Université de Montréal

EFFECTS OF INTERLEUKIN-27 ON HUMAN CD8 T CELLS

par

Teodora Yaneva

Département de microbiologie et immunologie

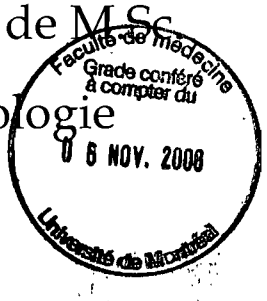
Faculté de médecine

Mémoire présenté à la Faculté des études supérieures

en vue de l'obtention du grade de M.Sc.

en microbiologie et immunologie

Juin, 2008



Université de Montréal

Faculté des études supérieures

Ce mémoire intitulé:

EFECTS OF INTERLEUKIN-27 ON HUMAN CD8 T CELLS

présenté par :

Teodora Yaneva

a été évalué par un jury composé des personnes suivantes:

Dr. Jean-Francois Gauchat

Président-rapporteur

Dr. Nathalie Arbour

Directrice de recherche

Dr. Nathalie Labrecque

Membre du jury

RÉSUMÉ EN FRANÇAIS

L'orchestration des médiateurs cellulaires et solubles du système immunitaire inné est essentielle pour le maintien de la santé. Les lymphocytes T contribuent de part leurs fonctions cytolytiques et la sécrétion de nombreux médiateurs solubles et à cet équilibre.

L'interleukine-27 (IL-27) est composée de deux sous-unités, EBI3 et p28, qui sont liées à une façon non-covalente. Des effets pro- et anti-inflammatoires sur des cellules T murines ont été décrits pour cette cytokine. Toutefois, peu d'informations sont disponibles pour les cellules T humaines.

L'impact potentiel de l'IL-27 sur les fonctions des cellules T humanes a été étudié en utilisant des cellules mononucléaires du sang périphérique (PBMC) des donneurs sains. Une petite quantité des cellules CD4 et CD8 exprimaient le récepteur de l'IL-27 (IL-27R), composé de deux chaines : TCCR et gp130, ex vivo, surtout les CD8, et cette proportion augmentait site à une activation in vitro. Des PBMC et des cellules T CD8 purifiées, naïves et mémoires, ont été activées brièvement in vitro et leur prolifération et production des médiateurs ont été étudiées par cytométrie en flux. L'ajout d'IL-27 à une stimulation polyclonale a augmenté de façon significative et dose-dépendante la prolifération et la production d'interféron-γ et de granzyme B par les cellules T. De plus, une analyse par RT-PCR a démontré que les cellules CD8 humanes ne peuvent pas produire d'IL-27 même après activation.

Ces résultats démontrent l'impact pro-inflammatoire de l'IL-27 sur des cellules T (CD8) humanes et leur plus grande susceptibilité aux effets de cette cytokine après activation via l'augmentation de l'expression de l'IL-27R.

Mots clés : cytokine, interféron, cytométrie en flux, granzyme, récepteur de cytokine

RÉSUMÉ EN ANGLAIS

Orchestration of the innate and adaptive immune systems through numerous cell types and their secreted mediators is crucial to maintain health. Cytokines, soluble protein mediators, have crucial role in shaping immune responses. T lymphocytes through the secretion of soluble mediators and cytolytic functions are important players of the adaptive immune responses.

Interleukin-27 (IL-27) consists of two non-covalently linked subunits: EBI3 and p28. This recently described cytokine has been shown to exert both pro- and anti-inflammatory effects, especially on mouse T cells. However, information on human T cells is lacking.

The potential impact of IL-27 on human T cell functions was assessed using, peripheral blood mononuclear cells (PBMC) from healthy donors. Surface expression of both chains of IL-27 receptor (IL-27R) (TCCR and gp130) was assessed on ex vivo and in vitro activated PBMC. A small proportion of CD4 and CD8 T cells expressed detectable IL-27R ex vivo, with the latter subset having a greater proportion and increased levels upon activation. PBMC or purified naïve and memory CD8 T cells were shortly stimulated in vitro and then analyzed using flow cytometry-based assays assessing proliferation and mediators. Addition of IL-27 to anti-CD3 stimulated cells led to a significant dose-dependent increase of proliferation, interferon-γ and granzyme B production by T cells. Moreover, in contrast to mouse cells, RT-PCR analysis showed that human CD8 T cells can not be a source of IL-27.

These results underscore the pro-inflammatory impact of IL-27 on human CD8 T cells and their increased susceptibility upon activation.

Key words: cytokine, interferon, flow cytometry, granzyme, cytokine receptor

TABLE OF CONTENTS

TABLE OF CONTENTS 1

LIST OF FIGURES 4

LIST OF TABLES 5

LIST OF ABBREVIATIONS 6

DEDICATION 10

ACKNOWLEDGEMENTS 11

LITERATURE REVIEW 12

1.0 T lymphocytes 13

1.1 Distinct T lymphocyte subsets 13

1.2 Transition from naïve to effector and memory T cells 15

1.3 Roles of cytokines in T cell polarization and acquisition of effector functions 20

2.0 ANTIGEN PRESENTING CELLS (APC) 24

2.1 Monocytes and Macrophages (Mφ) 24

2.2 Dendritic cells 26

2.3 B cells 27

3.0 INTERLEUKIN-27 (IL-27) 28

3.1 Characteristics of IL-27 protein 30

3.2 IL-27 expression in human tissues 33

3.3 Receptor of IL-27 34

3.4 Downstream signalling of IL-27R 36

3.5 Effects of IL-27 on T lymphocytes 37

3.6 Impact of IL-27 on monocytes, Mφ and dendritic cells 43

3.7 Impact of IL-27 on B lymphocytes 44

3.8 Impact of IL-27 on other cell types 45

3.9 Roles of IL-27 in mouse models of human diseases 46

3.10 Roles of IL-27 in human disorders 50

3.11 Interleukin-35: EBI3 + IL-12p35 51

HYPOTHESES AND OBJECTIVES 53

Hypotheses 53

Objectives 53

MATERIALS AND METHODS 54

1.0 Isolation of human blood cells 55

1.1 Peripheral mononuclear cell isolation 55

1.2 Total CD8 T cell isolation 56

1.3 Naïve CD45RA+ CD8 T cell isolation (negative selection) 56

1.4 Memory CD45RO+ CD8 T cell isolation 57

2.0 Functional assays on human T cells 58

2.1 CFSE labelling 58

2.2 Cell stimulation 58

2.2.1 PBMC 58

2.2.2 Naïve CD8 T cells (plate-bound) 59

2.2.3 Memory CD8 T cells (plate-bound) 60

2.3 Flow cytometry 60

2.4 Ex-vivo expression of IL-27R (gp130 and TCCR) 61

2.5 ELISA for IFN-γ 62

3.0 Determination of IL-27 mRNA expression by real-time PCR 63

3.1 RNA extraction 63

3.2 Complementary DNA (cDNA) 64

3.3 Real time-Polymerase Chain Reaction 64

4. 0 Statistical analysis 66

RESULTS 67

1.0 Optimization of anti-CD3 activation of human PBMC 68

1.1 Proliferation of human T cells in response to α-CD3 Ab 68

2.0 Expression of IL-27 signalling receptor (IL-27R) by human PBMC 70

2.1 Ex vivo expression of IL-27R by human T lymphocytes and monocytes 71

2.2 IL-27R expression upon activation 73

3.0 Effects of IL-27 on T cell functions. 75

3.1 PBMC 75

3.2 Naïve CD8 T cells 84

3.3 Memory CD8 T cells 88

4.0 CD8 T cells as a source of IL-27 91

DISCUSSION 93

1.0 Expression and modulation of IL-27R 94

1.1. Ex-vivo IL-27R expression 94

1.2 IL-27R modulation upon activation 98

2.0 Effects of IL-27 on T cell functions. 99

3.0 CD8 AS A SOURCE OF IL-27 107

4.0 FUTURE DIRECTIONS 108

BIBLIOGRAPHY 110

APPENDIX 136

LIST OF FIGURES

Figure 1: Effects of IL-27 signalling 42
Figure 2: α-CD3 Ab dose response by PBMC as assessed by proliferation 69
Figure 3: Expression of IL-27 receptor chains on ex-vivo monocytes (CD14), CD4 and CD8 T cells 72
Figure 4: Expression of IL-27R on T cells upon activation. 74
Figure 5: Illustration of IL-27 effects on the proliferation of, and IFN-γ and granzyme B production by human CD8 T cells 76
Figure 6: IL-27 boosts the proliferation of T cells 78
Figure 7: IL-27 augments the IFN-γ producing T cells 80
Figure 8: IL-27 increases granzyme B production by CD8 T cells 81
Figure 9: IL-27 augments IFN-γ production by α-CD3 stimulated PBMC 83
Figure 10: IL-27 enhances naïve CD8 T cell proliferation upon α-CD3 stimulation in the presence or absence of co-stimulation 85
Figure 11: Effects of IL-27 on effector functions of naïve CD8 T cells 86
Figure 12: IL-27 enhances memory CD8 T cell proliferation upon α-CD3 stimulation 89
Figure 13: Effects of IL-27 on effector functions of memory CD8 T cells 90
Figure 14: IL-27 is not produced by CD8 T cells upon stimulation 92
Figure 15: TCCR expression by different cell subsets via Western blot 136

