binds MICA and prevents its interaction with NKG2D [107]. Tumors may cleave and shed soluble MICA and MICB to interfere with NKG2D-mediated killing of tumor cells [108, 109]. HIV uses its Nef protein to evade NKG2D-mediated killing. The protein, in addition to down-regulating the expression of HLA-A, -B and CD1d, also binds to and degrades MICA, and ULBP-1 and -2 [110].

It is noteworthy that NKG2DL are usually induced in human cells upon genotoxic stress, which activates DNA damage response (reviewed in ref. [111]). The response arrests cell cycle until the damage is repaired. If the damage is not repairable, the response induces apoptosis in the cells. The response is initiated by two PI-3K-like kinases: ataxia telangiectasia mutated (ATM) and ATM and RAD-3-related (ATR). The two kinases are activated by dsDNA and ssDNA breaks, respectively. Stalled replication forks also activate ATR. The induction of NKG2DL by HIV implies that the infection causes genotoxic stress in the infected cells. It could be an unintended consequence of the functional activities of the viral protein R (Vpr). The protein is known to induce cell-cycle arrest by recruiting DCAF-1/VprBP and an E3 ligase Cul4-DDB1 in eukaryotic cells and activates ATM and ATR, which may result in the induction of NKG2DL [112]. **Figure 2** summarizes HIV-induced changes in the expression of MHC ligands in HIV-infected persons.

Changing the expression of NKRs

Viruses may evade NK cell-mediated killing by increasing the expression of inhibitory and/or by decreasing the expression of activating receptors on the surface of NK cells of the infected host. There is sufficient evidence to suggest that HIV uses this strategy to counter antiviral NK cell responses of the host. Several workers have documented an increase in the expression of inhibitory receptors (e.g., iKIR) and a decrease in the expression of activating receptors (e.g., NCR) in HIV-infected individuals. Interestingly, these dichotomous effects on inhibitory and activating NKRs were mainly observed in viremic patients and correlated with viral load. Only a transient decrease was observed in the expression of 2B4, whereas no effect was observed on the expression of NKG2D on NK cells from HIV-infected persons. These changes in receptor expression were often accompanied with decreased cytolytic activities of NK cells [1, 16, 49, 51] (reviewed in ref. [113]). The occurrence of these changes in viremic patients as well as their correlation with viremia suggest that the virus might have caused the receptor modulations. This is further supported by the fact that a stabilizing effect of the HAART treatment on the receptor expression was observed. However, the treatment was able to restore the expression to normalcy after a long period of administration, when it resulted in undetectable viral loads in the patients [51, 56]. In addition to direct effects of the virus, chronic activation of the immune system via antigens from HIV-1 and/or from other coinfecting pathogens may have

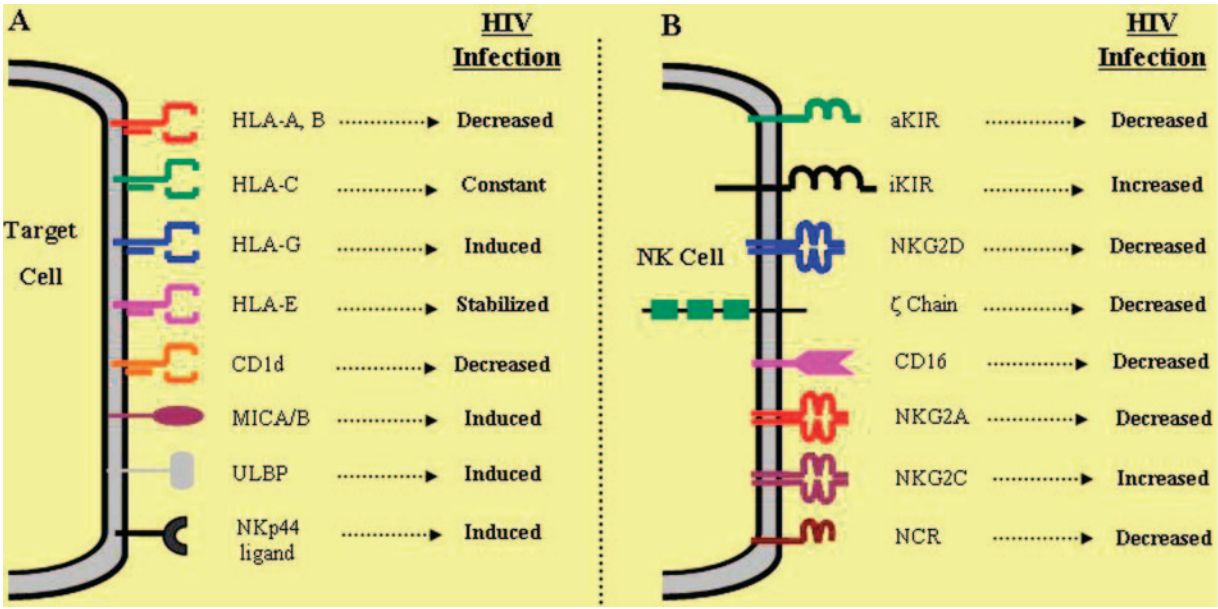

Fig. 2. HIV-induced changes in the expression of NKRs and their ligands. (A) Changes in the expression of various NKR ligands. (B) Changes in the expression of NKRs from HIV-infected persons. Collectively, these changes may protect HIV-infected cells from NK cell-mediated lysis. aKIR, Activating KIR; iKIR, inhibitory KIR.

caused perturbations in the expression of NKR. Repeated antigenic stimulations are known to induce expression of several inhibitory receptors including KIR on immune cells [114].

With respect to the NKG2/CD94 family receptors, an expansion of CD94/NKG2C$^+$ and a marked depletion of CD94/NKG2A$^+$ NK cells have been described in the peripheral blood of HIV-infected persons [53]. HAART did not reverse these changes despite reducing viremia to undetectable levels in these patients. These changes are also not observed in the individuals infected with HIV alone. It is noteworthy that similar changes in the expression of the CD94/NKG2 family of receptors have been reported in humans suffering from chronic infections with HCMV. It seems more likely that HCMV infection may be the real cause in driving these changes in HIV- and HCMV-coinfected patients. Indeed, HCMV-infected fibroblasts cause proliferation of NKG2C$^+$ human NK (HNK) cells in in vitro studies. However, the changes seem to be more pronounced in the coinfected individuals [53, 115, 116]. These observations suggest a possible role of HIV infection in these NKR perturbations. It may be relevant to mention here that NKG2A is an inhibitory and NKG2C is an activating NKR. Both of them bind to HLA-E on target cells and regulate NK cell functions. The two receptors usually occur on the CD56^{high}CD16^{low} subset of NK cells, which express low levels of KIR. It is believed that NKG2A may be important in maintaining self-tolerance in NK cells that do not express self-reactive KIR. It is noteworthy that a HCMV-encoded protein UL40 provides a peptide, which binds and stabilizes HLA-E. The HLA-E is also stabilized by a peptide derived from the HIV p24 protein [100, 117]. An increased expression of HLA-E on CD4$^+$ T cells in HIV-infected individuals has also been described [55]. The enhanced HLA-E expression may have caused proliferation of NKG2C$^+$ and/or an early depletion of NKG2A$^+$ NK cells. It is not clear how these receptor changes could affect progression of HIV infection. The fact that persons coinfected with HIV and HCMV progress more rapidly toward AIDS [118] suggests that NKG2C$^+$ NK cells may be involved in immunopathology. We speculate that these CD56$^+$NKG2C$^+$ NK cells may kill many different types of cells including mature DC and CD4$^+$ T cells, which express HLA-E [100, 117, 119]. However, it must be emphasized that there is no direct experimental evidence at this point in time to support this notion. The modulation of NKRs by HCMV provides an example of how this herpesvirus may affect the natural course of HIV infection in coinfected individuals.

NKp44 is an activating receptor, which is not expressed on resting NK cells. The receptor is induced on cytokine-activated NK cells. A group of researchers has shown that freshly isolated NK cells from HIV-infected viremic persons are aberrantly activated: They are CD69$^+$, HLA-DR$^+$ but do not express NKp44. Furthermore, they express relatively low levels of other NCR [52].

As mentioned above, HIV or its products have been implicated in the induction of changes in the expression of NKRs in HIV-infected patients. It is noteworthy that the infection causes a dysregulated production of many cytokines in the human body. It is not surprising that these cytokines have been implicated in this process. Two groups of researchers have suggested the involvement of IL-10. This immunosuppressive cytokine induces changes in the expression of NKRs in vitro similar to those seen in HIV-infected patients in vivo, i.e., increased expression of CD94, CD161, and CD158a or KIR2DL1 [41, 48]. It is noteworthy that concentrations of IL-10 are increased in the circulation of HIV-infected persons. The changes observed in the expression of NKRs in HIV-infected persons are summarized in Figure 2.

Changing the expression of NKRs on non-NK cells

Many NKRs are also expressed on non-NK cells. CD56 is usually expressed on activated $CD8^+$ T lymphocytes. Its expression has been associated with the acquisition of cytotoxic functions in these cells [120]. A decrease in the expression of CD56 has been described on NK and $CD8^+$ T cells in HIV-infected persons. Indeed, $CD56^+$ NK and $CD8^+$ T cell populations from HIV-infected persons express less perforin and are less cytolytic compared with their counterparts from HIV-seronegative, healthy subjects [10, 58, 121].

Normally, monocytes do not express CD16; they do so upon activation. TGF-β1 has been shown to induce its expression on monocytes in humans. Monocytes from HIV-infected AIDS patients express this marker, and this expression correlates with increased concentrations of this cytokine in the circulation of these patients [122]. This expression has implications for virus replication, as $CD16^+$ monocytes are highly permissive to HIV replication [123]. Furthermore, these cells may shed sCD16, which may interfere with killing HIV-infected cells via ADCC.

CD57 (Leu-7; HNK-1) is a 110-kD glycoprotein expressed on a subset of NK, $CD8^+$, and $CD4^+$ T cells, which plays a role in homotypic cell adhesion. It bears a sulfated carbohydrate epitope (glycotope), which is also present on several other glycoproteins and glycolipids expressed on the surface of different cell types. The epitope is regulated by two glucuronyltransferases (-P and -S) and a sulfotransferase (HNK-1; see ref. [124] for a review). In the immune system, CD57 is expressed on terminally differentiated effector cells. These cells can neither proliferate nor circulate; however, they do migrate to nonlymphoid tissues and secrete cytokines. In the case of $CD8^+$ T cells, CD57 expression is restricted to effector/memory phenotype. The marker is also expressed on aberrantly differentiated and clonally exhausted effector cells. Increased numbers of CD57-expressing NK and $CD8^+$ T cells occur in chronic viral infections including that of HIV [16, 124]. Repeated antigenic stimulation may lead to clonal exhaustion and increased CD57 expression in HIV-infected persons. Furthermore, aberrant differentiation of these cells as a result of a lack of CD4 help and/or dysregulated production of cytokines such as IL-2, IL-7, IL-15, IL-21, IL-10, etc., may also lead to an increased number of $CD57^+$ cells in this infection. Increased numbers of $CD57^+$ T and NK cells represent immune dysfunction.

In the course of normal differentiation into terminally differentiated effector cells, $CD8^+$ T cells acquire CD57 and lose CD27. However, in the case of HIV infection, they acquire relatively low levels of CD57 (compared with HCMV and EBV-specific effector CTL) and do not lose CD27 expression [125]. This suggests that HIV-specific CTL undergo aberrant and incomplete course of differentiation. This defective differentiation of HIV-specific CTL is further supported by their decreased expression of perforin, lower cytotoxicity, and increased expression of the inhibitory marker programmed death (PD)-1 and other phenotypic markers [126, 127]. It has also been proposed that in HIV infection, NK cells and CTL undergo premature senescence without undergoing complete physiological differentiation. This premature senescence has been proposed as the main reason of inability of HIV-infected persons to control the virus [128].