LIST OF TABLES

Table 1. Naïve and memory specific markers for human T cells 19
Table 2. Different effector T cell lineages 20
Table 3: Stimuli, inducing mRNA expression of both subunits of IL-27 32
Table 4. List of fluorochroms used for flow cytometry analysis 61
Table 5: List of antibodies used for flow cytometry analysis 62

LIST OF ABBREVIATIONS

A647	Alexa Fluor®647 (fluorochrome)
A700	Alexa Fluor®700 (fluorochrome)
Ab	Antibody
Ag	Antigen
APC	Antigen presenting cell
APC-Cy7	Allophycocyanin-Cy™7 (fluorochrome)
APCy	Allophycocyanin
BCR	B cell receptor
CD	Cluster of differentiation
CFSE	5-(and 6-) carboxyfluorescein diacetate succinimidyl ester
CNTF	Ciliary neurotrophic factor
CT-1	Cardiotrophin 1
CTL	Cytotoxic T lymphocytes ($CD8^+$)
d.s.	Double stranded deoxyribonucleic acid
DC	Dendritic cells
EBI3	Epstein-Barr virus-induced gene 3
ELISA	Enzyme linked immunosorbent assay
Eomes	Eomesodermin (transcription factor)
FITC	Fluorescein (fluorochrome)
GATA-3	"GATA" DNA sequence recognizing transcription factor 3
G-CSF	Granulocyte colony stimulating factor
GM-CSF	Granulocyte-macrophage colony stimulating factor
gp130	Glycoprotein 130
IFN-α	Interferon alpha
IFN-γ	Interferon gamma
IL-27	Interleukin 27
IL-27R	Receptor of interleukin-27
LIF	Lymphocyte inhibitory factor
LPS	Lipopolysaccharide
M-CSF	Macrophage-colony-stimulating factor
mRNA	Messenger ribonucleic acid
Mφ	Macrophages
NF-kB	Nuclear factor kB
NK	Natural killer cells
OSM	Oncostatin M
PAMPs	Pathogen associated molecular patterns
PB	Pacific Blue™ (fluorochrome)
PBMC	Peripheral blood mononuclear cells
PE	Phycoerythrin (fluorochrome)
PE-Cy7	Phycoerythrin-Cy™7 (fluorochrome)

PRRs	Pattern recognition receptors
ROI	Reactive oxygen intermediates
ROR-γ	Retinoid orphan receptor γ
RT-PCR	Real-time polymerase chain reaction
s.s. DNA	Single stranded deoxyribonucleic acid
sgp130	Soluble gp130
SOCS	Suppressor of cytokine signaling
STAT	Signal transducer and activator of transcription
T-bet	T-box expressed in T cells
Tc	Cytotoxic T cells ($CD8^+$)
TCR	T cell receptor
TGF-β	Transforming growth factor- beta
Th	T helper cells ($CD4^+$)
TLR	Toll-like receptor
TNF	Tumor necrosis factor
T_{reg}	Regulatory T cells
A647	Alexa Fluor®647 (fluorochrome)
A700	Alexa Fluor®700 (fluorochrome)
Ab	Antibody
Ag	Antigen
APC	Antigen presenting cell
APC-Cy7	Allophycocyanin-Cy™7 (fluorochrome)
APCy	Allophycocyanin
BCR	B cell receptor
CD	Cluster of differentiation
CFSE	5-(and 6-) carboxyfluorescein diacetate succinimidyl ester
CTL	Cytotoxic T lymphocytes ($CD8^+$)
d.s.	Double stranded deoxyribonucleic acid
DC	Dendritic cells
EBI3	Epstein-Barr virus-induced gene 3
ELISA	Enzyme linked immunosorbent assay
Eomes	Eomesodermin (transcription factor)
FITC	Fluorescein (fluorochrome)
FnIII	Fibronectin type III domain
GATA-3	"GATA" DNA sequence recognizing transcription factor 3
G-CSF	Granulocyte colony stimulating factor
GM-CSF	Granulocyte-macrophage colony stimulating factor
gp130	Glycoprotein 130
IFN-α	Interferon alpha
IFN-γ	Interferon gamma
IL-27	Interleukin 27
IL-27R	Receptor of interleukin-27

LIF	Lymphocyte inhibitory factor
LPS	Lipopolysaccharide
M-CSF	Macrophage-colony-stimulating factor
mRNA	Messenger ribonucleic acid
Mφ	Macrophages
NF-kB	Nuclear factor kB
NK	Natural killer cells
PAMPs	Pathogen associated molecular patterns
PB	Pacific Blue™ (fluorochrome)
PBMC	Peripheral blood mononuclear cells
PE	Phycoerythrin (fluorochrome)
PE-Cy7	Phycoerythrin-Cy™7 (fluorochrome)
PRRs	Pattern recognition receptors
ROI	reactive oxygen intermediates
ROR-γ	Retinoid orphan receptor γ
RT-PCR	Real-time polymerase chain reaction
s.s. DNA	Single stranded deoxyribonucleic acid
sgp130	Soluble gp130
SOCS	Suppressor of cytokine signaling
STAT	Signal transducer and activator of transcription
T-bet	T-box expressed in T cells
Tc	Cytotoxic T cells ($CD8^+$)
TCR	T cell receptor
TGF-β	Transforming growth factor- beta
Th	T helper cells ($CD4^+$)
TLR	Toll-like receptor
TNF	Tumor necrosis factor
T_{reg}	Regulatory T cells

DEDICATION

"You are never given a wish without being given the power to make it true."

Richard Bach

"The future belongs to those who believe in the beauty of their dreams. "

Eleanor Roosevelt

To my mother, ***Tsvete***, the magic flower with spirit of steel, who taught me to dream…

To my father, ***Yanko***, who taught me to fight for my dreams…

To my brother, ***Yavor***, who leads me in the invisible world of beliefs…

And to my sister, ***Vlada***, who knows me better than anybody else!

ACKNOWLEDGEMENTS

I would like to express my gratitude to my supervisor, Dr. Nathalie Arbour, for giving me the opportunity to work with her, for teaching me and being so patient and full of love, for believing in me and for keeping the guiding light in sight till the very end.

I would like also to thank Neuro-Immuno team at CHUM Notre-Dame Hospital:

Our lab technician, Diane Beauseigle, for being my second pair of eyes and for providing me with help and guidance…

My colleagues Camille Pittet, Dr. Raphael Schneider and Lama El-Khoury for their love, assistance and moral support….

Dr. Alexandre Prat and his team – Monique Bernard, Igal Ifergan, Hania Kébir, Romain Cayrol, Aurore Dodelet-Devillers, Dr. Mike Sabbagh and Simone Terouz for their support, for making the lab my home and for turning the time of work into such an incredible experience…

Thank you, all!

LITERATURE REVIEW

The immune system comprises two complementary arms: the innate and the adaptive systems. The innate immune system acts as the first line of defence and consists of cells that recognize pathogen associated molecular patterns (PAMPs) through pattern recognition receptors (PRRs), for example Toll-like receptors (TLRs). Although it is rapidly activated in response to insult or pathogen, the innate system lacks the ability to improve the host's resistance upon re-encounter of the same Ag (Ag). This immune system compartment is composed of mediators including the lysosyme, the complement, acute phase proteins, and cells such as phagocytes (dendritic cells (DC), macrophages (Mφ) and neutrophils) and natural killer cells (NK). When this first line of defence does not successfully get rid of the pathogen threat in the first few days, the innate immune system activates the adaptive immune system, which recognizes specific Ag of the infectious agent through the B cell receptor (BCR) and the T cell receptor (TCR) leading respectively to antibodies (Ab) secretion by B lymphocytes and cellular immune responses by T lymphocytes. The adaptive immune system through the activation and expansion of specific B cells and T cells is responsible for the immunological memory towards the pathogen-derived Ag.