A subset of CTL has been shown to express KIR, NKG2/CD94, killer lectin-like receptor (KLR)-G1, and ILT-2. These markers are usually expressed at distinct stages in the course of development and differentiation of naïve CTL into effector/memory cells. For example, KLR-$G1^+$ $CD57^+$ CTL represent terminally differentiated effector CTL, and $KLRG1^+CD57^-$ CTL represent long-lived memory CTL [129]. Developing T cells acquire these markers after completion of their TCR gene rearrangements. Therefore, CTL with similar TCRVβ genes may have different repertoires of KIR, NKG2, and KLR-G1 receptors [130]. The level of expression of these receptors on CTL determines their antigenic threshold for activation and is "fine-tuned" to avoid autoimmunity and to mount an effective immune response against invading pathogens [131, 132]. The expression of KIR in humans (and of Ly49 in mice) seems to confer survival advantage in CTL and prevents them from undergoing activation-induced cell death in response to TCR stimulation. KIR^+ CTL express higher levels of the antiapoptotic protein Bcl-2 as compared with the ILT-2^+ CTL [133–136]. It appears that KIRs are expressed on long-lived memory T cells having monoclonal or oligoclonal expression of TCRVβ genes. ILT-2, on the other hand, are expressed earlier than KIR in the course of differentiation of CTL. Consequently, they are expressed on a larger percentage of antigen-specific CTL with a broader use of TCRVβ genes. Interestingly, ILT-2^+ but not KIR^+, HIV-specific CTL could be easily detected in HIV-infected AIDS patients, which again suggests their defective differentiation. KIR^+ CTL express perforin and secrete IFN-γ, whereas ILT-2^+ CTL can only secrete cytokines and contain little perforin [133]. Expansions of $CD8^+$T cells expressing these receptors usually occur in viral infections, which subside upon resolution of the infection. However, increased frequencies of the cells bearing these receptors persist in chronic infections [137–140]. The expression of inhibitory NKRs on CTL may be essential for the development of virus-specific memory responses. This expression raises the activation threshold of CTL and prevents indiscriminate killing of host cells but still allows killing of virus-infected cells. However, coengagement of inhibitory receptors inhibits TCR-mediated activation of CTL [141]. It has also been observed in in vitro studies that a blockage of KIR markedly increases CTL-mediated killing of HIV-infected, autologous cells [40]. In mice, which do not have KIR genes but express their functional orthologs (LY49 genes) on their NK cells and a subset of CTL, it was also demonstrated that blockage of LY49 receptors increases anti-tumor activities of NK cells resulting in tumor regression [142, 143]. Interestingly iKIR, CD94/NKG2, and KLR-G1 could also be detected but sparsely on $CD4^+$ T cells in human peripheral blood. Percentage of these cells increases with age.

It is noteworthy that many of the observations concerning the expression of inhibitory receptors on $CD8^+$ T cells have been verified in vivo in mice infected with chronic lymphochoriomenengitis virus infection [144].

It has been well documented that HIV infection induces a vigorous antiviral CTL response in the host (reviewed in ref.

[145]). The frequency of virus-specific CTL in the circulation of HIV-infected persons is usually higher as compared with that seen in several other viral infections. Consequently, HIV-specific CTL can be readily demonstrated in the peripheral blood of HIV-infected individuals without prior stimulation and expansion. Despite this, cellular immune response is unable to control HIV infection in humans. There could be several reasons for the inability of the antiviral CTL responses to clear HIV infections: high mutability of HIV-1, depletion of $CD4^+$ T cells and consequent loss of CD4 help, incomplete differentiation of CTL, increased expression of proapoptotic molecule PD-1, impaired proliferative capacity of HIV-specific CTL, decreased expression of CD3 on CTL, etc. (reviewed in refs. [145–147]). An increased expression of inhibitory NKR on these cells may also play a role in the ultimate failure of this antiviral immune response in controlling HIV infection in humans. This is supported by the facts that long-term, nonprogressors do not express increased levels of these receptors on their $CD3^+CD8^+$ peripheral blood cells, and in vitro blocking of these receptors causes increased killing of the CTL against autologous, HIV-infected cells [45, 148].

Disturbing NK cell interactions with other immunocytes

As mentioned earlier, NK cells interact intimately with DC. These interactions have important implications for the ensuing innate and adaptive immune responses against viral infections and malignancy. During these interactions, the two types of cells form an immune synapse with each other. NK cells induce polarized secretion of IL-12, IL-18, and membrane-bound IL-15 from DC. The polarized secretion from DC requires tubulin rearrangement and activation of calcium-calmodulin-dependent kinase II (CAMK-II) [149, 150]. These cytokines activate NK cells, which in return, secrete IFN-γ, TNF-α, and high mobility group box-1 (HMGB1), which cause DC maturation [151, 152] (reviewed in ref. [153]). It is noteworthy that HMGB1 is the most potent proinflammatory cytokine that causes DC maturation. The DC-maturing capacity of different NK cell clones depends on their ability to secrete this cytokine [154, 155]. The physical contact between the two cell types involves interactions among several receptor-ligand pairs, which include LFA-1, NKp30, NKp46, 2B4, DNAX accessory molecule 1 (DNAM-1), NKG2D, TNFRII, and NKG2A [150, 156, 157]. NK cells also perform the task of quality control and kill immature DC if they do not undergo proper maturation. It has been demonstrated that NKp30, DNAM-1, and LFA-1 are involved in the NK cell-mediated killing of autologous, immature DC [158]. Mature DC are not killed, as the maturation process induces expression of HLA antigens, which protect them from NK cells. It is noteworthy that it is the $CD56^{high}CD16^{dim}$ NK cells that interact with and cause maturation or killing of immature DC. These NK cells express little KIR and express CD94/NKG2A as the main inhibitory receptors. It is not yet fully understood how NK cells choose between killing and causing maturation of immature DC. It probably depends on the profile of expression of several molecules on the surface of immature DC. If DC fail to express HLA antigens upon maturation, they may be killed by NK cells. The NK cell-activating potential of DC also depends on the milieu in which they differentiate. For example, immature DC, differentiated in the presence of IL-4, selectively activate NK cells but not T cells. IL-4 induces the expression of triggering receptor expressed on macrophage-2 on DC [159, 160]. The ratio between NK cells and their interacting DC is also a factor: A greater ratio tends to favor the killing rather than maturation.

After maturation, DCs express CCR7 and migrate to secondary lymph organs, e.g., lymph nodes, where they interact with T cells as well as with activated NK cells, which control and determine T cell-priming capabilities of DC. The DC generated from monocytes in the absence of NK cells are unable to prime $CD8^+$ T cells. The NK/DC interactions may allow DC to prime T cells without help from $CD4^+$ T cells. The speculation is that NK cells may themselves provide this help. As mentioned earlier, properly activated NK cells express molecules that may enable them to interact with T cells. The cross-talk also involved cell–cell contact via CD161/Clr-b, 2B4/CD48, DNAM-1/Poliovirus receptor, NKG2D/NKG2DL, as well as soluble mediators, e.g., TNF-α, IFN-γ, IL-12, and others [161–163].

Depending on these interactions, DC may emerge that could prime naïve $CD4^+$ T cells into TH1-type cells. The interactions may also lead to the generation of DC, which may favor the generation of immunosupressive regulatory T cells (Tregs).

As a result of the importance of NK/DC interactions in mediating effective antiviral immunity, viruses may target these interactions for immune evasion. For example, it has been shown that monocyte-derived DC (MDDC) from hepatitis C virus (HCV)-infected persons and their autologous NK cells fail to induce reciprocal activation. This failure results from the inability of these MDDC to express MICA and MICB in response to IFN-α. The MDDC generated from the infected persons produce more IL-10 and TGF-β [164, 165], and TGF-β promotes induction of IL-10-secreting Tregs by inducing forkhead box P3 (FoxP3) expression in $CD4^+$ precursor cells [166].

The NK/DC interactions also become aberrant in HIV-infected persons. The NK cell-editing function seems to be lost in HIV-infected persons. Activated NK cells from viremic persons are unable to kill autologous, immature MDDC [51, 167, 168]. This defect was more profound in the $CD56^-CD16^+$ NK cell subset, as it could not be overcome even after masking NK cell inhibitory receptors. The mature DC from HIV-infected persons produced less IL-12 and could not activate interacting NK cells. Consequently, these NK cells produce less IFN-γ. Defective NKp30- and TRAIL-mediated killing was blamed on the escape of the immature DC from NK cell-mediated killing in HIV-infected persons [167]. Aberrant NK cell/DC interactions may result from overall defective NK cell functions, depletion of certain functional NK cell subsets, and changes in the expression of NKRs and coreceptors. Certain viral proteins have also been shown to interfere in these interactions. It was demonstrated in in vitro studies that LFA-1-mediated activation of CAMK-II and microtubule rearrangement are essential for NK cell activation by mature DC. Tat inhibits this activation by interfering with Ca^{++} influxes and activation of CAMK-II. More specifically, the C-terminal domain of Tat was found to be responsible for this interference [150]. In another study, Nef was shown to dysregulate DC/NK

interactions. Nef-pulsed DC inhibit chemokine secretory capacity as well as the cytotoxic ability of NK cells, including the $CD56^{low}$ $CD16^{high}$ subset, possibly by inducing TGF-β and IL-10 [169].

NK cell/DC interactions determine T cell-priming characteristics of DC. For example, IFN-α-activated NK cells induce type 1 DC. These DC, which produce IL-12 upon stimulation via CD40, are efficient in priming TH1-type $CD4^+$ effector T cells. IFN-α is necessary for inducing this kind of helper function in NK cells [170]. Treatment of NK cells with IL-2 or polyinosinic:polycytidylic acid has similar effects [152, 171]. Improperly "helped" DC may induce tolerance in the interacting T cells and/or may cause their differentiation into suppressive Tregs. The DC, which fail to prime T cells, frequently express tryptophan-catabolizing enzyme indoleamine 2,3-dioxygenase (IDO), which converts tryptophan (an essential amino acid) into kynurenine and other catabolites [172, 173]. In the absence of tryptophan, T cells cannot proliferate. Furthermore, tryptophan catabolites induce apoptosis in T cells [174]. This results in decreased serum concentrations of tryptophan. It has also been shown that individuals with increased IDO activity are compromised in the production of 5-hydroxytryptamine in their brains. This mediator is important for signaling across neuron synapses. Its decreased production leads to decreased cognitive abilities, loss of memory, depression, and other psychiatric abnormalities. In the case of HCV-infected persons, it has been shown that decreased serum concentrations of tryptophan correlate with psychiatric symptoms in these patients. Decreased serum concentrations of tryptophan have also been reported in HIV-infected persons [175]. These concentrations correlate with neoptrin as well as with depression, psychiatric, and neurological symptoms. Abnormal NK/DC interaction could play a role in these manifestations.

As mentioned above, NK cells also interact with macrophages. NK cell/macrophages could play an important role in protecting host from pathogens (reviewed in ref. [176]). Nothing is known about NK cell/monocyte interactions in HIV infections.

Tregs are $CD4^+CD25^+FoxP3^+$ and glucocorticoid-induced TNFR-related $protein^+$ T cells known for their immunosuppressive properties. They can inhibit immune responses by suppressing T and NK cell functions. Enhanced numbers and functional activities of Tregs have been reported in the tissues of HIV-infected persons (reviewed in ref. [177]). It has been demonstrated that Tregs suppress NK cell functions. Activated Tregs express membrane-bound, functionally active TGF-β. In vitro incubation of Tregs with NK cells leads to down-regulated expression, which is TGF-β-dependent, of NKG2D and other activating receptors on NK cells (reviewed in ref. [178]). Depletion of Tregs may represent a novel way for enhancing NK cell functions in HIV-infected persons. It also leads to enhanced, HIV-specific CTL activity [177].