1.0 T LYMPHOCYTES

1.1 Distinct T lymphocyte subsets

T lymphocytes are cells of the adaptive immune system expressing the cluster of differentiation (CD) marker CD3, which consists of the invariable signal transducing subunits (CD3γ, CD3δ, CD3ε and CD3ζ [CD247]) (Lin and Weiss, 2001; Werlen and Palmer, 2002; Risueno et al., 2008). The CD3 chains have negatively charged acidic residues in their transmembrane domains which ensure interaction with the positively charged ligand-binding subunits (TCRα and TCRβ) of the T cell receptor (TCR). The TCR of most T cells consists of an α- and a β-chains, whereas a minor population expresses an alternative form made of a γ- and a δ-chains. These ligand-binding chains are immunoglobulin like and have a constant and a variable region. The αβTCR recognizes peptides presented by

major histocompatibility complex (MHC). αβTCR T lymphocytes fall into two classes based on distinct cell-surface co-receptors: CD4 and CD8, thus are called CD4 T lymphocytes or CD8 T lymphocytes. CD4 is a single chain molecule binding the classical MHC class II molecule at a different site than the TCR. Thus, CD4 T lymphocytes, also named T helper cells (Th), recognize peptidic Ag presented by MHC class II molecule. The CD8 molecules in most T lymphocytes is a heterodimer consisting of an α- and a β-chains and interacts with MHC class I molecules in a way similar to the interaction CD4 ↔ MHC class II. Thus, CD8 T lymphocytes, also named cytotoxic T cells (Tc), recognize peptidic Ag presented by classical MHC class I molecules or non-classical ones such as HLA-G (Gomes et al., 2007) and HLA-E (Godfrey et al., 2008). However, T cells can also recognize glycolipids, complexed to CD1d molecule (Godfrey et al., 2008). CD1 molecules are cell surface glycoproteins expressed mainly by B lymphocytes, macrophages and dendritic cells. They consist of two chains: β2 microglobulin (β2m), similarly to MHC class I molecules, and a heavy chain containing three extracellular domains (α1–α3). The α1–α2 super domain forms the antigen-binding groove consisting of two ahelices (α1 and α2) whereas the membrane proximal α3 domain binds β2m. Humans have five CD1 isotypes (CD1a–e). Many natural killer T cells are specific for CD1d presenting several types of glycolipids (Zajonc and Kronenberg, 2007).

Both classes of MHC molecules are heterodimers anchored in the cell membrane and bear a groove where the presented peptide binds. The MHC class I molecule consists of a larger α-chain spanning the membrane, and a smaller, non-covalently linked β_2–microglobulin, which does not have a transmembrane domain. The peptide that fits in the groove is usually 8–10 amino acids (aa) long and gets anchored at both ends by its free amino- and carboxy- termini. The MHC class II molecule comprises two non-covalently associated chains (α- and β-) both spanning the cytoplasmic membrane. The peptide-binding groove is more open than the cleft of the MHC class I molecule resulting in presentation of longer peptides (13-17 aa) and lack of anchoring at the ends of the peptide. Both classes of

MHC molecules are differentially expressed throughout the body: almost all nucleated cells express MHC class I molecules, whereas MHC class II molecules are restricted to professional Ag presenting cells (APC) (e.g. B lymphocytes, DC and Mφ). MHC class I and class II expression could be enhanced upon activation and inflammatory conditions.

1.2 Transition from naïve to effector and memory T cells

Based on experimental observations mainly obtained using mouse models of infection, the in vivo T cell response has been dissected into four main phases: i)initial activation ii) expansion phase iii) contraction phase, and iv) memory phase.

The initial activation consists in the activation of rare naïve T cells specific for the particular Ag into effector T cells. Three signals are required for initiation of a program leading to strong expansion, development of effector functions, and survival of an efficient memory cell population. The first signal is the MHC-peptide complex recognized by the TCR, the second signal is provided by co-stimulatory molecules (e.g. CD80 or CD86) concurrently provided with the MHC-peptide complex on the surface of the APC. Cytokines present near-by the T cell-APC interaction provide the third signal (Mescher et al., 2006; Harty and Badovinac, 2008). Stimulation with these three signals synergize and cause the naïve T cells to enter an irreversible differentiation program through activation of specific transcription factors (T-bet, GATA-3, Foxp3 or RORγt) leading to the acquisition of tissue homing receptors and distinct effector functions. Cytokines provided in the vicinity have a tremendous impact on the differentiation of T cells. Skewing towards different T cell responses will be described in more details below.

The expansion phase consists in the proliferation and differentiation of specific T cells, which will become effector or memory T cells that will then travel throughout the host's body to find their specific cognate peptide-MHC complex. Chromatin remodeling of effector cytokine genes can be inherited through mitosis

and can contribute to the maintenance of specific states of gene activity between cell generations (Agarwal et al., 1998; Sallusto et al., 2004) through a process, called "cycling" in the absence of cognate Ag (demonstrated for memory CD8 T cells).

The contraction phase describes the transition from a large effector population to a smaller population of memory T cells in order to maintain long-lasting immunological memory. This is necessary given the limited space and resources available for all T cells expanded throughout the host's life. Moreover, protection from the highly active effector cells, which can turn into detrimental autoimmune weapon once the pathogen has been cleared (Harty and Badovinac, 2008) is provided by this contraction phase. The mechanisms involved in this phase are not fully elucidated but could include apoptosis of Ag-specific T cells and a balance between pro- and anti-apoptotic BCL-2 family members. In addition, after each division, chromosomal telomeres shorten determining the cellular lifespan and limiting the number of divisions that cells can undergo (Harty and Badovinac, 2008).

The memory phase describes the long-term maintenance of memory T cells within the host. Cytokines play crucial roles in this process especially the common γ chain cytokines: IL-2, IL-7 and IL-15. IL-7 provides the second signal required for survival of both memory and naïve T cells, which makes it a limiting factor (Schluns et al., 2000; Vivien et al., 2001; Guimond et al., 2005; Boyman et al., 2007; Prlic et al., 2007). The memory T cells are highly heterogeneous in terms of surface markers, cytokines they are able to produce as well as their lytic enzyme content. This heterogeneity could be ascribed to several factors including the signals provided during the priming (type of pathogen, presence of cytokine milieu, and APC) determining the bouquet of cytokines produced by the effectors and thus by the memory T cells (Seder et al., 2008), and survival signals received later on (including survival cytokines).

Some of the markers used for phenotyping T cells according to their state of activation are shown in Table 1 including the function of these markers with (+)

and (-) representing the relative expression in a given subpopulation. Thus, (+) means that the marker is usually expressed by the entire subpopulation, (++/-) means that most cells in the subpopulation express the marker, whereas (+/--) means that most cells in the subpopulation do not express the marker, but that a small subset do. One of these markers is CD45 which has two isoforms: CD45RA, expressed on naïve T cells, and CD45RO typical for memory T cells. A small subset of CD8 T cells called effector memory CD45RA+ (T_{EMRA}) express CD45RA, are devoid of CCR7, and are characterized by the largest content of perforin among the memory subsets. The transition between both isoforms occurs upon T-cell activation with a shift from RA to RO as this change is reversible. CD62L is a lymph node homing receptor, which in combination with the CC-chemokine receptor 7 (CCR7) is used for distinguishing the central memory (T_{CM}, expressing $CD62L^{hi}CCR7^{hi}$) from the effector memory (T_{EM}, expressing $CD62L^{low}CCR7^{low}$) subset. T_{CM} represent the long-term memory cells which maintain the memory pool due to their good proliferative capacity. Human T_{CM} express CD45RO, CCR7, and are found mainly in secondary lymphoid organs (lymph nodes, spleen) and tissues. They have high sensitivity to Ag stimulation, are less dependent on co-stimulation than naïve T cells, and up-regulate CD40L to a greater extent to provide more effective co-stimulatory feedback to DC and B cells. Upon activation they produce mainly IL-2, but after proliferation they differentiate into effector cells and produce large amounts of cytokines. The other subset (T_{EM}) have less efficient proliferative capacity upon re-encounter of the Ag but can rapidly and efficiently respond to it via cytokine production (Sallusto et al., 2004). This compartment consists of effector memory T cells that have lost the constitutive expression of CCR7, are heterogeneous for CD62L, show different chemokine receptor and adhesion molecule expression that lead them to inflamed tissues. These cells reside mainly in the spleen, blood, and non-lymphoid tissues (lung, liver, and gut) but are not found in the lymph nodes and have rapid effector functions because are charged with great amounts of perforin. Both CD4 and CD8 T_{EM} produce cytokines very shortly after reactivation (Sallusto et al., 2004;

Badovinac and Harty, 2006). Other markers used are the co-stimulatory molecules CD27 and CD28. In addition, memory T cells are characterized by their effector functions including their cytokine profile and lytic enzyme content. Thus, to identify the memory phenotype a combination of multiple markers is used. However, regardless of their phenotype, good memory T cells: (i) persist and outnumber the naïve T cell repertoire; (ii) are able to rapidly respond to re-infection by vigorous proliferation and various effector mechanisms (cytolysis and cytokine production) in an Ag dose-dependent manner; (iii) provide rapid protection after pathogen re-encounter (Badovinac and Harty, 2006).

Table 1. Naïve and memory specific markers for human T cells

	CD4			CD8				Functions	
Marker	T naïve	T_{CM}	T_{EM}	T naïve	T_{CM}	T_{EM}	T_{EMRA}		
CD197 (CCR7)	(+)	(+)	(-)	(+)	(+)	(-)	(-)	Chemokine receptor binding CCL19 and CCL21; Homing to lymph nodes; Down-regulated upon naïve T cell activation; Re-expressed after stimulation.	
CD45RA	(+)	(-)	(+/--)	(+)	(-)	(-)	(+)	high-molecular weight	Transmembrane phosphate regulating the TCR-CD3 complex signalling; T-cell activation leads to a reversible shift from RA to RO.
CD45RO	(-)	(+)	(+)	(-)	(+)	(+)	(+/-)	low-molecular weight, increases efficiency of TCR-CD3 signalling	
CD62L	(+)	(++/-)	(+/-)	(+)	(++/-)	(+/--)	(+/--)	Cellular adhesion molecule from selectin family, homing to lymph nodes via interaction with 6-sulpho-Lewis X,	
CD27	(+)	(++/-)	(+/-)	(+)	(++/-)	(+/-)	(+/-)	Binds CD70; Present on activated T cells and DC; Promotes effector functions and memory cell formation; CD70 engagement or prolonged stimulation leads to loss of CD27 expression; no re-expressed upon activation.	
CD28	(+)	(+)	(+)	(+)	(++/-)	(+/-)	(+/-)	Binds CD80 and CD86 expressed by APC; Potent transducer of co-stimulatory signals enhancing IL-2 production, proliferation, survival, and effector functions.	