Dysregulating production of NK cell-activating cytokines in HIV-infected individuals

NK cells bear receptors for a variety of cytokines, e.g., IL-2, IL-12, IL-15, IL-18, IL-21, TGF-β, type I IFNs (IFN-α/β), etc. An optimum production of these cytokines is needed to maintain NK cell homeostasis and ready-to-kill state in the body. Several studies have shown that hosts (including humans) respond to a viral infection with the enhanced production of several cytokines, e.g., IFN α/β, IL-12, IL-15, and IL-18 [179, 180]. A coordinated production of these cytokines is essential for mediating an effective antiviral NK cell response of the host. Immediate activation of NK cells following a viral infection is, to a large extent, a consequence of this virus-mediated cytokine production. Each of these cytokines plays a distinct role in NK cell activation and expansion following a viral infection. In chronic viral infections, e.g., HIV-1, this coordinated production of cytokines is dysregulated, which may be responsible, at least in part, for defective NK cell responses. **Table 3** shows how these cytokines affect NK cell function and what happens to their production in this viral infection. HIV and its proteins play a role in the dysregulated production of cytokines. For example, gp120 induces IL-10, IFN-β, and TNF-α, and Tat induces TGF-β1 and IL-6 but inhibits IL-12 production in human PBMC [181–183]. The viral protein Nef induces IL-15 and decreases IL-18 production in the human cells [184, 185]. Overall, it has been well-documented that HIV-infected persons become compromised in their ability to produce IL-2, IL-12, IL-15, and IL-21 [179, 186–189]. Their

TABLE 3. Effects of Different Cytokines on Human NK Cells

Cytokine	Development Maturation	Cytotoxicity	Survival Homeostasis	Cytokine Production	Proliferation	Status in HIV[a]
IFN-α/β	–	↑	–/+	↑	–/+	↓
IL-1	–	↑	–/+	↑	–/+	↓
IL-2	–	↑	–/+	↑	↑	↓
IL-7	–	↑	+	↑	↑	↓
IL-4	D2	–/+	–	–/+	↑	↑
IL-10	–	↑	–	↑	–/+	↑
IL-12	D1	↑	↑	↑	↑	↓
IL-15	+	↑	↑	↑	↑	↓
IL-18	M	F	↑	↑	↑	↑
IL-21	M	↑, N	↑	↑	↑	↓
TGF-β	–	↓	↓	↓	↓	↑

It is noteworthy that cytokines usually act in synergism with each other; e.g., IL-2 and IL-12 act synergistically with IL-12 and IL-18, respectively. ↑, Increased effect, ↓, decreased effect; –, no effect; –/+, the cytokine may have an enhancing effect in combination with another cytokine; D1, differentiation into NK1; D2, differentiation into NK2; F, increased cytotoxicity as a result of increased expression of Fas ligand (FasL); N, decreased expression of NKG2D and decreased cytotoxicity against NKG2DL-positive target cells; M, cytokine needed for full maturation of NK cells. [a]Overall production in HIV-infected persons.

type I IFN-producing cells (pDC) also produce less of the cytokine and are progressively depleted [190, 191]. A lack of these cytokines affects differentiation, survival, and cytolytic functions of NK cells. On the other hand, the concentrations of some immunosuppressive cytokines, e.g., TGF-β and IL-10, are increased in the circulation of HIV-infected patients [192, 193]. Parato et al. [48] have proposed that increased IL-10 induces similar changes in NK cells as observed in HIV-infected persons. They observed a normalizing effect of HAART on IL-10 and NK cell phenotypes in a limited number of HIV-infected persons. Contrary to IL-12 and IL-15, whose production decreases in HIV-infected persons, we and others [194, 195] have reported increased concentrations of IL-18 in the sera from HIV-infected persons. Interestingly, the PBMC from these persons were found to produce less of this cytokine with or without stimulation with LPS. Interestingly, cells other than monocytes also produce the cytokine: Keratinocytes, adrenal cortex, and platelets also are rich sources of this cytokine. We have shown that activated platelets contribute toward increased concentrations of this cytokine in HIV-infected persons [196]. It is noteworthy that IL-18 concentrations also increase in chronic inflammatory conditions. The cytokine increases FasL expression on NK cells, and FasL-positive NK cells may be involved in fratricidal killing of other NK cells. The cytokine appears to hasten NK cell death via Fas/FasL interactions. In constrast to the concentrations of various cytokines, little is known whether there is any change in the expression of cytokine receptors on NK cells in HIV-infected patients. In this regard, a group has shown decreased expression of the IL-7R on NK cells in HIV-infected persons [197]. IL-7 promotes proliferation of the $CD56^{bright}$ subset of NK cells, which express this receptor. The viral protein Tat is known to down-regulate this receptor in $CD8^+$ T cells [198] and is probably also responsible for this effect in NK cells. The protein is released from HIV-infected cells and is actively taken up by other cells in the body. Another research group has documented that NK cells from HIV-infected persons do not respond to IFN-α [43]. The authors did not find out whether the lack of response was a result of a decrease in the cytokine receptor and/or a result of a defective signaling pathway of the cytokine. The decreased expression of cytokine receptors may adversely affect NK cell functions in a variety of ways: causing aberrant expression of NKRs, inducing apoptosis, etc.

Altering NK cell-secreted cytokines and chemokines in HIV infection

The profile of NK cell-secreted cytokines appears to be modified in HIV-infected persons. As stated above, NK cells are known to secrete several cytokines and soluble mediators: IFN-γ, TNF-α, TNF-β, GM-CSF, IL-3, IL-4, IL-5, TGF-β1, IL-10, IL-13, etc. They do so upon interaction with the target cells, which trigger NK cell cytotoxicity as well as upon activation with an appropriate combination of other cytokines, e.g., IL-12 and IL-15. Interestingly, IL-15 appears to be required by NK cells for their production of TH2-type cytokines [199–203]. NK cells also express constitutive, but not inducible, endothelial NO synthase (NOS) and secrete NO. Interestingly, NOS inhibitors can significantly inhibit functions of HNK cells [204]. Several studies have shown that the profile of NK cell-secreted cytokines depends on the milieu in which they develop and differentiate. In analogy to TH1- and TH2-type $CD4^+$ T cells, NK cells could differentiate into type 1 or type 2 NK cells (NK1 or NK2). NK1 cells predominantly secrete IFN-γ, whereas NK2 cells predominantly secrete IL-5 and IL-13 [205, 206]. It is noteworthy that existence of the two types of NK cells has been demonstrated in vivo in humans, and they may affect the course of certain disease conditions. For example, NK1 and NK2 cells have been associated with episodes of relapses and remissions in multiple sclerosis, respectively [207]. It has also been shown that NK2 cells play a role in the immunopathogenesis of asthma and in the maintenance of normal pregnancy in humans [208, 209].

Surprisingly, we could not come across any study in literature about the profile of NK cell-secreted cytokines in HIV-infected persons. However, Chan et al. [210] have shown that NK cells from these persons are of type 2. Their study relied on two cell surface markers belonging to the IL-1R superfamily, IL-18R and ST2L, which are expressed on the surface of cells producing TH1- and TH2-type cytokines, respectively [210, 211]. These results support earlier reports implicating TH2-type cytokine responses in the immunopathogenesis of AIDS [193, 212, 213]. NK cells may be contributing to the predominance of TH2 cytokine responses in HIV-infected AIDS patients. However, studies are needed to investigate NK cell-secreted cytokines in humans in the course of HIV infection.

In addition to cytokines, NK cells produce abundant amounts of several chemokines, e.g., CCL3 (MIP-1α), CCL4 (MIP-1β), and CCL5 (RANTES), which play an important role in initiating NK cell-mediated inflammation. These chemokines are also important in the context of HIV infection, as they bind to CCR5 and block entry of CCR5-using M-tropic HIV strains from entering into human cells. It is important to note that primary HIV infections usually result from M-tropic viral strains. This may also explain why persons with high-activity NK cells may be relatively protected from contracting HIV infections [12, 13]. It has been demonstrated that NK cells from HIV-infected individuals produce relatively less amounts of these chemokines and may be less efficient in blocking CCR5 and suppressing HIV replication [6, 8]. Not surprisingly, culture supernatants of NK cells from HIV-infected persons are less efficient in suppressing HIV replication than similar supernatants obtained from the cells of HIV-seronegative, healthy persons. Interestingly, viremia seems to directly suppress chemokine production from NK cells [11].

Infecting NK cells

Infecting the very immune cells that may inhibit viral replication is a clever strategy to evade host immunity. By infecting an immunocyte, the virus could cripple its immune effector functions. HIV-1 can infect many types of immune cells, e.g., $CD4^+$ T cells, macrophages, DC, etc. In vitro studies have shown that the virus can also infect NK cells [214, 215]. The $CD8^+$ NK cell subset was found to be more susceptible to HIV infection than the $CD8^-$ subset. The two cell subsets varied in the production of cytokines: the former producing more TNF-α and the latter producing more IFN-γ. This differential production of the cytokines was shown to be responsible for the differential susceptibility of the NK cell subsets to HIV infec-

tion [214, 215]. This preferential infection of $CD8^+$ NK cells with HIV-1 may also explain why the $CD8^+CD16^+$ NK cells are frequently depleted in the circulation of HIV-infected individuals [14, 27, 216]. The infected NK cells become impaired in their cytolytic functions. Remarkably, NK cells can also be infected with HIV with help from human herpesvirus 6 (HHV-6). The latter virus infects human NK cells and induces the expression of CD4 in these cells, rendering them susceptible to infection with HIV-1 [217]. It is noteworthy that HIV-infected individuals suffer from frequent reactivations of herpes viruses, and HHV-6 infection is considered an important cofactor in the development of AIDS. Moreover, CD8-tropic HIV-1 strains have also been isolated from HIV-infected AIDS patients. Interestingly, these strains use CD8 and not CD4 as a primary receptor in human cells and infect $CD8^+$ T cells [218]. Little is known about these CD8-tropic HIV strains. Theoretically, they could potentially infect $CD8^+$ NK cells. It is noteworthy that in vivo infection of NK cells in HIV-infected persons has also been demonstrated [219]. A small percentage (0.3–6.5%) of circulating $CD3^-CD56^+$ HNK cells expresses CD4 and HIV coreceptors, CXCR4 and CCR5. Proliferative activation of NK cells causes an increase in the expression of CD4 and CCR5 on these cells. $CD4^+$ NK cells can be infected in vitro with T- and M-tropic HIV-1 strains. A more efficient way of infecting NK cells is their coculture with HIV-infected cells. This suggests that in vivo, cell-to-cell infection of NK cells may be more important. NK cells seem to be relatively resistant to killing by HIV infection. The infected NK cells may persist in vivo despite treatment of the infected persons for several years with HAART [219–221]. Thus, NK cells may provide a sanctuary to HIV, and the virus-infected NK cells may represent important viral reservoirs. The virus may persist in these cells even when HAART may have reduced viremia to very low or undetectable levels. It may be relevant to mention here that NK cells express a higher level of P-glycoprotein compared with other lymphocytes. Therefore, HIV-infected NK cells may be relatively more resistant to antiretroviral drugs, e.g., protease and RT inhibitors [219, 222]. These findings have implications for therapeutic strategies being used for elimination of the virus from HIV-infected persons.

Enhancing apoptosis in NK cells

NK cells from HIV-infected individuals have a reduced capacity to proliferate upon in vitro culture. It is noteworthy that the expression of a senescence marker CD57 is significantly increased on the surface of NK cells in HIV-infected persons. NK cells also undergo enhanced, spontaneous apoptosis as compared with the cells from healthy, control subjects. The enhanced apoptosis was ascribed to their relatively low expression of the antiapoptotic proteins Bcl-2 and Bcl-X_L. It has been shown that Tat induces TGF-β and apoptosis in NK cells. It also down-regulates Bcl-2 expression in other hematopoietic cells [183, 223]. IL-10 is known to enhance serum starvation-induced apoptosis in human cells by decreasing transcription of antiapoptotic proteins Bcl-2 and Bcl-X_L. As mentioned above, increased concentrations of this cytokine in the circulation of HIV-infected persons have been well documented. The viral glycoprotein gp120 from T-tropic viral strains has also been shown to increase expression of proapoptotic genes and decrease expression of antiapoptotic genes in NK cells [224]. In this connection, another study has shown that gp120 interaction with the viral coreceptor CXCR4 induces cell death via autophagy: a kind of programmed cell death in which large chunks of cellular material and cytoplasmic organelles are degraded in lysosomes [225]. NK cells constitutively express this receptor, and its interaction with gp120, which is present in virions and/or the circulation, may induce autophagic death of NK cells. As mentioned above, recombinant gp120 has, in fact, been shown to induce up-regulation of several proapoptotic genes in NK cells [224]. So, it is not surprising that viremia is associated with decreased NK cell numbers as well as with decreased functional capability of NK cells in HIV-infected persons [1].

Addition of the prosurvival cytokine IL-15 to in vitro NK cell (and T) cell cultures increases their survival by up-regulating the expression of Bcl-X_L [226]. In normal NK cells, which constitutively express high amounts of Bcl-2 and Bcl-2-like proteins, IL-15 increases NK survival by down-regulating Bim and maintaining antiapoptotic protein Mcl-1 [227]. Bim is the only-BH-3 domain-containing, proapoptotic member of the Bcl-2 family of proteins. It binds with and inactivates Mcl-1, another member of the Bcl-2 family having antiapoptotic functions (reviewed in ref. [228]). Recombinant human IL-15 may represent a useful immunotherapeutic tool and vaccine adjuvant for HIV-infected AIDS patients because of its prosurvival and antiapoptotic effects on NK cells, less toxicity, and minimal enhancement of HIV replication (reviewed in ref. [229]).

A small proportion of NK cells from normal, healthy persons undergoes apoptosis when they are used as effector cells in in vitro NK cell cytotoxicity or ADCC assays [230, 231]. It has also been demonstrated that NK cells can undergo apoptosis after activation, as in the case of T cells. For example, IL-2 and IL-12-stimulated NK cells undergo apoptosis when they were incubated with immobilized antibodies directed against CD16, CD2, or CD94 [232–234]. It was also learned that incubation of NK cells with high concentrations of certain activating cytokines, e.g., IL-15 and IL-12, induced production of TNF-α, which caused apoptosis of NK cells [235]. In fact, it is a negative-feedback mechanism by which NK cells control and limit self-activation and secretion of IFN-γ. As mentioned above, we and others [194, 195] have reported increased concentrations of IL-18 in the sera from HIV-infected persons. The cytokine induces FasL expression on NK cells, which could lead to fratricidal killing of NK cells via Fas/FasL interactions. This may explain a negative correlation between serum concentrations of IL-18 and NK cell numbers reported in patients suffering from chronic autoimmune disorders [236]. Indeed, we have also observed a significant negative correlation between serum IL-18 concentrations and NK cell numbers in these individuals (unpublished data). These studies suggest that IL-18 may be associated with compromised NK cell functions in HIV infections. The HIV protein Tat, secreted from HIV-infected cells, has been shown to induce FasL expression on NK cells and CTL [237]. It is tempting to speculate that Tat and IL-18 may act in concert to induce FasL expression on NK and CTL in HIV-infected persons. Fas/FasL interactions have

been implicated in the immunopathogenesis of AIDS in HIV infection (reviewed in ref. [238]).

NK CELL FUNCTIONS BECOME COMPROMISED IN HIV-INFECTED PERSONS

Although humans respond to HIV infection with activation of their NK cells, the virus uses many different strategies to neutralize this host response. As a consequence, NK cell function becomes compromised in these infections. Several workers have reported that NK cell functions (killing of target cells, ADCC effector function, editing of DC, and production of cytokines and chemokines) become defective in HIV-infected persons [26, 33, 34, 37, 38, 63, 239, 240]. The defects in the NK cell compartment usually occur in early stages of the infection. A decreased expression of LFA-1 on cells from HIV-infected persons has been reported [241]. Furthermore, it has also been shown that immune activation leads to an increase in shedding of soluble ICAMs and CD16 in the circulation of HIV-infected persons. The soluble forms of these molecules interfere with their membrane-inserted forms. The role of LFA-1 and its ligands in cell adhesion, conjugate formation, and polarization of cytotoxic granules is crucial for NK cell-mediated killing. Thus, NK cells from HIV-infected persons may be impaired in their ability to form immune synapses with target cells. Furthermore, the HIV protein Tat was found to inhibit NK cell-mediated lysis by blocking L-type Ca^{++} channels [242]. Ca^{++} infuxes are essential for activation of CAMK-II, rearranging microtubules and triggering degranulation of NK cells following activation of cells via LFA-1 [150]. Furthermore, gp120 binding to CD4 also inhibits LFA-1-mediated cell–cell interactions by causing dissociation of the integrin from its cytoplasmic partner cytohesin [243]. Few studies have been undertaken to investigate functional capabilities of NK cells from these persons for conjugate formation and triggering their cytolytic machinery. It was demonstrated that NK cells from the infected persons may form conjugates with target cells but are defective in triggering their cytolytic mediators onto the target cells [19, 24]. The inability of NK cells from HIV-infected individuals to establish and maintain an effective immune synapse and trigger its cytolytic mediators may represent a fundamental reason for compromised NK cell functions in HIV-infected persons.

It appears that absolute numbers and percentages of NK cells decrease over time in HIV-infected persons. $CD8^+CD16^+$ and $CD56^+CD16^+$ NK cell subsets have been reported to decrease in percentages and in absolute numbers in these individuals. These decreases are often accompanied by the expansion of a functionally defective subset of $CD16^+CD56^-$ NK cells, which express KIR. It is noteworthy that it is the $CD16^-CD56^+$ subset of NK cells that expands in primary viral infections. The changes in NK cells are more severe with the onset of AIDS and correlate with clinical condition of the patients [27, 32, 35, 216]. The decreases in NK cell subsets correlate significantly with depletion of the $CD4^+$ T cells in these patients [14, 16], suggesting that $CD4^+$ T cell-secreted cytokines (e.g., IL-2, IL-21) may be important in vivo in maintaining NK cell survival. Alternatively, the declines in the numbers of these two types of immune cells may reflect immune dysfunction independently of each other. It may be relevant to mention here that $CD56^+$ NK cells develop and differentiate in thymus and secondary lymphoid organs in T cell-rich areas. A progressive destruction of the architecture of these organs as well as depletion of $CD4^+$ T cells in HIV-infected persons may result in depletion of this subset of NK cells. Overall, NK cells from HIV-infected persons express lower levels of perforin and higher levels of SHIP, which may be responsible for their poor cytolytic and activating potentials [58]. As mentioned elsewhere, the envelope glycoproteins of HIV can up-regulate proapoptotic genes and reduce survivability and vigor of NK cells (see the next section). Recombinant gp120 inhibits NK cell functions when added to in vitro microcytotoxicity assays. Furthermore, certain peptides derived from the protein also have NK cell inhibitory properties [80, 244, 245]. The exact mechanism of inhibition of the peptides remains unknown. Finally, stress could be a factor in suppressing NK cell functions in HIV-infected persons. Cortisol has been shown to act in synergism with HIV proteins in mediating the suppressive effects on NK cells [244].

In vitro studies have shown that several exogenous cytokines, e.g., IL-2, IL-12, IL-15, IFN-α, etc., increase cytolytic and ADCC effector function of NK cells from HIV-infected individuals. However, the responses were significantly lower in HIV-infected individuals as compared with HIV-seronegative, healthy controls [19, 34, 43, 239, 246]. These observations suggest that NK cells from HIV-infected persons may have decreased expression of cytokine receptors and/or may have defects in cytokine-induced signaling pathways. This may explain why NK cells from these patients produce defective LAK cells when they are incubated with cytokines, e.g., with IL-2 [25].

EFFECT OF HAART ON ANTIVIRAL NK CELL RESPONSES

HAART suppresses HIV replication to undetectable limits in the circulation of HIV-infected persons. Over time, this leads to improvement in the NK cell functions. However, a prolonged treatment is needed for tangible improvements in the NK cell compartment. In most of cases, the recovery is only partial. NK cells and the receptor expression tend to normalize in the treated persons; however, certain NK cell functions, e.g., their ability to produce IFN-γ in response to IL-2 and IL-15, remain compromised [50]. In one study, HAART reversed expression of iKIR on NK cells after 2 years' administration, but the reduced expression of activating receptors persisted [51]. Similarly, a normalizing effect of HAART was observed on the expression of 2B4 on NK cells [56]. HAART, for more than 6 months, caused a differential disappearance of iKIR on virus-specific CTL but usually had no effect on ILT-2 expression [45]. HAART also does not have any effect on the expansion of NKG2C on NK cells and CTL in HIV- and HCMV-coinfected patients. As mentioned earlier, this can be ascribed to the fact that HCMV and not HIV causes expansion of these cells [115, 116, 247]. In primary HIV infection, an early start of HAART may normalize changes in the NK cell compartment within 6

months [61]. The baseline activation of the immune system and viral load determines the extent to which innate immune parameters could be reconstituted by HAART in HIV-infected AIDS patients. Continued viral suppression and reduction in immune activation for more than 1 year resulted in recovery of pDC, better NK/DC interactions, and partial restoration of NK cell numbers and functions [248].

If NK cells become infected, they may act as latent reservoirs for the virus, as the infection could persist in these cells even after years of HAART [219, 221]. Thus, immunotherapy should be considered for invigorating NK cell responses along with chemotherapy.

NOVEL APPROACHES FOR ENHANCING ANTI-HIV NK CELL RESPONSES

A better understanding of interactions between HIV and NK cell responses of the host has led to novel, rational approaches for boosting antiviral immunity in HIV-infected persons and for designing more effective anti-AIDS vaccines. These approaches are listed in **Table 4** and are discussed in the following sections.

Blocking inhibitory NKRs

As stated above, KIR, NKG2A, and ILT-2 are the main inhibitory receptors that control NK cell activities in an individual. They are also expressed on a subset of antigen-experienced effector/memory CTL, in which they increase the antigen-mediated activation threshold. Cumulative data have shown that the expression of iKIR increases on NK cells and CTL in HIV-infected persons, especially under viremic conditions [16, 40, 148]. Blocking the functional activities of these receptors with receptor- or MHC-specific antibodies or with small molecular weight inhibitors increases cytolytic activities and cytokine secretion from NK cells and CTL. Studies in animal models have shown that blocking of the inhibitory NKR LY49 in vivo also augments the anti-tumor effects of NK cells and CTL and results in tumor regression [143]. This strategy may boost antiviral effects of NK cells and CTL in HIV-infected individuals. In this regard, in vitro studies have shown that masking of iKIR by mAb increases the cytolytic activities of HIV-specific CTL from HIV-infected patients against autologous, virus-infected cells [40]. The receptors could also be blocked by soluble MHC antigens. However, they are more likely to bind TCR preferentially than KIR, and hence, they may block CTL functions. Small molecular weight chemical compounds could be synthesized to specifically block KIR–MHC interactions. These immunotherapies will have to be tailored individually, as the patients may differ in their KIR–HLA combination repertoires. As the blocking of inhibitory receptors on NK cells and CTL may promote killing of autologous cells and uncontrolled cytokine production, the treatment could cause immunopathology. Furthermore, the strategy may interfere with the development of long-term, virus-specific memory and even may promote apoptosis of these effector/memory cells. Admittedly, such treatments could be risky, and the treated patients will have to be carefully monitored for any untoward effects.

TABLE 4. Novel Ways of Boosting Anti-HIV Adaptive and Innate Immunity

1. By manipulating the activity of NKRs.
2. By adoptive transfer of autologous or alloreactive NK cells.
3. By the adoptive transfer of HLA-C- and HLA-E-restricted, antiviral CTL.
4. Using HLA-C- and -E-restricted, immunogenic viral peptides.
5. Using NK cell activity-enhancing, novel adjuvants.
6. Targeting NK cells to HIV-infected cells.

Novel ways of anti-HIV cell therapy

In the past, the infusions of the in vitro-expanded, autologous, HIV-specific CTL have been used as immunotherapeutic tools in HIV-infected AIDS patients without much success [249, 250]. A better strategy may involve expansion and infusion of the CTL specific for HLA-C-restricted viral peptides, as the virus does not down-regulate the expression of this MHC antigen in the infected cells. Similarly, in vitro-expanded, lymphokine-activated, autologous NK cell clones that express inhibitory receptors for HLA-A or -B but not for HLA-C or -E could be considered as immunotherapeutic tools in these patients. These cells should kill only HIV-infected cells that have down-regulated HLA-A and -B but not HLA-C or -E. Similarly, lymphokine-treated, heterologous NK cells that express one or more iKIR specific for the recipient HLA-A or -B could also be beneficial. Alloreactive NK cells are known to preferentially kill hematopoietic cells in MHC-disparate recipients without causing graft-versus-host disease (GvHD). The beneficial effects of alloreactive NK cells have been well documented in leukemia patients receiving bone marrow transplants (reviewed in refs. [251, 252]). The potential of alloreactive NK cells as therapeutic tools for viral infections including HIV is worth investigation.

Selecting epitopes for anti-HIV vaccination

As stated above, HIV-1 differentially down-regulates the expression of MHC class I antigens on the surface of infected cells for evading NK and CTL-mediated killing. The viral protein Nef causes degradation of most of the HLA-A, HLA-B, and CD1d antigens but leaves HLA-C and HLA-E to intact on the cell surface [96–98]. As HLA-C and -E act as ligands for inhibitory receptors on NK cells, the virus-infected cells maintain their resistance to NK cells by maintaining their expression on the surface of infected cells. The virus, in fact, increases the expression of HLA-E by providing a peptide (within the viral protein p24) that can bind to this nonclassical MHC antigen. It is noteworthy that HLA-C is not exclusively used as ligands for NKRs. Several HIV peptides are presented to T cells via this MHC antigen [253–255]. These peptides may serve as better immunogens for inducing anti-HIV CTL, as HLA-C are not degraded from the surface of the virus-infected cells, and therefore, virus may not be able to hide from the peptide-specific CTL. The notion is supported by the reported association between the presence of HLA-C-restricted viral peptides in HIV-infected individuals and their long-term nonprogression toward AIDS [256]. Furthermore, the existence of

CTL, which recognize HLA-E-restricted viral peptides, has also been demonstrated for different viruses [257]. We could not find any study in literature about HLA-E-restricted HIV peptides presented to CTL. These HIV peptides could also be considered as immunogens for vaccination against HIV.

It is noteworthy that HLA-A and -B antigens mainly present viral peptides recognized by HIV-specific CTL. Many studies have shown that several "protective" HLA-B allotypes can present broadly reactive, immunodominant peptides to CTL [258, 259]. On the other hand, only a few HLA-C-restricted HIV epitopes have been described [253–255]. In part, it could be a result of the fact that this HLA antigen is expressed at relatively lower levels on human cells [260]. CTL may not be able to detect the peptide-complexed antigen. Using knowledge-based algorithms, Tong et al. [261] have shown that HLA-C-restricted peptides could be found in most HIV proteins. Further studies are needed to evaluate the functional significance of these epitopes.