Adapted from (Sallusto et al., 2004) and (van Lier et al., 2003)

1.3 Roles of cytokines in T cell polarization and acquisition of effector functions

The timing and the intensity of several signals (signal 1: TCR, signal 2: co-stimulation, signal 3: cytokines) regulate the development of effector as well as memory T cells (Harty and Badovinac, 2008). Cytokines produced by innate immune cells significantly contribute to shape subsequent T cell activation by regulating the expression of many genes in T lymphocytes promoting optimal proliferation, survival, commitment to a lineage (a summary of Th1/Tc1, Th2/Tc2, and Th17/Tc17 lineages is shown in Table 2), effector functions, adhesion and trafficking (Mescher et al., 2006). The skewing of T cells towards type 1, type 2 or type 17 has been considerably studied for CD4 T cells but much less is known for CD8 T cell differentiation. Moreover, numerous publications suggest that factors dictating the activation, expansion, and development of memory differ between these two T cell subsets (Seder and Ahmed, 2003). Thus, the following description of lineage commitment has been mainly described for CD4 T cells, but when appropriate information regarding CD8 T cells is mentioned. In addition to the Th1/Tc1, Th2/Tc2, or Th17/Tc17 lineages, other T cell differentiation patterns can be observed. For example, presence of TGF-β during TCR activation can induce the expression of forkhead box transcription factor Foxp3 by CD4 T cells leading to the development of a T cell subset bearing regulatory functions (Shevach et al., 2008), but these regulatory T cell subsets will not be discussed herein.

Table 2. Different effector T cell lineages

T lineage	Cytokine profile	Role in immunity
Th1/Tc1	IFN-γ, TNF	Proinflammatory action against intracellular pathogens (e.g. viruses)
Th2/Tc2	IL-4, IL-5, IL-10, IL-13	Stimulate humoral immunity by activating B cells, contribute to fight extracellular pathogens
Th17/Tc17	IL-17 IL-17F, IL-21, IL-22,	Implicated in autoimmunity, inflammation, cancer, protect from extracellular pathogenic bacteria

Presence of IL-12 in the vicinity of CD4 T cells being activated and the signalling and induction of transcription factor 'T-box expressed in T cells' (T-bet) and STAT-4 (Signal Transducer and Activator of Transcription-4) lead to the development of Th1 cells (Seder and Ahmed, 2003). Similarly, it has been shown that naïve CD8 T cells exposed to their cognate Ag in the presence of co-stimulation but in absence of IL-12 expand but these cells do not acquire cytolytic activity (Mescher et al., 2006), supporting the crucial role of IL-12 also in efficient CD8 T cell effector functions. In Table 2 are represented the basic T subsets according to their secreted cytokines. Type 1 T cells (Th1/Tc1) produce pro-inflammatory cytokines such as IFN-γ and TNF as well as lytic enzymes and are implicated in clearance of viral infections and extracellular pathogens. An uncontrolled expansion of Th1 cells has been associated with the development of autoimmune diseases (Gonzalez-Rey and Delgado, 2006; Guilherme et al., 2007; Skurkovich and Skurkovich, 2007).

In contrast, presence of IL-4 and signalling and induction of the transcription factor GATA-3 (the name comes from the "GATA" DNA sequence recognized) and activation of STAT-6 (Signal Transducer and Activator of Transcription-6) favour the development of Th2 cells. Type 2 T lymphocytes (Th2/Tc2) produce cytokines such as IL-4, - 5, -10 and IL-13, suppressing pro-inflammatory Th1 cytokines, and promoting humoral immunity and responses to intracellular pathogens. An uncontrolled expansion of Th2 cells has been linked with the development of allergy (Robinson, 2000; Bullens et al., 2004; Haczku, 2006).

Differentiation of naïve CD4 T cells into Th17 occurs in different conditions in mouse and human (McGeachy and Cua, 2008). Naïve mouse CD4 T cells activated in the presence of TGF-β and IL-6 up-regulate the transcription factor ROR-γ (Retinoid-related orphan receptor-gamma) and adopt the Th17 lineage (Bettelli et al., 2006; Mangan et al., 2006; Veldhoen et al., 2006). Cytokines implicated in the differentiation of human CD4 T cells into Th17 cells is not fully elucidated. Contradictory results have been published regarding the role of TGF-β and IL-6.

According to one group (Yang et al., 2008) Th17 lineage results from stimulation in the presence of TGF-β and IL-21, whereas others (Acosta-Rodriguez et al., 2007a; Wilson et al., 2007; McGeachy and Cua, 2008) observed that the combined action of IL-23 and IL-1 is necessary for the differentiation of human Th17 which can be further enhanced by IL-23 and/or IL-6. Th17/Tc17 secrete IL-17, IL-17F, IL-21 and IL-22 and have been implicated in many human diseases like multiple sclerosis, systemic lupus erythematosus, asthma, rheumatoid arthritis (Afzali et al., 2007; Aujla et al., 2007; Hirota et al., 2007; Kebir et al., 2007; Roark et al., 2007; McGeachy and Cua, 2008; Wong et al., 2008). IL-22 and IL-17 have been related to bacterial protection in lungs and gut, and regulate immune responses of cells in non-lymphoid tissues. IL-21 has been also implicated in humoral immunity in the lymph node germinal center reactions (Dong, 2008). It has been shown that Th17 cells, especially human Th17 cells expressing specific chemokine receptors (CCR6 and CXCR3) can also produce IFN-γ suggesting that the segregation of cytokine profile is not complete (Acosta-Rodriguez et al., 2007b; McGeachy and Cua, 2008).

There is extensive cross-talk between each T cell subsets through the secretion of cytokines that can negatively regulate the differentiation of other subsets. For example, IL-12, IFN-γ or IL-4 can prevent the differentiation induced by IL-23 or TGF-β+IL-6 of human or mouse Th17 respectively (Murphy et al., 2003; Harrington et al., 2005; Annunziato et al., 2007; Wilson et al., 2007; McGeachy and Cua, 2008).

Cytokines are also crucial in the survival and maintenance of naïve and memory T cells, but CD4 and CD8 T cells have different requirements, probably because each subset has intrinsic proliferative capacity (more prominent in the CD8 T cell compartment) (Seder and Ahmed, 2003). The homeostatic proliferation of both naïve CD4 and CD8 T cell populations is strongly reduced when transferred into IL-7-deficient mice (Schluns et al., 2000; Tan et al., 2001; Goldrath et al., 2002) demonstrating that this cytokine is crucial for the maintenance of these cells in mice. IL-15 deficiency selectively affects the homeostasis of naïve mouse CD8 T cells leading to a diminished expansion of these cells but does not affect

naïve CD4 T cells, supporting a selective role for IL-15 in the survival and proliferation of murine naïve CD8 T cells (Zhang et al., 1998; Tan et al., 2001; Judge et al., 2002; Tan et al., 2002; Berard et al., 2003). IL-7 and IL-15 have non-redundant roles in murine naïve CD8 T cell proliferation as treatment of IL-15 deficient mice with IL-7Rα blocking mAbs completely abolishes naïve CD8 T cell division (Goldrath et al., 2002). Similarly, using in vitro studies, human naïve CD4 and CD8 T cell subset survival and expansion are induced by IL-7, whereas IL-15 favors mainly naïve CD8 T cell (Geginat et al., 2001; Alves et al., 2003; Geginat et al., 2003).

IL-7 has been shown to be important for the survival but not for the homeostasis proliferation of murine memory CD4 T cells (Lantz et al., 2000; Tan et al., 2002; Kondrack et al., 2003; Li et al., 2003; Seddon et al., 2003). Although IL-15 is not crucial in the maintenance of this cell subset, it can also promote their survival (Mueller et al., 2003). On the other hand, homeostasic proliferation of murine memory CD8 T cells is induced by both IL-7 and IL-15, but IL-15 seems to play a more crucial role (Schluns et al., 2000; Goldrath et al., 2002; Judge et al., 2002; Kieper et al., 2002; Tan et al., 2002). Homeostatic proliferation of human memory CD4 and CD8 T cells is driven by IL-7 and IL-15 respectively although IL-7 can also affect human CD8 T cells (Alves et al., 2007). Furthermore, IL-15 enhances cytotoxic T lymphocyte responses by inducing IFN-γ, perforin and granzyme B, and up-regulating co-stimulatory receptors (Alves et al., 2007).