Invigorating NK cells with cytokines/anticytokines

Use of cytokines for enhancing innate and adaptive immunity of the host has been a cherished goal of immunologists since the discovery of IL-2 in the early 1980s. However, the toxicities associated with their use have always been prohibitive. Potential cytokines that can be used to enhance NK cell activity in vivo include IL-2, IL-15, IL-21, as well as ligands for c-Kit and FMS-like receptor tyrosine kinases (Flt-3). It may be relevant to mention here that IL-2 and/or IL-2-activated killer cell infusions have not been promising as therapeutic tools in cancer patients [262, 263]. In the context of HIV infection, these immune enhancers may pose another complication. They may increase HIV replication and act as paracrine growth factors in AIDS-related malignancies. In this regard, IL-15 has been shown to be relatively less mitogenic and less toxic and to have minimal effects on HIV replication. Furthermore, it inhibits spontaneous apoptosis in NK cells and CTL from HIV-infected patients by increasing the expression of antiapoptotic protein Bcl-X_L (reviewed in ref. [229]). The cytokine is an absolute necessity for normal development, differentiation, and homeostasis of HNK cells. IL-21 is another relatively recently discovered cytokine produced mainly from activated $CD4^+$ T cells. It increases cytolytic potential of NK cells and is even less mitogenic than IL-15. However, no data are yet available about its effects on HIV replication and cytotoxicity.

Enhancing immunogenicity of viral immunogens

NK cell activation in the beginning of a viral infection has a strong adjuvant effect. Activated NK cells kill virus-infected cells, whose products send a "danger signal" to the host for initiating antiviral inflammatory and immune responses [264]. The role of NK cell-secreted IFN-γ in this connection has been well documented. The studies about interactions between activated NK cells and DC interactions also testify to the role of NK cells in the generation of adaptive immunity. DC pulsed with tumor cell lysates are effective in mediating anti-tumor immunity in vitro and in vivo in animal models. It has been shown that these DC mediate these adjuvant effects by activating NK cells [265]. It was also demonstrated that the presence of IL-18 in in vitro cultures of NK cells, DC, T cells, and tumor cells leads to rapid generation of tumor-specific CTL [266]. These studies show that activating NK cells at or prior to immunization may lead to effective antiviral immunity. This activation may be achieved by cytokines and/or TLR agonists, which also cause release of cytokines. α-Galactosyl ceramide has also been used as an adjuvant. It is presented by APC via CD1d to NKT cells, which in turn, activate NK cells [267, 268]. Based on our present understanding of NK cell biology, inhibiting KIR–MHC interactions and/or inducing expression of ligands for activating NKRs may produce better adjuvant effects than our currently used adjuvant formulations in vaccination regimens. Indeed, better antigen-specific, immune responses were induced when vectors expressing ligands for NKG2D were used along with immunogen [269]. These novel approaches should contribute to better and more effective vaccine strategies against HIV infection and AIDS.

PERSPECTIVE AND FUTURE DIRECTIONS

Despite spectacular advances made in understanding NK cell biology, there still remain unknown aspects of these cells, which should be addressed in future research. For example, we are still far from discovering all NKRs. An area that needs immediate attention concerns finding ligands for aKIR and NCR. Furthermore, we need to know better how the NKR repertoire of the host is shaped and what effects the host MHC has in shaping this repertoire. A lot has been learned about interactions between NK cells and DC. It appears that NK cells could also interact directly with T cells. This could be an extremely productive area of research.

It is now evident from several studies that different NKRs, particularly of the inhibitory type (e.g., KIR, ILT, KLR-G1), are expressed frequently on antigen-experienced $CD8^+$ T cells and less frequently on $CD4^+$ T cells. These receptors seem to be expressed at distinct stages in the course of differentiation and development of these cells. They may serve important functions; e.g., they may prevent apoptosis and increase survival of the cells and/or may increase the activation threshold of the effector cells to prevent autoaggression. These receptors could serve as important markers to distinguish different developmental stages of these cells. This knowledge may allow us to identify exact defects, which appear in these cells in viral infections and malignancy.

Activating NKRs of the KIR family have been reported to occur on $CD4^+$ T cells under certain disease conditions. Their expression has been described on CTL in HIV-infected viremic persons [62]. It is not known what triggers their expression and what are the consequences of this expression—how the receptor-positive cells differ from the receptor-negative cells in terms of their proliferation, cytokine production, and interaction with other cells in the body. It would also be of great interest to see if and how HIV induces the expression of their ligands on infected human cells.

In the context of HIV infections, future efforts should be directed at knowing which of the NKRs are aberrantly expressed on the surface of NK cells as well as on other immu-

nocytes, e.g., monocyte/macrophages, DC, and B and T cells ($CD4^+$ and $CD8^+$ subsets). As mAb are not available for all of these receptors, and the ones that exist may not distinguish between the activating and inhibitory forms of these receptors, therefore, one may have to use alternate methods. Fortunately, the NKR genes, which have been studied so far, seem to be regulated at the transcriptional level. This suggests that real-time RT-PCR and/or oligonucleotide microarrays with appropriate controls may give a fair idea of the genes whose expression may be dysregulated in HIV-infected individuals.

As discussed above, modulation of interactions between NKRs and their ligands may represent an important tool of immunotherapy. Studies should be performed in animal models to see the long-term effects of these interventions on the resistance of the host to pathogens and development of tumors. Small, antagonist chemical molecules, peptides, and humanized receptor-specific mAb should be developed for their potential use in boosting innate and adaptive immunity in HIV-infected individuals.

We also need to develop innovative means to target NK cells toward HIV-infected cells. In this connection, fusion proteins combining intracellular ζ chains with the extracellular region of CD4 or with HIV-specific single-chain antibody have been developed. Transduction of these fusion proteins into primary human NK cells via retroviral vectors redirects their killing toward HIV-infected cells [270]. Another group has made a fusion protein combining gp120-specific antibodies of IgA and IgG isotypes. It is meant to kill HIV-infected cells by linking the viral envelope protein with FcR on NK and other immune cells [271]. Finally, the potential of alloreactive NK cells as therapeutic tools in viral infections, particularly with HIV, is worth exploring. These cells have benefited leukemia patients undergoing bone marrow transplantation and do not seem to cause GvHD (reviewed in ref. [252]). Fortunately, now, the technology exists for obtaining fully differentiated and functional NK cells from human stem cells [272].

Invigorating and activating NK cells may benefit HIV-infected persons in controlling the infection. However, it should not be forgotten that activated NK cells are equipped with a lot of destructive potential. Their excessive activation may cause tissue destruction and contribute toward pathogenesis of the disease. Therefore, NK cell activity-enhancing treatments will have to be closely monitored for undesirable consequences.

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CHAPITRE 2. HYPOTHÈSE ET OBJECTIFS

2.1 HYPOTHÈSE

Lors de cette étude nous avons proposé les hypothèses suivantes :

1. Il existe une relation entre l'IL-18 et le nombre des cellules NK chez les personnes infectées par le VIH.

2. Il y a une différente régulation de l'IL-18BP et de l'IL-18 circulant chez les patients infectés par le VIH.

3. L'IL-18 joue un rôle important sur l'homéostasie des cellules NK

4. L'IL-18 cause l'apoptose des cellules NK par la voie Fas/FasL

2.2 OBJECTIFS

L'objectif global de cette présente étude est d'approfondir les connaissances sur le rôle de l'IL-18 dans les infections par le HIV. Donc les objectifs spécifiques sont d'étudier :

1. S'il existe une corrélation entre la concentration d'IL-18 et le nombre et les fonctions des cellules NK chez les personnes infectées par le VIH.
2. L'effet potentiel de l'IL-18 sur les cellules NK.
3. L'effet potentiel de l'IL-18 sur la réplication virale du VIH.
4. La production de l'IL-18BP chez les personnes infectées par le VIH.

En dépit de l'effet pro inflammatoire de l'IL-18 qui peut réguler les réponses immunitaires innées et adaptatives. Cette cytokine est contrôlée par son antagoniste IL-18BP. Relativement, à nos études qui ont démontré des niveaux élevés de l'IL-18 chez les patients infectés par le VIH. Cependant, aucune étude à ce jour n'a été réalisée concernant la production de l'Il-18BP chez ces patients. Due à sa relevance dans la régulation de l'IL-18 nous avons étudié l'effet de l'infection par le VIH sur l'équilibre entre ces deux facteurs et l'impact de cet équilibre sur l'homéostasie des cellules NK. En vue de l'importance de l'équilibre IL-18/IL-18BP, le déséquilibre de ce ratio peut contribue vers l'immunopathogénèse de la maladie de l'immunodéficience, tout en diminuant les fonctions des cellules NK, aussi bien en augmentant la réplication virale.

CHAPITRE 3. RÉSULTATS

Les résultats obtenus de ces expériences sont présentés sous le format d'un article de recherche présenté dans les pages qui suivent.

3.1 Article 4:

3.1.1 Potential role of IL-18 in the immunopathogenesis of AIDS: Involvement in fratricidal killing of NK cells and enhanced HIV replication.

Iannello.A, Samarani.S, Debbech.O, Ahmad.R, Boulassel.MR, Tremblay.C, Toma.E, Routy.JP, and Ahmad.A. 2009. Journal of Virology. 83(12): 5999-6010

RÉSUMÉ

Le virus de l'immunodéficience humaine ou VIH est l'agent qui cause le SIDA. Le VIH donne lieu à une dérégulation dans la production de certaines cytokines qui ont un rôle immunologique très important chez les patients infectés. L'IL-18 est une de ces cytokines, qui a un effet pro inflammatoire très puissant, et qui peut réguler les deux systèmes immunitaires, innées et adaptatifs. Son activité est strictement régulée in vivo par "*IL-18Binding Protein* "(IL-18BP), une cytokine qui se lie spécifiquement et neutralise IL-18 dont elle a une très forte affinité. Des études ultérieures ont montré que la concentration d'IL-18 augmente de façon très significative dans la circulation des patients Sidéens infectés par le VIH comparé à un nombre similaire de personnes saines. Cependant, il n'est pas clair jusqu'à maintenant comment le niveau élevé de cette cytokine affecte le développement

de la maladie chez les patients infectés par le VIH. Concernant la production de l'antagoniste IL-18BP, jusqu'à date nos connaissances sont limitées chez ces patients. Alors, ces issues ont été abordées dans les études présentées dans ce mémoire. Nous avons mesuré le taux de l'IL-18 et de l'IL-18BP dans le sérum des patients infectés par le VIH, en utilisant des Kits d'ELISA commerciaux, puis en les comparants avec le même nombre des personnes en santé qui sont séronégatives. Nous avons déterminé aussi, le nombre total et absolu des différents sous-types des cellules NK (*Natural Killer*) et l'activité des cellules NK chez les cellules mononuclées du sang périphérique. Finalement nous avons déterminé les effets de l'IL-18 humaine recombinante, aussi bien que le sérum très riche en IL-18 provenant des patients sidéens, sur la survie et l'effet cytolytique des cellules NK humaines. Nos résultats démontrent que les patients infectés par le VIH ont trois fois plus de l'IL-18 que les donneurs sains. Cependant le niveau de l'IL-18 BP est plus bas chez les patients infectés comparés aux donneurs sains. Ce qui résulte d'une augmentation de l'IL-18 libre qui est biologiquement active. Nos résultats démontrent qu'il y a une corrélation négative entre la concentration de l'IL-18 et le nombre des cellules NK, aussi bien que leur activité cytotoxique. Donc, ces résultats suggèrent l'implication de l'IL-18 dans l'apparition des cellules NK. Ceci nous incite à voir l'effet cytocidal potentiel de l'IL-18 sur les cellules NK. Nos résultats *in vitro* montrent que l'IL-18 humaine recombinante et le sérum de patients malades, causent l'apoptose dans la lignée cellulaire, aussi bien que les cellules primaires des cellules NK. L'anticorps antagoniste anti Fas Ligand peut inhiber cette mort cellulaire. De même, *in vitro*, L'IL-18 stimule la transcription du promoteur de Fas Ligand. Cette cytokine, augmente la susceptibilité de la mort des cellules NK mediés par Fas, et induisant l'expression de la protéine anti apoptotique Bcl-XL. Finalement, nos études nous permettent de conclure, que l'augmentation de la bio activité d'IL-18 chez les patients infectés cause le dérèglement de l'homéostasie des cellules NK qui pourrait donc contribuer à la pathogénèse du SIDA.

Contribution

J'ai exécuté les expériences et analysé les résultats. J'étais également impliqué dans la rédaction de cet article**.**

N.B : Une petite moitié des résultats fait désormais partie d'une autre publication (Iannello A et al. 2009, « HIV causes imbalance in production of interleukin-18 and its natural antagonist in HIV-infected AIDS patients: implications for enhanced viral replication »), qui a été soumise au Journal of Infectious Diseases et accepté provisoirement. L'article n'est pas inclut dans ce mémoire.

Severe imbalance between interleukin-18 and interleukin-18 binding protein in patients with HIV/AIDS: implication for enhanced viral replication

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Running title: IL-18/IL-18BP in HIV-infected patients

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Abstract

Interleukin-18 (IL-18) is a proinflammatory cytokine playing an important role in immune responses against viruses and is regulated by a neutralizing cytokine named interleukin-18 binding protein (IL-18BP). In order to study the balance between these two factos in HIV-infected AIDS patients, we measured IL-18 and IL-18BP in the sera of thirty-two HIV-infected AIDS patients and compared them with a similar number of sera from age-matched HIV-seronegative healthy subjects. Confirming earlier results, our data show that sera from HIV-infected patients had 2.5 to 3 fold higher levels of IL-18 as compared to the sera from healthy subjects. In contrast to this, the levels of IL-18BP were significantly decreased in the sera from HIV-infected persons as compared to healthy subjects. Consequently the mean ratio between IL-18 and IL-18BP was significantly increased in the infected subjects leading to elevated concentrations of biologically active free IL-18. We show here that in vitro infection of monocyte-derived macrophages with HIV resulted on elevated IL-18 production and reduced production of IL-18BP leading to increased IL-18/IL-18BP ratio and concentrations of biologically active free IL-18. We also demonstrate here the impact of cytokines treament on IL-18BP production by monocyte-derived macrophages with or without in vitro infection with HIV. We further show that IL-18 had increasing effect on HIV replication in isolated human $CD4^+$ T cells. This uncoordinated production of IL-18 and IL-18BP may contribute further to IL-18-induced viral replication and increase the HIV-induced pathogenesis.

Key words: Interleukin-18, Interleukin-18 binding protein, HIV, AIDS, innate immunity

Introduction

Interleukin-18 (IL-18), originally discovered and named as the IFN-g-inducing factor, is a multifunctional and pleiotropic cytokine, which acts in synergism with other cytokines (e.g. IL-12 and IL-15) to induce IFN-g from T and natural killer cells [Dinarello, 1999]. This cytokine is mainly produced by activated macrophages but also by many other cell types like dendritic cells, Kupffer cells, keratinocytes, and enterocytes as well as by the adrenal cortex and neurohypophysis. In the circulation, IL-18 is bound specifically to a natural antagonist, IL-18 binding protein (IL-18BP) and for the most part is functionally inactive. IL-18BP is constitutively produced from a wide variety of body cells and has much higher affinity for IL-18 than what IL-18 has for its receptor (12-14). The protein exists in humans in four different isoforms, designated as “a”, “b”, “c” and “d”, that result from alternate splicing of the IL-18BP mRNA. Of these isoforms, the “a” isoform accounts for the major IL-18-neutralizing activity in the circulation in humans. Given that IL-18 is a proinflammatory cytokine, it is not surprising that increased concentrations of this cytokine have been reported in many chronic inflammatory conditions in humans, e.g., Rheumatoid Arthritis, Mutiple Sclerosis, Crohn’s disease, Graft-versus-Host disease, atherosclerosis, etc (4).

Human Immunodeficiency Virus type 1 (HIV-1) is the etiological agent of AIDS in humans. The infections with this virus are usually accompanied by changes in the production of several immunologically important cytokines, e.g., IL-15, TNF-α, IL-4, IL-12, IL-10, TGF-β1, etc (15-17); reviewed in (18-20)). These cytokine disturbances play an important role in the immunopathogenesis of AIDS in HIV-infected persons. Concerning IL-18, we and others have demonstrated increased concentrations of this cytokine in HIV-infected persons ((21-23); see ref (24) for a review). The Simian Immunodeficiency Virus (SIV), which is a close cousin of HIV-1, also induces IL-18 production in Rhesus monkeys (25). However no information exists concerning serum concentrations of IL-18BP in these patients. Since in the circulation, IL-18BP binds and inactivates IL-18, it would be important to know the status of its concentrations in the circulation of HIV-infected persons. A concomitant increase in its concentrations would offset the biological effects of the increased IL-18 concentrations observed in the sera of HIV-infected persons. On the other hand, if IL-18BP production decreases in these patients, this would translate in enhance concentrations of free and biologically active IL-18. We addressed this issue in this study and show that the two cytokines are quite differentially regulated in HIV-infected

AIDS patients. We show here that IL-18 levels increase while those of IL-18BPa decrease in the sera from the infected persons. The serum levels of the two cytokines show significant positive correlation in the control subjects when this correlation was not observed in sera from HIV-infected AIDS patients. Consequently the mean ratio between IL-18 and IL-18BP was significantly increased in the infected subjects leading to increased levels of free IL-18 in HIV/AIDS patients. We provide experimental evidences that in vitro infection of monocyte-derived macrophages (MDM)with HIV results on increased IL-18 levels and reduced production of IL-18BP leading to increased IL-18/IL-18BP ratio and concentrations of free IL-18. We also show here the impact of cytokines treament on IL-18BP production by MDM infected or not with HIV. We finally show that IL-18 had increasing effect on HIV replication in isolated human $CD4^+$ T cells. The increase in IL-18 production accompanied by a relative decrease in the production of IL-18BP represents a severe imbalance between these two soluble mediators in HIV-infected patients.

Materials and Methods

Cell culture

All cells used in this study were cultured at 37°C in 5% CO_2 humidified atmosphere. The PBMC were obtained from the peripheral blood from HIV-seronegative healthy subjects. For this purpose, blood was collected in heparinized vacuum tubes and PBMC were isolated by centrifugation over Ficoll-Hypaque (Pharmacia, Montreal, Quebec, Canada) as described earlier. The buffy coat at the interface of Ficoll-Hypaque and plasma was collected, washed, and resuspended in RPM1-1640 medium containing 10% FCS, 2 mM L-glutamate, 100 μg/ml penicillin, and 100 μg/ml streptomycin as described previously. To generate monocyte-derived macrophages, monocytes present in total PBMC preparations were isolated by adherence to the plate and differentiated in macrophages in culturing them with RPMI 10% FCS, 5% human AB serum and 2 ng/ml GM-CSF for 5 days.

Antibodies and recombinant cytokines

The recombinant human cytokines used for this study were purchased: rhIL-2 from (), rhIL-18 from Biosource Inc. (Camarillo, CA), rhIL-10 from (), rhTGF-β from (), rhIL-12 from (), rhGM-CSF from (), Phytohemagglutinin (PHA) was purchased from Sigma-Aldrich (Saint-Louis, MI).

Patients

Peripheral blood was obtained for serum collection from 32 HIV-infected persons of both genders from local AIDS clinics after their written informed consent. All the patients had one or more AIDS-defining condition and were receiving HAART that comprised at least one protease inhibitor (saquinavir, ritonavir, or indinavir) and one or two reverse transcriptase inhibitors (D4T, 3TC, AZT or didanosine). Blood samples from 32 age-matched HIV-seronegative healthy persons were used as controls. Serum was obtained from each blood sample after letting it clot at room temperature for 2 to 3 h. Each serum sample was aliquotted and stored at −80°C until used.

Measuring IL-18 and IL-18BPa concentrations

The concentrations of IL-18 and IL-18BPa were determined in serum samples and experimental samples by using commercial ELISA kits from Bender Medsystems (Burlingame, CA) and R&D Systems (Minneapolis, MN), respectively. The detection limits for these kits are 12 pg and 60 pg/ml, respectively. The kit for IL-18 measures the total IL-18 (both free as well as IL-18BP-bound forms).

HIV-1 replication assay

PBMC from healthy HIV-seronegative donors were activated with 100 U/ml IL-2 and 10 μg PHA/ml in the culture medium for 3 days at 37°C in humidified 5% CO_2 atmosphere. $CD4^+$ T cells were isolated by negative selection using a commercially available kit (Stem Cell Technology; Vancouver, British Columbia, Canada). The purity of the isolated cells was verified by fluorescence-activated cell sorting (FACS) analysis using FITC-conjugated anti-CD4 antibodies (BD Biosciences), and was always more than 90% positive for CD4. The purified $CD4^+$ T cells were infected *in vitro* with HIV-1 (NL4.3). The cells were infected for 2 hours at 37°C with an m.o.i. of 1, and were extensively washed with the culture medium to remove the residual virus. Recombinant human cytokines were added for different lengths of time (detailed in individual experiments). Cell culture supernatants were harvested, clarified by centrifugation and stored at -20°C until they were analysed for their HIV-1 p24 contents by ELISA purchased from AIDS & Cancer Virus Program (NCI-Frederick, MD).

Statistical analysis

Standard statistical methods like Student's unpaired *t* test, Pearson's correlation and linear regression from GraphPad (Prism, San Diego, CA) were used for data analysis.

Results

Concentrations of IL-18 are increased but those of IL-18BPa are decreased in the sera of HIV-infected/AIDS patients

It has been reported earlier that IL-18 concentrations increase significantly in the sera from HIV-infected persons as compared to the sera from HIV-seronegative healthy subjects. However it is not known whether this increase is accompanied by any change in the levels of IL-18BPa in the circulation. In order to address this issue, we measured IL-18 and IL-18BPa in the sera obtained from the peripheral blood of 32 HIV-infected AIDS patients and from an equal number of age-matched HIV-seronegative healthy subjects. As shown in Figure 1A, the sera from HIV-infected patients had increased levels of IL-18 as compared to the sera from healthy subjects (294 ± 38 vs. 125 ± 25 pg/ml, respectively). The average concentration in the infected group sera was approximately 2.5 to 3 fold higher than that of the control sera and the difference between the two groups was statistically significant ($p = 0.0006$). The analysis of the same sera for their IL-18BPa contents is shown in Figure 1B. Surprisingly and unlike IL-18, the average concentration of IL-18BPa was significantly lower ($p = 0.0089$) in the sera of HIV-infected persons as compared to the control sera (2.7 ± 0.09 vs. 3.8 ± 0.4 ng/ml, respectively).

Absence of correlation between IL-18 and IL-18BPa in HIV-infected patients

In view of the importance of IL-18BPa in regulating the biological activity of IL-18, we sought to determine whether the serum concentrations of the two cytokines were mutually correlated. As shown in Figure 2A, a positive correlation was found between the two parameters in healthy persons ($p = 0.0014$; $r = 0.5407$). These results are consistent with the idea that IL-18 activity is tightly controlled by its natural antagonist in the human body and therefore the production of these two cytokines may be coordinated. Interestingly, no significant correlation (Figure 2B; $p = 0.5880$; $r = -0.09949$) was found between these two cytokines in the sera of HIV-infected patients. These data suggest that the coordination, observed in the production of the two cytokines in control subjects, is lost in HIV-infected patients.

Imbalance between IL-18 and IL-18BP levels results in increased concentrations of free IL-18 in HIV-infected patients

We further determined ratios between IL-18 and IL-18BPa concentrations the sera of the patients and control subjects. As shown in Figure 3A, the average ratio increased 3 to 4 times in HIV-infected patients as compared to healthy persons ($p < 0.0001$). These data suggest a decreased concentration of the IL-18BPa in the face of increased concentrations of IL-18 in the circulation of HIV-infected patients. A decrease in the concentrations of IL-18BP with concomitant increase in IL-18 concentrations in the circulation of HIV-infected persons could lead to increased concentrations of biologically active, free (not bound with IL-18BP) IL-18. To address this issue, we calculated concentrations of free IL-18 in the sera of our study participants using the law of mass action as described in Materials & Methods. As shown in Figure 3B, we observed an increase in the serum concentrations of free, biologically active IL-18 in HIV-infected patients as compared to healthy subjects. Consequently, the difference in free IL-18 concentrations between HIV/AIDS patients and healthy donors is more pronounced as compared to the concentrations of total IL-18.

In vitro infection of monocyte-derived macrophages with HIV results in an imbalance production of IL-18 and IL-18BP

Cytokines for which concentrations are elevated in HIV/AIDS patients decrease the levels of IL-18BP independently or in synergy with HIV infection

IL-18 treatment of $CD4^+$ T cells infected with HIV increases viral replication in vitro

In accord with our earlier results, no significant correlation existed between total serum IL-18 concentrations and viral load in HIV-infected persons (data not shown). Nevertheless, we were interested in determining the effect of IL-18 on HIV replication, since any replication-enhancing effects may have been suppressed in the HIV-infected patient by HAART. In order to determine the effect of rhIL-18 on HIV replication, we added the cytokine alone as well as in combination

with IL-10 or TGF-β to microcultures of isolated human $CD4^+$ T cells infected with HIV-1. These latter two cytokines were chosen because their concentrations are frequently increased in the circulation of HIV-infected patients (16, 21, 34). As shown in Figure 3B, we observed little effect of recombinant human IL-18 on HIV replication. However, it increases HIV replication in concert with equimolar concentrations of IL-10 and TGF-β1 ($p < 0.001$) (Figure 3B). These data suggest that increased biological activity of IL-18 may contribute towards increased HIV replication in concert with other cytokines.