T lymphocytes can also acquire the capacity to kill target cells via two distinct ways: lytic enzymes (perforin-granzyme)-mediated and Fas-mediated (including FADD-recruitment, caspase-8, TNFR1 and TRAILR) mechanisms. The lytic enzyme-mediated pathway is mainly used by cytotoxic T lymphocytes (CD8 Tc) and NK cells (Russell and Ley, 2002). NK cells contain storage of lytic enzymes in specific granules, but naïve CD8 T cells lack such enzymes. However, upon complete activation (TCR, co-stimulation and cytokine activation such as IL-2) CD8 T cells express granule components, including perforin and granzymes. In human five types of granzymes are known. It has been demonstrated that upon its

release, perforin rapidly polymerizes in the presence of Ca^{2+} in a ring-like structure that is inserted into the target cell's membrane. First, perforin-induced damage of the target cell's membrane was thought to facilitate entry of granzymes. It was further clarified that perforin is needed not only for creating an entry pore for granzyme into the target cell's membrane, but also to release lytic enzymes from the endocytosed membrane in the created endosome (Barry and Bleackley, 2002). Granzyme A and granzyme B are released by cytotoxic T lymphocytes bound to proteoglycans which protect it from inactivation (Russell and Ley, 2002). Granzyme H can enter target cell in a perforin-independent way, whereas granzyme K is dependent on perforin (Barry and Bleackley, 2002).

2.0 ANTIGEN PRESENTING CELLS (APC)

Although most nucleated cells have the capacity to present Ag from the translated polypeptides they produce in the context of MHC class I, they do not have the capacity to activate naïve CD8 T cells. However, professional Ag presenting cells (APC) have the capacity to take-up external Ag, process them, and then efficiently present them via the MHC class II molecules to naïve CD4 T cells and via MHC class I to naïve CD8 T cells leading to the activation of these adaptive immune cells. In addition to provide TCR stimulation, professional APC supply the necessary extra signals for a full activation of T cells: co-stimulatory molecules and cytokines.

2.1 Monocytes and Macrophages (Mφ)

Monocytes are a subset of peripheral white blood cells that can be distinguished by their bean-shaped nucleus. They originate from a bone marrow myeloid progenitor common with neutrophils. They circulate for several days in the peripheral blood and then enter in different tissues to replenish local macrophage (Mφ) populations or DC. Circulating monocytes and Mφ, which they give rise to, are heterogeneous populations and different monocyte subsets appear to represent different developmental stages with distinct physiological roles

(recruitment to inflammatory sites or entry to normal tissues). Monocytes typically express the differentiation marker CD14, which is part of the receptor for lipopolysaccharide (LPS). Additional markers are used to distinguish different monocyte subsets: including CD16 (Fc receptor), chemokine receptors such as CD192 (CCR2 - receptor for CCL2, a chemokine involved in monocyte infiltration in inflamed sites), CXC3CR1 (receptor for the chemokine CX3CL1), CCR5 (receptor for CCL3, CCL4, and CCL5) (Dorner et al., 2002)). The classical monocytes (Strauss-Ayali et al., 2007) also called inflammatory monocytes by other authors (Gordon and Taylor, 2005) represent the vast majority of circulating monocytes and bear the following phenotype: $CD14^{hi}CD16^{-}$MHC class $II^{+}CCR2^{+}CX3CR1^{lo}$. The second subset of monocytes, less abundant in the circulation is called the non-classical or resident monocytes and has the phenotypic profile $CD14^{+}CD16^{+}$MHCclass$II^{hi}CCR2^{-}CX3CR1^{hi}CCR5^{+}$. Both monocyte subsets can differentiate into DC or Mφ in the presence of adequate stimuli.

Recruitment of monocytes to peripheral sites can be enhanced by pro-inflammatory (which activates them through the classical pathway) or anti-inflammatory cytokines like IL-4 and IL-13 (this leads to their activation through the alternative pathway, leading to inflammation relief and persistence of infections with intracellular pathogens (Ruckerl et al., 2006)), metabolic or immune stimuli leading to their differentiation into Mφ and DC. Tissue Mφ are responsible for clearance of senescent cells, remodeling and repair of tissues after inflammation (Gordon, 1986; Gordon and Taylor, 2005). It is still unknown whether tissue Mφ are derived from random monocytes or from particular lineage-committed precursors (Gordon and Taylor, 2005). They are maintained both by entry of new monocytes and local proliferation. Heterogeneity within Mφ results from the tissue specialization that these cells undergo upon each particular microenvironment (Gordon and Taylor, 2005). Tissue Mφ include osteoclasts in bones, alveolar Mφ in the lungs, microglia, perivascular and meningeal Mφ in the

central nervous system (CNS), and Kuppfer cells in the liver (Gordon and Taylor, 2005).

2.2 Dendritic cells

Dendritic cells (DC) are a heterogeneous population of specialized migratory APC and are dispersed throughout the body. They belong to the myeloid phagocyte system together with Mφ, brain microglia and osteoclasts (Jung, 2004) or have a lymphoid origin like Langerhans' cells and plasmacytoid DC. These cells are very mobile allowing them to carry Ag from peripheral tissues to lymphoid organs and thus becoming highly immunogenic.

Immature DC very efficiently uptake Ag sample from their environment by phagocytosis, macro-pinocytosis, or receptor mediated endocytosis, without discrimination between foreign and self-Ag (Jung, 2004). These cells contain numerous intracellular MHC molecules in the non-lysosomal compartments ready to be loaded with these processed Ag. Immature DC express a variety of sensors of inflammation and PAMPs, and upon stimulation via these sensors stop their Ag up-take activities and enter a maturation process leading to mature DC. High expression levels of MHC molecules and co-stimulatory molecules of the B7 superfamily characterize mature DC, that will then migrate to draining lymph nodes in order to very efficiently activate naïve T cells. In fact, mature DC are the only cells capable of fully activating naïve T cells by efficiently presenting MHC-peptide complexes and co-stimlatory molecules. The initial stimulation of the latter is restricted to the tissue-draining lymph nodes. DC can be both Ag carriers and bystander stimulators and thus can prime directly, cross-prime or cause cross-tolerance. Apart from being immunogenic in the context of complete maturation signalling in the presence of danger/foreign signalling, DC are also responsible for tolerance towards self-Ag. Whether DC will cause tolerization or immunostimulation depends on many factors including the maturation/activation status of the DC such as the expression level of co-stimulatory molecules and tolerogenic cytokine production (such as TGF-β and IL-10) (Jung, 2004), the

turnover of Ag and the amounts of ingested Ag. When low levels of Ag is uptaken, cross-tolerance could take place (Melief, 2003) depending on other conditions present. Fully activated mature DC upon danger/foreign signals are the most efficient APC.

2.3 B cells

B cells originate from the bone marrow and their name initially came from the fact that they were found to mature in birds in a specific organ, called "Bursa of Fabricius". In human or mouse, after reaching a certain stage of maturation (a successful expression of μ heavy chain with a concomitant expression of κ or λ light chain) in the bone marrow, they migrate to the spleen where they reach their full mature state. Each B cell has a unique receptor protein (B cell receptor (BCR)) on its surface, which is an immunoglobulin by nature and binding with good affinity to Ag leads to B cell activation. However, to become fully activated B cells need co-stimulation through CD40 provided by CD40L (CD154) on the surface of activated CD4 T cells (Rodriguez-Pinto, 2005). B cells can also serve as APC and though being less efficient than DC, can still be very effective APC upon appropriate stimulation (Banchereau and Steinman, 1998; Gagro et al., 2006). Ag ligation on the surface of naïve B cells induces signalling through the BCR, leading to the internalization of the complex through the endocytic pathway towards the MHC class II rich compartments where peptide-MHC class II complex is formed, leading to up-regulated MHC class II expression and peptide presentation. The BCR signal also leads to growth, proliferation, survival, and expression of co-stimulatory molecules (e.g. CD86). CD40 signalling enhances the Ag processing (Faassen et al., 1995), increases the MHC class II expression and induces CD86 (Ranheim and Kipps, 1993; Kennedy et al., 1994; Wu et al., 1995; Mackey et al., 1998; Evans et al., 2000; Roy and Chaudhuri, 2008). Upon such activation, B cells can become efficient APC and subsequently activate T lymphocytes. Fully activated B cells divide and become plasma cells, producing different classes of antibodies through different splicing of mRNAs. It has been shown that cytokines

influence the class switching. In mouse, under Th1 conditions (IFN-γ) isotype switching is from IgM to IgG2a and IgG3, in Th2 conditions (IL-4) the switch is towards IgG1 and IgE, while in the presence of TGF-β the switch is towards IgG2b and IgA.

3.0 INTERLEUKIN-27 (IL-27)

Cytokines are soluble proteins released by cells acting as intercellular mediators in the generation of an immune response, and playing roles in cell and body homeostasis. The latter is the ability of the immune system to maintain normal cell counts following depletion or cell expansion. Cytokines include numerous proteins coined lymphokines (produced by lymphocytes), monokines (produced by monocytes/Mφ), interleukins (first seen to be expressed by white blood cells (leukocytes, hence "leukin") as a means of communication ("inter-") and interferons.