Discussion

The results presented in this paper confirm earlier reports from this and other laboratories (21-23); reviewed in (24)) showing increased expression of IL-18 in the sera of HIV-infected patients as compared to those of HIV-seronegative healthy subjects. These results further show that concomitant with an increase in IL-18 concentrations, IL-18BPa levels are reduced in these patients. Since the "a" isoform is responsible for almost all IL-18 neutralizing activity in the circulation in humans, we interpret these results as decreased production of IL-18BP in these

patients. A practical consequence of the decrease in the levels of this IL-18 antagonist is that the concentrations of free, biologically active IL-18 become even more increased than total IL-18 in the circulation of these patients. To the best of our knowledge this is the first report about the levels of IL-18BP in HIV-infected patients. This further confirms our earlier results that it is not only total IL-18 levels, but its biological activities (e.g., its ability to induce IFN-γ from NK and T cells) are also increased in these patients (21). The increase in the biological activities is, at least in part, due to a decrease in the levels of IL-18BP.

As mentioned in the Introduction section, the biological activities of IL-18 are controlled by IL-18BP. IL-18 induces production of IL-18BP as a negative feed back mechanism and controls towards regulation of its biological effects. It does so indirectly via inducing IFN-γ production from NK and T cells. It is noteworthy that IFN-γ is a powerful stimulus for production of IL-18BP. The gamma-interferon-activated sequences (GAS) occur in the promoter region of the IL-18BP gene (36). In this connection, we observed a correlation between IL-18 levels and those of its antagonist in the sera from healthy persons. This suggests a coordinate production of these two soluble mediators under physiological conditions. However this coordinate production seems to be lost in HIV-infected patients, as we could not find a significant ($p > 0.05$) positive correlation between their levels in the sera of these patients. At least in part it could be due to a decreased ability of the PBMC from HIV-infected persons to produce IFN-γ in response to a variety of stimuli. Interestingly, the decreased production occurs despite an increase in the expression of the interferon receptors (37). Although IL-18 is a powerful IFN-γ inducer from NK and T cells, it does so only in combination with other cytokines, e.g., IL-12, IL-15, IL-2, etc. Alone IL-18 induces little of this interferon. Since production of IL-12, IL-15 and IL-2 markedly decreases in HIV-infected persons (15, 19, 38), it is not surprisingly that the production of IFN-γ decreases in these patients. Furthermore, immunosuppressive cytokines like TGF-β and IL-10 also inhibit production of this interferon in response to IL-18 and other stimuli. The concentrations of these immunosuppressive cytokines are increased in HIV-infected persons (16, 19-21, 39). Decreased production of TH1 type cytokines, of which IFN-γ is the representative one, has been implicated in the pathogenesis of AIDS. Taken together these observations suggest that decreased production of IFN-γ may be responsible for a decreased production of IL-18BP in HIV-infected patients. In addition to this, a direct inhibitory effect of immunosuppressive cytokines, e.g., IL-10 and/or TGF-β1, on the production of IL-18BP could also play a role.

An imbalanced production of IL-18 and IL-18BPa is not unique to HIV infections. It has been reported to occur in the patients suffering from secondary haemophagocytic syndrome (SHS) leading to four-fold increased concentrations of free IL-18 (26). Consequently, the SHS patients have decreased NK cell numbers, impaired NK cell-mediated cytotoxicity and activated macrophages. The levels of free biologically active IL-18 are also increased in chronic liver diseases (especially in cirrhosis), Crohn's disease and Lupus Nephritis (40-42). Novick et al. (31) have shown that in septic patients the levels of total IL-18 increase 20 fold but those of IL-18BP increase only by 10 fold causing an increase in the levels of free IL-18. In these conditions recombinant human IL-18BP may have the potential to act as a therapeutic tool. Compared with these conditions, the serum levels of IL-18BP are decreased in HIV-infected persons.

It is noteworthy that cytokines like IL-2, 12, 15 and 18 have the potential of enhancing innate and adaptive immunity against cancer and infectious diseases, and are being used or considered as adjuvants as well as prophylactic and therapeutic tools. Our results suggest that careful consideration should be given to the *in vivo* use of these cytokines either alone or in different combinations due to their negative feed back mechanisms and their effects on immune responses and viral replication.

Conflicting results have been published in literature concerning the effects of IL-18 on HIV replication (24). It is not yet clear whether the cytokine has proviral or antiviral role. The cytokine stimulates HIV replication *in vitro* in chronically infected human monocytic and T cell lines but not in primary human cells (48, 49). In one study, IL-18 was reported to inhibit replication of both M- and T-tropic HIV-1 in human PBMC that was ascribed to IL-18-induced production of IFN-γ (50). The authors also noted a decrease in the expression of CD4 on IL-18-treated PBMC. Sailer et al (51) have suggested that early in the course of HIV infection in humans, IL-18 suppresses viral replication by increasing TH1 responses (IFN-γ production) and by reducing the expression of CXCR4. Their suggestion was based upon the results, which showed increased levels of IL-18 in total blood from HIV infected persons early in the course of infection. The levels correlated with decreased expression of CXCR4 and unexpectedly also with decreased levels of IFN-γ in the circulation of these patients (51). Furthermore in animal models of the infection (simian/human chimeric immunodeficiency viruses; SHIV), high plasma levels of IL-18 were associated with more pathogenic viruses, rapid decline of $CD4^+$ T cells and higher viral set points (52). Our present results show that rhIL-18 alone has minimal effects on HIV

replication in human PHA blasts as well as in purified $CD4^+$ T cells. However, the cytokine increases HIV replication, when added to these cell cultures in combination with TGF-β or IL-10. It is noteworthy that increased levels of these two cytokines have been reported in HIV-infected persons (16, 19, 21, 34, 39). Therefore, we conclude that IL-18 promotes HIV-replication in HIV-infected persons. For that reason, it is not surprising that some researchers have found association of AIDS progression with raised serum levels of IL-18 in HIV-infected subjects (22, 53, 54). They reported that the cytokine levels were higher in progressors but remained rather stable in non-progressors. HAART caused a decrease in viral load, increased $CD4^+$ T cell counts, and decreased IL-18 levels in the therapy-responsive patients. However the levels never became normal and were not correlated with $CD4^+$ T cell counts.

Apart from increasing HIV replication, increased serum levels of IL-18 may also be adversely affecting HIV-specific adaptive immunity. It is noteworthy that the ability of IL-18 to induce IFN-γ production from T cells depends upon IL-12, which induces expression of IL-18R on TH1 type $CD4^+$ T cells (5). Consequently, TH1 type $CD4^+$ T cells proliferate in response to IL-18 and secrete this interferon. In the absence/deficiency of IL-12, IL-18 acts on mast cells, basophils and NKT cells, and induces production of IL-4, which promotes TH2 type responses in the body (reviewed in (5, 6)). Naïve and TH2 type $CD4^+$ T cells do not express IL-18R and are unresponsive to this cytokine. As mentioned above, IL-12 is also needed to induce production of this interferon from NK and T cells, as IL-18 alone induces little of it from these cells. Furthermore, it has been shown that in the presence of IL-18, antigen-specific memory TH1 type $CD4^+$ T cells also produce TH2 type cytokines, when they encounter their specific target cells or are cross-linked via anti-CD3 antibodies (55). It is noteworthy that a marked deficiency of IL-12 production occurs in HIV-infected persons (17-19). Therefore it is very likely that in the face of decreased IL-12 production in HIV-infected persons, IL-18 promotes TH2 type immune responses. TH2- type cytokine-skewed immune responses are known to control intracellular viral infections poorly. These types of immune responses have been shown to predominate in an overwhelming majority of HIV-infected persons and may be responsible for ultimate failure of the immune response to control and eliminate the viral infection (18, 19, 38, 55). Our results suggest that increased biological activity of IL-18 in the circulation of HIV-infected persons may play a role in inducing TH2 type immune responses in HIV-infected patients.

HIV-associated lipodystrophic syndrome (HALS), which is characterized by redistribution of fat (wasting of subcutaneous fat at face, limbs and trunk and ectopic accumulation in places like dorsocervical region), dyslipidemia, and metabolic disturbances occur frequently in HIV-infected persons undergoing HAART (see ref (56) for a review). It has been observed that serum IL-18 levels are often higher in the HIV-infected persons with HALS than in those not having this syndrome (8, 57). Furthermore, the levels were reduced in the subcutaneous tissue, where fat wasting had occurred (8, 57). These data suggest that along with other proinflammatory cytokines, e.g., TNF-α, IL-18 plays a role in lipolysis, depletion of adipocytes and the development of HALS.

In summary our study advances our understanding about the mechanisms involved in the immunopathogenic role of IL-18 in the progression of HIV infection towards AIDS. Given that increases HIV replication, its increased biological activities could be a factor in the progression of HIV infection towards AIDS. Furthermore, the cytokine plays a role in the development of HALS in HIV-infected patients. Therefore it should be seriously considered as a molecular target for therapeutic intervention in AIDS patients. Fortunately, its natural antagonist is constitutively produced in human body and therefore could represent a relatively safe therapeutic agent.

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Figure legends

Figure 1. Concentrations of serum IL-18 and IL-18BPa in HIV-infected/AIDS patients. IL-18 and IL-18BPa concentrations were determined in serum samples by using commercial ELISA kits. (A) Average ± standard error concentrations of IL-18 in the sera of HIV-infected/AIDS patients (P) and control subjects (N). The average concentrations differed significantly between the two groups of donors ($p = 0.0006$). (B) Average ± standard error concentrations of IL-18BPa

in the same 32 sera used for IL-18 determinations. The average concentrations of IL-18BPa also differed significantly between the two groups of donors ($p = 0.0089$).

Figure 2. Correlations between serum IL-18 and IL-18BPa in HIV-infected/AIDS patients. (A) Pearson correlation between concentrations of IL-18 and IL-18BPa in 32 sera of healthy persons. The two parameters showed a statistically significant correlation ($p = 0.0014$; $r = 0.6407$). (B) Pearson correlation between concentrations of IL-18 and IL-18BPa in 32 sera of HIV-infected/AIDS patients. The two parameters did not show a statistically significant correlation ($p = 0.5880$; $r = -0.09949$). (C) IL-18/IL-18BPa ratios in the 32 sera of healthy persons (N) and AIDS patients (P). A dot indicates an individual IL-18/IL-18BPa ratio and the horizontal line in each column indicates group mean. The mean of ratios differed significantly between the two groups of donors ($p < 0.0001$).

Figure 3. Increased concentrations of free IL-18 in HIV-infected/AIDS patients. (A) Concentrations of free IL-18 (nM) in the sera of healthy persons (N) and AIDS patients (P) calculated using the law of mass action as described in Materials & Methods. A dot indicates an individual free IL-18 concentration and the horizontal line in each column indicates group mean. The mean differed significantly between the two groups of donors ($p < 0.0001$). (B) Effect of rhIL-18 on HIV replication in human $CD4^+$ T cells. Human $CD4^+$ T cells were purified from the PBMC, infected *in vitro* with HIV and treated with 10 ng/ml IL-18, alone or in combination with equimolar concentrations of IL-10 and TGF-β1, for different lengths of time. The HIV-1 p24 was measured in the culture supernatants with a commercial ELISA kit.

CHAPITRE 4. DISCUSSION

Comme d'autres maladies pro inflammatoires chroniques, nous avons observé qu'il existe une corrélation négative entre la concentration et le nombre des cellules NK chez les personnes infectées par le VIH. Ceci s'applique également à tous les sous types comme, les $CD3^-$ $CD16^+$, les $CD3^-$ $CD56^+$ et les $CD16^+$ $CD56^+$. De même, cette étude implique le rôle de l'IL-18 dans la perte de ces cellules chez les personnes infectées par le VIH et éclaircit le mécanisme qui cause cette mort. Le traitement des cellules NK92 et des cellules NK primaires avec l'IL-18, induit la mort fratricide de ces cellules, tout en induisant l'expression de FasL et la production de TNFα.

Il existe une expression constitutive de FAS, et sans aucun changement même après traitement de l'IL-18. Alors, ceci nous permet de déduire une plus forte sensibilité des cellules NK, qui est dû probablement aux stimuli apoptotique. Tout en remarquant une diminution de l'expression de la protéine anti apoptotique Bcl-XL, qui peut être cruciale pour leur apoptose causé par FasL. C'est la première fois qu'on démontre la "down régulation" de cette protéine anti apoptotique chez les cellules NK humaines. Alors ce sera très intéressant et pertinent de démontrer si cette protéine induit les mêmes effets sur d'autres types cellulaires.

Il a été démontré que la mort des cellules NK dépend de l'augmentation du taux de l'IL-18. Pour confirmer cela, nous avons détecté une mort très significative chez les cellules NK des patients infectés par Le VIH, dont la concentration est plus élevée ou égale à 500pg/ml. Tandis que chez les personnes dont la concentration est plus petite que 500pg/ml et chez les personnes saines, un faible taux de mortalité est détecté. Ceci nous mène à expliquer cette différence, tout en déduisant, la probabilité d'interférence d'autres facteurs, comme l'antagoniste l'IL-18BP, et les facteurs de

croissance. Après la neutralisation de TNF, la combinaison de l'IL-15 et de l'IL-12, aussi bien que l'IL-2 et l'IL-12 causent la mort des cellules NK, mais sans aborder l'implication de l'interaction Fas/FasL [149]. La mort des cellules NK murins, se fait par l'intermédiaire de FasL, bien qu'il n'ait pas mesuré l'expression de FasL [150-152]. Notre étude démontre et pour la première fois que l'IL-18 induit l'expression de FasL, qui augmente la cytotoxicité de ces cellules. Ces cellules peuvent tuer toutes les cellules qui sont Fas positif, et causer des dommages tissulaires, comme une étude qui implique FasL et TNF-α pour causer des dommages dans le foie des souris [153, 154]. Alors, notre étude contredit celle de Kalina et collaborateur [157] en démontrant que les cellules NK ne tuent pas les cellules cibles, K562, dont on remarque l'absence de l'expression de Fas. Ceci est probablement est dû à la différence des techniques utilisées dans ces deux études. Il faut noter que l'IL-12, l'IL-15 et l'IL-18 possèdent un potentiel pour augmenter la réponse immunitaire adaptative ou innée contre les maladies infectieuses ou contre le cancer, et elles étaient utilisées comme des outils thérapeutiques. Notre étude suggère la prudence avant l'utilisation de ces cytokines toutes seules ou en combinaison avec d'autres cytokines, dues à leur effet de supprimer la réponse immunitaire.

Une étude démontre l'effet positif de l'IL-18 sur les cellules NK, par la prévention de l'apoptose de ces cellules, tout en induisant TRAF et c-IAP [155]. Une autre étude montre que l'IL-18 induit l'expression de CD28, CCR7 et CD25 sur les cellules NK, et augmente leur migration vers les nodules lymphoïdes et permettent leur interaction avec d'autres immunocytes [156]. Alors comment ne pas contredire leurs études, quand nos expériences démontrent une forte mortalité des cellules NK, après traitement de l'IL-18

Comme déjà mentionné, il y a une augmentation de la concentration de l'IL-18 [69, 70, 158], aussi bien qu'une diminution de son antagoniste l'IL-18 BP. C'est déjà connu, que chez l'humain, l'activité de l'IL-18 est neutralisé par l'isoforme *a* de son antagoniste. Ce qui mène à une augmentation de la concentration de l'IL-18 libre biologiquement active plus que l'IL-18 total dans la circulation sanguine des patients et ceci est dû en partie à la diminution de la concentration de l'IL-18BP [69]. Par respect à d'autres laboratoires, nous sommes les premiers a mesuré et dévoilé le niveau de l'IL-18BP chez les patients infectés par le VIH Il existe une corrélation entre le niveau de l'IL-18 et son antagoniste chez les personnes saines. Tandis que cette corrélation n'existe plus chez les personnes infectées et la cause sera la diminution de la production de l'INF-γ, mais on remarque une augmentation de l'expression du récepteur de l'INF-γ [159]. La production de l'IFN-γ n'est pas induite par l'IL-18 seul, mais en combinaison avec d'autre cytokine comme l'IL-15, l'IL-2, et l'IL-12, à partir des cellules T et NK. Toutefois, la diminution de la concentration de l'IL-2, de l'IL-15, et de l'IL-12 chez les personnes infectées, garde l'IL-18 seul, et la production de l'IFN-γ est faiblement induite [138, 159, 160]. Cependant la concentration des cytokines immunosuppresseurs comme l'IL-10 et/ou TGF-β augmentent chez les patients infectés par le VIH [69, 138, 161, 162], et ceci inhibe aussi la production de cet interféron en réponse à l'IL-18 et d'autres stimuli. Il y a aussi une diminution de la production des cytokines de type Th1, et qui sont représentés par l'IFN-γ. Alors, nous pouvons conclure, que la diminution de l'IFN-γ, et l'effet inhibiteur des cytokines immunosuppresseurs peuvent jouer aussi un rôle dans la diminution de la production de l'IL -8BP, chez les personnes infectées par le VIH. Aussi bien, ce déséquilibre existe dans d'autre maladie, comme le SHS, dont on observe une forte concentration de l'IL-18 libre, accompagné d'une diminution du nombre et de l'activité des cellules NK. Bien que le niveau

de l'IL-18 libre active augmente dans la maladie chronique du foi, et la maladie de crohn [93, 163]. Finalement, la recombinante humaine IL-18BP peut devenir un outil thérapeutique. D'après Torre [132,158], une grande exclamation existe si cette cytokine a un rôle pro viral ou antivirale. Cette cytokine stimule la réplication virale in vitro chez les monocytes et les cellules T humains, mais pas chez les cellules T primaires [124, 125]. Il a été rapporté, par une seule étude, que l'IL-18 inhibe la réplication du VIH dans les PBMC, en induisant la production de l'INF-γ, et une diminution de l'expression du récepteur CD4 [123]. Tandis que Sailer et collaborateurs [128] suggère que l'IL-18 supprime la réplication virale, durant la phase précoce. Ceci est causé par l'augmentation de la réponse du type Th1 et la diminution de l'expression du corécepteur CXCR4 [128]. Tandis que dans le modèle animal SHIV (simian/human chimeric immunodeficeincy virus), un taux élevé de l'IL-18 dans le plasma est combiné avec une augmentation de la charge virale, accompagné d'une déclinaison rapide du nombre des cellules $TCD4^+$ [121] . Alors notre étude montre que l'IL-18 recombinant humain seul a un faible effet sur la réplication virale, tandis que la réplication augmente en présence de l'IL-10 et TGF-β. De même, l'augmentation du niveau de ces deux cytokines a été rapportée déjà chez les patients infectés [69, 138, 161, 162, 164]. Ceci conclut que l'IL-18 peut promouvoir la réplication virale chez ces patients. De plus, certains chercheurs ont trouvé une association entre la progression de cette maladie et l'augmentation du taux de l'IL-18 dans le sérum des patients infectés par le VIH [165-167]. Le taux cette cytokine est plus élevée chez les progresseurs et stable chez les non progresseurs. En plus d'agir sur les cellules NK et sur la réplication virale, cette cytokine agit sur la réponse immunitaire adaptative, spécifique au VIH. Elle induit la production de l'INF-γ en combinaison avec l'IL-12, ce qui induit l'expression du récepteur de l'IL-18 (IL-18R) sur les cellules Th de type 1 [97]. En

absence de l'IL-12, l'IL-18 agit sur les mastocytes, les NK, les basophiles, et induit la production de l'IL-4, qui va promouvoir la réponse Th de type 2 [97, 152]. Les cellules T naïves et de type 2 n'expriment pas l'IL-18R et sans aucune réponse à cette cytokine. Cependant, on remarque une déficience de la production de l'IL-12 chez les personnes infectés [138, 168]. Comme déjà connu, les cellules Th de type 2 ne contrôlent pas bien l'infection virale intracellulaire. Alors ce type de réponse peut être responsable de l'échec de la réponse immunitaire à contrôler et à éliminer la charge virale [49, 138, 159, 169]. De même nos résultats suggèrent que l'augmentation de l'activité biologique de l'IL-18 dans la circulation des personnes infectées induit la réponse des cellules Th de type 2.

Un autre point à soulever, c'est l'utilisation d'une forte concentration de l'IL-18 dans nos expériences. La concentration physiologique connue, c'est celle dans la circulation. On ne connaît pas le taux dans les tissus ou dans les zones inflammatoires, et elle est sûrement plus élevée. En plus durant des études de recherche concernant une cytokine, il n'est pas inapproprié d'utiliser une concentration plus forte que la concentration physiologique. Il y a plusieurs recherches qui utilisent des nano grammes par millilitre, voir référence 157.

En somme cette étude, améliore nos connaissances dans l'immunobiologie de l'IL-18 et son agoniste et met le point sur un point, comment le taux élever de cette cytokine trouvé, contribue dans la pathogenèse de la maladie de l'immunodéficience humaine.

CHAPITRE 5. CONCLUSIONS

Nous avons réalisé des expériences pour comprendre le rôle de l'augmentation de la concentration d'IL-18 dans le sérum des patients sidéens dans le développement de cette maladie. En contraste de l'IL-18, la concentration de son antagoniste l'"*IL-18 Binding Protein*" diminue dans la circulation sanguine. Alors, ceci nous permet de conclure une nette augmentation de l'activité biologique d'IL-18 libre chez ces patients malades. De même, nous sommes les premiers à démontrer l'augmentation de la concentration d'IL-18BP chez les personnes sains et une diminution de la concentration de cet antagoniste chez les patients infectés par le VIH. Normalement, il y a une corrélation entre l'IL-18 et son antagoniste dans la circulation des donneurs sains, mais cette corrélation n'existe pas chez les patients malades.

Ce qui est intéressant, c'est notre résultat qui montre une corrélation négative entre la concentration d'IL-18 et le nombre et la cytotoxicité des cellules NK chez les patients. Ceci suggère que cette cytokine soit impliquée dans la disparition de ces cellules chez les patients et nous mène à voir l'effet direct d'IL-18 sur les cellules NK. Dans les expériences *in vitro,* cette cytokine cause la mort cellulaire de la lignée cellulaire NK92, aussi bien que chez les cellules NK primaires humaines. Cette mort cellulaire a été inhibée de façon significative par l'ajout de l'anticorps anti FasL et anti-TNFα. Cependant, nos résultats, montre que cette cytokine induit l'expression de FasL sur les cellules NK humaines, qui se tuent entre eux par l'interaction Fas/FasL. En plus, les sérums des patients malades et riches en IL-18, nous mènent aux-mêmes conclusions. De plus, cette cytokine, induit la transcription d'un gène rapporteur fusionné avec le promoteur humain FasL. En contraste de FasL il n'y a aucun effet observé sur l'expression chez ces cellules. IL-18 n'induit pas seulement

l'expression de FasL sur les cellules NK, mais il augmente la susceptibilité des cellules NK de mourir par FAS. Les cellules NK traités meurent en grand nombre par rapport aux cellules non traitées en présence de la même concentration d'un anticorps anti-Fas agoniste. Au moins, ceci peut être expliqué en partie qu'IL-18 diminue l'expression de la protéine anti apoptotique BCL-XL chez les cellules NK. Aucun effet de cette cytokine n'a été vu sur l'expression de BCL-2 dans ces cellules. De même, des résultats contradictoires ont été publiés regardant l'effet d'IL-18 sur la réplication virale du VIH dans les cellules humaines, nos résultats montrent que cette cytokine augmente la réplication virale. Alors nos résultats nous permettent de suggérer qu'IL-18 puisse contribuer vers l'immunopathogénèse du SIDA en diminuant les fonctions des cellules NK, aussi bien en augmentant la réplication virale.

En addition de ces résultats, une revue de littérature nous montre que l'augmentation de la concentration de cette cytokine a été impliquée dans le syndrome métabolique, la lipodystrophy, et la *"démence*" chez les personnes infectées. En outre, IL-18 est considéré comme une cytokine antivirale, qui peut augmenter les réponses antivirales en induisant l'INF - γ des cellules NK et T. Cependant, l'IL-18 seul induit la production de cet interféron, en combinaison avec l'IL-12, l'IL-15 ou l'IL-21. Les concentrations de l'IL-12, l'IL-15 et l'IL-21 diminuent dans la circulation des personnes infectées par le VIH. En absence ou l'insuffisance de cette collaboration de cette cytokine, l'IL-18 est connu pour promouvoir les réponses du type Th2 en induisant IL-4 des cellules mastocytes et des basophiles. Ainsi, dans le contexte de l'infection par le VIH, l'IL-18 peut diminuer l'immunité anti virale, par la promotion de la réponse immunitaire de type 2. Finalement, on conclue, que l'augmentation de la concentration de l'IL-18 contribue dans la pathogénèse du SIDA chez les personnes infectées par le VIH. Alors cibler cette cytokine par son

antagoniste naturel, peut ralentir la progression de l'infection par le VIH vers la maladie, le SIDA

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