Several members of class I helical cytokines share little primary sequence identity but have a common tertiary structure. It is characterised by a bundle of four tightly packed α-helices arranged in an "up-up-down-down" fashion. In most of the class-I cytokines the helix bundle is stabilized by up to three disulphide bridges or in some cases – by hydrophobic interactions (Huising et al., 2006). This group includes: interleukins-2 to 7, -9, -11 to -13, -15, -27, granulocyte-macrophage colony-stimulating factor (GM-CSF), granulocyte-colony stimulating factor (G-CSF), erythropoietin (EPO), ciliary neurotrophic factor (CNTF), leukaemia inhibitory factor (LIF), oncostatin M (OSM), cardiotropin-1 (CT-1), cardiotropin-like cytokine (CLC), thrombopoietin (TPO), leptin and others.

Class-I helical cytokines signal through surface receptors that share the same modular structure. The extracellular domain of the receptor includes at least one cytokine-binding domain of around 200 aa arranged in Fibronectin type -III domains (FnIII). Very often there is a second FnIII or immunoglobulin-like domain associated to the first one. The cytokine FnIII domain that is close to the membrane usually contains a characteristic WSXWS motif. Some of these receptors have long

signal trnsducing intracellular tails whereas others have short ones with reduced capacity for signalling. Thus, the class-I helical cytokine receptor complex contains at least one chain with long intracellular domain responsible for signalling (Huising et al., 2006).

The multimerisation of the cytokine receptor upon ligand binding brings together the long intracellular tails of two receptor chains. Each intracellular domain is constitutively associated with a tyrosine kinase from the Janus Kinase (JAK) Family and upon dimerisation JAKs get activated via transphosphorylation. The mammalian JAK family consists of four members: JAK1, 2, 3 and TYK2 (Shuai and Liu, 2003). Phosphorylated JAKs phosphorylate some intracellular membrane-distal tyrosine residues of the receptor chain. These phosphorylated tyrosines serve as docking sites for members of the signal transducer and activator of transcription (STAT) family. It includes seven members in mammals: STAT1, 2, 3, 4, 5a 5b and 6. Upon docking to a phosphorylated tyrosine, STATs get phosphorylated through their Src homology 2 (SH2) domains. This leads to their dissociation from the receptor chain, with consecutive formation of homo- or heterodimers and translocation into the nucleus to induce transcription (Huising et al., 2006). The types of genes targeted by STATs depend on the composition of the STAT dimer. STATs can be divided into two groups. The first one comprises STAT-2, -4 and -6 which are implicated in the T cell development and in IFN-γ signalling. The second group consists of STAT-1, -3 and -5 which are activated by different ligands playing role in IFN signalling and embryogenesis (Calo et al., 2003). In mice, STAT-1 mediates IFN-γ signaling, STAT-2 plays anti-apoptotic role in IFN-γ responses, STAT-3 mediates IL-10 effects (Matsukawa, 2007), STAT-4 plays role in Th1 differentiation (Matsukawa, 2007), whereas STAT-5 is activated by γc cytokines, EPO, IL-3, IL-5 and different growth factors (Ross et al., 2007).

3.1 Characteristics of IL-27 protein

Interleukin-27 (IL-27) belongs to the IL-6/IL-12 family, together with IL-12 and IL-23. Cytokines of this family are composed of two distinct subunits not linked by a disulphide bond. This lack of covalent link theoretically permits production of the two subunits by different cells with a subsequent extracellular association (Batten and Ghilardi, 2007) or association with different partners. IL-27 subunits are named EBI3 and p28.

The EBI3 subunit of IL-27 has been described for the first time in 1996 as a lymphocyte - specific G-protein coupled receptor family member related to the IL-12p40 subunit (Devergne et al., 1996) and ciliary neurotrophic factor receptor (CNTFR) (Becker et al., 2005). It was identified from Epstein-Barr virus (EBV) transformed B lymphocytes from which the name was coined Epstein-Barr Induced molecule 3. This secreted 34-kDa glycoprotein lacks membrane anchoring motif thus resembles a soluble cytokine receptor. Together with several other proteins involved in hematopoietic cell growth and differentiation, it is encoded on chromosome 19. EBI3 contains two pairs of conserved cysteine residues (positions 35, 46, 79, and 89), implicated in intramolecular disulfide linkage but lacks the cysteine that could mediate heterodimerization with other cytokine subunits. The mouse and human predicted aa sequences share 62% identity as estimated using BLAST from the NCBI webpage.

mRNA encoding for EBI3 is expressed at low levels in human lymphoid tissues, in higher levels in placenta, but is undetected on resting PBMC but significantly expressed in the latter upon activation (Devergne et al., 1996; Pflanz et al., 2002). Murine APC (DC, Mφ, and B lymphocytes) express basal levels of EBI3 mRNA, but such expression is strongly boosted by signalling through the TLRs, via MyD88 and NF-kB p50/p65 (Wirtz et al., 2005). The promoter of EBI3 contains binding sites for the transcription factors NF-κB and PU-1, which have been shown to control its transcription (Wirtz et al., 2005). EBI3 association with IL-27p28 was demonstrated for the first time in 2002 (Pflanz et al., 2002) and its

association with IL-12p35 subunit to form IL-35 has been reported (Devergne et al., 1997; Collison et al., 2007; Niedbala et al., 2007).

The p28 subunit was identified computationally through search for IL-6 helical cytokine homologues (Pflanz et al., 2002) and was named according to its apparent molecular mass, determined by SDS-PAGE. By sequence comparison p28 is very close to IL-11, novel neurotrophin-1 (NNT-1), cardiotrophin-like cytokine (CLC), and ciliary neurotrophic factor (CNTF). It is also structurally related to IL-6, IL-12p35 subunit (Molle et al., 2007), and IL-23p19 subunit. The human p28 (hp28) gene is located in chromosome 16 and encodes a 243 aa polypeptide with a calculated molecular mass of 24.5 kDa (the mouse gene encodes 234 aa and a 23.6 kDa calculated molecular mass). Whereas there are several O-glycosilation sites and no N-glycosilation sites on hp28, there are several N-glycosilation sites on the mouse p28 (mp28), which share 73% identity with its human counterpart (Pflanz et al., 2002). Four SNPs have been determined so far in the IL-27p28 gene: one in the promoter region (g. -964 A>G), one in exon 2 (g.2905 T>G), one between exons 3 and 4 (g.4603 G>A), and one in exon 4 (g.4730 T>C). The prevalence of the haplotype ATT to GGT in the regions of the g. -964 A>G, g.2905 T>G, and g.4730 T>C has been associated with asthma. However, this polymorphism in IL-27p28 gene is not related to changes in serum IgE levels, neither to counts of peripheral blood eosinophils (Chae et al., 2007) and remains to be confirmed by other groups.

IL-27 is rapidly produced by APC (DC or monocytes, or activated microglia) after stimulation, as decribed in Table 3 (Sonobe et al., 2005).

Table 3: Stimuli, inducing mRNA expression of both subunits of IL-27

Stimuli (Monocyte derived -DC)	EBI3 mRNA	p28mRNA
E.coli	++	++
Flu strain PR-8	+/-	-
Pam3Cys (TLR2)	-	-
PIC (TLR3)	+	+
LPS (TLR4)	+	+
R-848 (TLR7-8)	+	-
CpG (TLR9)	-	-
TNF, IL-1β, IL-6, PGE_2	-	-
CD40L	+	-

14-18 hours of stimulation (Schnurr et al., 2005)

APC, including mouse microglia, do not express p28 under basal condition but upon stimulation with IFN-γ, CD40L, LPS, or viruses quickly express the mRNA, such expression also rapidly declines (Pflanz et al., 2002). For example LPS-stimulated human monocyte-derived DC express the maximal p28 mRNA levels after 3-6 h of stimulation and such expression rapidly disappears after 24h (Pflanz et al., 2002). The expression of mouse p28 is mainly controlled at the mRNA levels (Liu et al., 2007) via multiple distinct pathways. Both LPS (the ligand of TLR4) and IFN-γ can induce p28 expression with LPS having a stronger effect, but both can further synergize (Sonobe et al., 2005; Liu et al., 2007). Whereas LPS-induced p28 expression is totally dependent on the TLR4-MyD88 (myeloid differentiation factor 88) pathway and partially dependent on the NF-kB c-Rel (nuclear factor kB c-Rel), the action of IFN-γ on p28 expression is partially dependent on MyD88 and c-Rel-independent (Liu et al., 2007). The p28 promoter contains at least two LPS response elements (Liu et al., 2007) and one interferon-stimulated response

element (ISRE), on which the transcription factor IRF-3 (interferon regulatory factor 3) binds. IFN-γ needs the transcription factor IRF-1 (the major IFN-γ response element (IRE) is localized between -57 and -48) for p28 expression, LPS is less dependent on it. IRF-3 was suggested to be a 'master switch' for p28 synthesis both in mouse and human myeloid cells (Molle et al., 2007).

IL-27p28 is not secreted by human cells in the absence of EBI3 (Pflanz et al., 2002). While mp28 can be secreted independently, hp28 requires the presence of EBI3 (Pflanz et al., 2002). Co-expression at both mRNA and protein levels of both subunits has been shown in DC (Pflanz et al., 2002; Coulomb-L'hermine et al., 2007), mouse NK and NKT cells, mouse CD8 T cells and $CD4^+CD25^+$ regulatory T cells (T_{reg}) (Villarino et al., 2005).

3.2 IL-27 expression in human tissues

In situ histochemical staining showed that in normal lymph nodes, co-expression of EBI3 and p28 was observed only in cells bearing a Mφ or an endothelial cell like morphology (Larousserie et al., 2004). However, in granulomatous diseases of Th1 type (tuberculosis, sarcoidosis, and Crohn's disease) cells positive for p28 and EBI3 were more abundant and had morphology consistent with epithelioid, multinucleate giant cells, endothelial, plasma cells, and Mφ (Larousserie et al., 2004). Stainings were not convincing in all cases and no co-staining was performed to confirm any cell type. The same group looked at the expression of IL-27 subunits in human fetal-maternal interface by looking at trophoblasts. They found that both subunits were detected in cells having the morphology of myeloid, endothelial, plasma cells and by the cells with fetal origin, that have direct contact with the maternal immune system - syncitiotrophoblasts and extravillous trophoblasts (Coulomb-L'hermine et al., 2007). Syncitiotrophoblasts expressed highest levels of both subunits of IL-27 during the first trimester of pregnancy and lower although detectable levels during the following stages (Coulomb-L'hermine et al., 2007). And while EBI3 and p28 mRNA are detected in the placenta through all gestational stages, in the

serum of pregnant women only EBI3 can be detected at the protein level (ELISA), but no significant levels of p28 protein could be detected (Coulomb-L'hermine et al., 2007), but appropriate tools may be lacking. The role of IL-27 during human pregnancy remains to be elucidated. Both IL-27 subunits have been detected at the mRNA in human and mouse retina (Amadi-Obi et al., 2007) suggesting a role for this cytokine in immunopriviledged organs or situations (such as pregnancy).

3.3 Receptor of IL-27

The typical type I cytokine receptor is a multimeric complex, usually consisting of several cytokine receptor chains: a ligand-binding α-chain, with or without intracellular signalling capacity, and a signal transducing β-chain, which is shared by multiple receptor complexes (Daniel G. Remick, 1997; Huising et al., 2006). The complete signalling receptor for IL-27 (IL-27R) consists of two membrane chains: TCCR (also named WSX-1) and gp130, as neither subunit is sufficient to transduce a signal (Pflanz et al., 2004). "WSX-1" comes from the WSXWS protein motif found in the carboxyl terminus of many type I cytokine receptor and TCCR stands for T cell cytokine receptor. The complete signalling receptor (as it will be called here-in the heterodimer of TCCR and gp130) is expressed on NK cells, monocytes, DC, T and B lymphocytes, mast cells and endothelial cells (Szabo et al., 2003; Pflanz et al., 2004; Li et al., 2005).

The glycoprotein 130 receptor (gp130 or CD130) belongs to the structural family of IL-6/IL-12 signal transducing type I transmembrane proteins together with WSX-1, granulocyte colony stimulating factor receptor (G-CSFR), IL-12Rβ1, IL-12Rβ2, Lymphocyte inhibitory factor receptor (LIFR), Oncostatin M receptor β (OSMRβ) and gp130-like monocyte receptor (Pflanz et al., 2004). It is a 918 aa polypeptide with a single transmembrane domain (Hibi et al., 1990) and in human is encoded in chromosome 5 (Rodriguez et al., 1995). The extracellular domain (597 aa) consists of six fibronectin type III modules, followed by a membrane spanning region of 22 aa, and a cytoplasmic region of 277 aa. There are four conserved cysteins (aa 134, 144, 172, and 182) and a WSXWS motif (aa 310-314).

The intracellular domain of gp130 contains the typical protein kinase sequence Gly-X-Gly-X-X-Gly-X-Val without the usual catalytic domain motifs. gp130 is expressed by most cells in the body (Scheller et al., 2005). Receptor association of JAKs (particularly JAK1 and JAK2) to gp130 is mediated by the membrane-proximal box1/box2 regions. Box1 is a proline-rich motif of eight aa residues essential for JAK association. Box2 is a cluster of hydrophobic aa residues followed by positively charged aa and is necessary for JAK association only with some receptors. In some cases the region between these two boxes can also be important for the signalling (Heinrich et al., 1998). A naturally occurring soluble murine form (sgp130) has been shown to bind to IL-6 and thus inhibit its signalling. However, this soluble chain does not inhibit IL-27 signalling (Scheller et al., 2005).

TCCR, a class I cytokine receptor, has been described for the first time as a result of gp130-sequence homology cDNA search (Sprecher et al., 1998). It is encoded in chromosome 19 (human) (like IL-12Rβ1 (Pflanz et al., 2002)) and has been identified in human infant brain after a search for the WSXWS motif. It encodes a receptor of 636 aa, consisting of a hydrophobic signal peptide, a conserved 200aa WSXWS-cytokine binding domain, three fibronectin type III domains, a 26 aa transmembrane domain and a 96 aa cytoplasmic domain. Apart from the conserved cysteine residues in the cytokine binding domain, there are other conserved resides ($proline_{131}$, $tryptophan_{151}$ and $tyrosine_{202}$), as well as a box 1 motif $(Apolar\text{-}X\text{-}X\text{-}X\text{-}Aliphatic\text{-}Pro\text{-}X\text{-}Pro)_{554\text{-}561}$ which are important for association with JAKs, and conserved tyrosine residues in the cytoplasmic domain, 2 for the human receptor and 3 for the murine one, with 1 tyrosine residue (Y609 (Takeda et al., 2003)), conserved between both species. The identity between the human and the murine WSX-1 is 63% (Sprecher et al., 1998). The only tyrosine-based phosphorylation motif in the cytoplasmic domain of this chain is G<u>Y</u>EKHF which closely resembles the STAT motif found in the cytoplasmic region of IFN-γR (G<u>Y</u>DKPH) (Takeda et al., 2003; Pflanz et al., 2004). However, this molecule is not capable of transducing a signal on its own (Pflanz et al., 2004).

TCCR mRNA is expressed at low levels in all tissues (including bone marrow and brain) but at much higher levels in the lymphoid tissues (thymus, spleen, lymph nodes and peripheral blood leukocytes). In mouse, higher levels of TCCR mRNA have been described in CD4 than in CD8 T lymphocytes especially following activation such as 7 day mixed leukocyte reaction (MLR) (Sprecher et al., 1998).

3.4 Downstream signalling of IL-27R

The downstream signalling of IL-27R has been mainly studied in the mouse system. Like all cytokines that bind to type I family cytokine receptors, IL-27 signals via the JAK/STAT pathway. It causes phophorylation of JAK1 (through WSX-1 (Takeda et al., 2003)), JAK2, and TYK2 (tyrosine kinase 2) (Becker et al., 2005), which further activate STAT-1, -2, -3, -5, and to a lesser extent STAT-4 in mouse naïve CD4 (Hibbert et al., 2003; Lucas et al., 2003; Takeda et al., 2003; Kamiya et al., 2004) and CD8 T cells (Morishima et al., 2005). It has been suggested that STAT-2 and STAT-5 activation depends on STAT-1 whereas STAT-3 is independent of that transcription factor (Kamiya et al., 2004). Downstream STAT-1 in mouse T cells, the cascade continues with the induction of T-bet, IL-12Rβ2, granzyme B and perforin in naïve CD8 T cells (Morishima et al., 2005). Moreover, IL-27 synergizes with IL-12 to boost IFN-γ production in a T-bet dependent manner while IL-27 alone does not induce much IFN-γ production (Lucas et al., 2003) in naïve (but not in memory) CD4 T cells (Pflanz et al., 2002; Takeda et al., 2003). It has been shown that T-bet and TCCR are critical for the IFN-γ production by CD8 T cells during infections such as influenza A virus and *Toxoplasma gondii* (Mayer et al., 2008). CD8 T cells require both T-bet and IFN-γR for optimal IFN-γ production in neutral conditions whereas the presence of IL-12 renders both molecules dispensable (Mayer et al., 2008), suggesting an important role for IL-27 in CD8 T cell priming. Using chimeric models in which bone marrow derived cells from either IFN-γR$^{-/-}$, T-bet$^{-/-}$ or TCCR$^{-/-}$ Yeti IFN-γ reporter mice were transplanted into sub lethally irradiated wild type recipients, Mayer and colleagues dissected the contribution of each factor to IFN-γ expression (at both

mRNA and protein levels) by CD8 T cells following different infections (e.g. influenza virus, Sendai virus, *T. gondii*) (Mayer et al., 2008). They observed that T-bet induced by TCCR is necessary, whereas IFN-γR-mediated signals are not essential for priming, expansion or dissemination of Ag-specific CTLs in these infection models (Mayer et al., 2008). In addition, other STAT-1 activating receptors (e.g. IFN-γR, type I IFNRs) can not compensate for direct IL-27R signals (Mayer et al., 2008).

3.5 Effects of IL-27 on T lymphocytes

Like all other cytokines, IL-27 has effects on multiple cell types including NK, NKT, and T cells. Most studies describing the impact of IL-27 on immune cells have been performed using mouse cells and very few studies on human cells are available. More than half of murine NK and NKT cells from naïve mice express TCCR but upon activation such as an in vivo *T. gondii* infection, these cells lose TCCR surface expression (Villarino et al., 2005). However, in the same infected animals TCCR surface expression on CD4 and CD8 T lymphocytes is enhanced compared to naïve mice and both effector and memory T cells express comparable levels of TCCR (Villarino et al., 2005). Similarly, TCR signalling (anti-CD3 cross-linking antibodies) enhances the proportion of TCCR expressing T cells regardless of polarizing conditions (Villarino et al., 2005). TCR-ligation leads to IL-2 production and proliferation of cells (at least one round is necessary for optimal expression of TCCR), which over a prolonged period of time leads to down-regulation of TCCR (but not of gp130) on T cells. These observations suggest that immune cells can be distinctly affected by IL-27 depending on their activation status.

Upon binding to its receptor, IL-27 activates JAK1, JAK2, TYK2, STAT-1, -2 slightly but constantly (Kamiya et al., 2004), -3 and STAT-5 in naïve murine CD4 T cells (Hibbert et al., 2003; Lucas et al., 2003; Kamiya et al., 2004) . Further more, STAT-2 and STAT-5 (but not STAT-3) activation depends on STAT-1 as in STAT-1 deficient mice the activation of these transcription factors is greatly reduced

(Kamiya et al., 2004). IL-27 favours Th1 skewed response in the early stages of activation by synergizing with IL-12, secreted later than IL-27 by APC, and promoting IFN-γ production (by phosphorylation of both STAT-1 and -3). However, IL-27 suppresses IFN-γ production in fully activated murine CD4 T cells (by preferentially activating STAT-3) (Yoshimura et al., 2006). It has been suggested that IL-27 synergizes with TCR-signalling pathways as a co-stimulatory signal (Brender et al., 2007). Presence of IL-2 decreases the enhancing effect of IL-27 on IFN-γ production (Villarino et al., 2005). IL-27 has been related to Th1 differentiation in the early stages of the immune response (Lucas et al., 2003; Takeda et al., 2003; Owaki et al., 2005; Owaki et al., 2006a) also by mediating a strong and rapid up-regulation of ICAM-1 (after α-CD3+α-CD28 stimulation) on naïve CD4 T cells via STAT-1-dependent but T-bet-, IFN-γ-, and STAT-4-independent mechanisms, even in the absence of IL-12 (Owaki et al., 2005). This rapid up-regulation of ICAM-1 leads to activation of ICAM-1/LFA-1/ERK 1/2 signalling pathway, though IL-27 can also activate p38 MAPK/T-bet. The p38 MAPK pathway is dependent on GADD45γ and can lead either to T-bet induction or to STAT-4 activation, whereas ICAM-1/LFA-1 signalling pathway is downstream of STAT-1 phosphorylation (which also leads to T-bet expression) and up-regulation of IL-12Rβ2 chain as well as ERK 1/2 activation. Both pathways (ICAM-1/LFA-1 and T-bet) are involved in IL-27-mediated Th1 differentiation (Owaki et al., 2006a).

The transcription factors T-bet and GATA-3 are responsible for the development of Th1 and Th2 subsets respectively and are mutually suppressive. Addition of IL-27 even to already developed murine Th2 cells converts these cells into Th1 cells via IL-12-independent up-regulation of T-bet and IFN-γ-independent suppression of IL-5 and IL-13 production (Shainheit et al., 2007; Yoshimoto et al., 2007). In a STAT-1 dependent manner, IL-27 induces SOCS-3 expression by T cells after α-CD3+α-CD28 co-stimulation, which inhibits IL-2 production (and its proliferative effect through STAT-5) without affecting CD25 expression (Owaki et al., 2006b). However, other published data (Brender et al.,

2007) have shown no difference in IL-2 production in the absence of SOCS-3, which suggests an IL-2 independent mechanism of SOCS-3 regulation of T-cell responses. Yet, in the absence of SOCS-3, IL-27 induces prolonged STAT-1, STAT-3 and STAT-5 activation (Brender et al., 2007).

Like IL-6, IL-27 augments the allospecific murine CTL generation where the role of T-bet appears to be nonessential (Morishima et al., 2005). IL-27 can increase the expression of the transcription factor Eomesodermin (Eomes), which also enhances the expression of IFN-γ, granzyme B and perforin (Pearce et al., 2003). Most probably, through Ag-specific stimuli, T-bet is activated, whereas through allogeneic stimuli, Eomes is activated (Morishima et al., 2005). As an overall, IL-27 enhances the type 1 cell-mediated immunity, related to IFN-γ and IL-12 production.

IL-27 increases the production of IL-10 by murine CD4 and CD8 T cells in either Th1/Tc1 or Th2/Tc2 but not in Th17/Tc17 conditions (Stumhofer et al., 2007). Its effect is stronger on CD8 T cells according to one group (Fitzgerald et al., 2007b), while according to another group (Stumhofer et al., 2007) CD4 T cells produce more IL-10. This IL-27 mediated induction of IL-10 production can be further enhanced by TGF-β (Awasthi et al., 2007), combination of TGF-β and IL-6 (which can induce IL-10 production independently) (Stumhofer et al., 2007), IL-10 or IL-12 (Fitzgerald et al., 2007b). TGF-β and IL-27 cumulatively up-regulate the expression of T-bet transcription factor in murine naïve CD4 T cells (Awasthi et al., 2007).

Although IL-27 does not change the number of murine IFN-γ^+ CD4 T cells, it modifies the percentage of IL-10^+IFN-γ^+ CD4 T cells in Th1 conditions and increases the number of IL-10^+ CD4 T cells while reducing the number of IL-13^+ and IL-10^+IL-13^+ CD4 T cells in Th2 conditions (Stumhofer et al., 2007). In Th17 conditions, there are three distinct subsets of cells: IL-10^+, IL-17^+, and IL-10^+IL-17^+. IL-27 inhibits the expression of IL-17 but does not increase the percentage of murine IL-10^+ CD4 T cells (Stumhofer et al., 2007). In line with these observations,

IL-27 has been shown to reduce the levels of IL-17 in murine T cells prior to their adoptive transfer to induce experimental autoimmune encephalomyelitis, without changing the levels of IL-12, IL-6 and TNF (Fitzgerald et al., 2007a). However, IFN-γ production was reduced (Fitzgerald et al., 2007a; Shainheit et al., 2007).

Controversially, IL-27 has also anti-inflammatory effects as it negatively regulates the responses of murine Th17 pro-inflammatory immune cells in IFN-γ/STAT-1 - dependent, IL-10-dependent and independent ways (Batten et al., 2006; Stumhofer et al., 2006; Amadi-Obi et al., 2007; Fitzgerald et al., 2007b). In addition, IL-27, like IL-10 but independently of it, suppresses murine IL-2 production by activated mouse splenocytes (Fitzgerald et al., 2007b). In non-polarized murine cultures, it also suppresses granulocyte-macrophage colony-stimulating factor, IL-1β, IL-3, CCL3, CCL4 and lymphotactin, without influencing the production of IL-4 (Lucas et al., 2003; Owaki et al., 2005), IL-6, -7, and -18, CCL2, CCL7, macrophage colony-stimulating factor and matrix metalloproteinase 9 (Stumhofer et al., 2007).

IL-27 alone inhibits the acquisition of $Foxp3^{+}CD25^{+}CD152^{+}$ (CTLA-4) T_{reg} phenotype and their suppressive function via a STAT-3 dependent mechanism (Huber et al., 2008). Contrary to TGF-β-induced T_{reg} cells, murine CD4 T cells differentiated in the presence of TGF-β and IL-27 are able to produce IL-2 and TNF. The inhibitory effect of IL-27 on T_{reg} generation is at least partially STAT3-dependent. STAT3-dependent inflammatory effect and T_{reg} inhibition of IL-27 seem to be opposed by STAT1 signals. Conversely, TGFβ can block IL-27-induced Th1 differentiation which suggests that IL-27 and TGFβ mutually control their effects (Huber et al., 2008).

IL-27 induces STAT-1 and STAT-3 phosphorylation and activation in primary human T cells (Hibbert et al., 2003; Lucas et al., 2003; Takeda et al., 2003). In contrast to IL-12, which shares structural homology with IL-27, the latter does not induce STAT-4 phosphorylation neither in human lymphocytes (Jurkat cells and peripheral blood lymphocytes), nor in human NK cells (Hibbert et al., 2003). Further more, primary human naïve but not memory CD4 T cells proliferate dose

dependently in response to IL-27 after α-CD3+α-CD28 stimulation in the presence of IL-2 blocking Ab (Pflanz et al., 2002). In the absence but not in presence of co-stimulatory signal, IL-12 synergizes with IL-27 in inducing proliferation in naïve (but not in memory) CD4 T cells. This suggests that IL-27 effect on CD4 naïve T cells proliferation can be enhanced either through CD28 or through IL-12 receptors with IL-27 being a stronger proliferative stimulus than IL-12 (Pflanz et al., 2002). The main lines of IL-27 signalling are represented on Fig. 1.

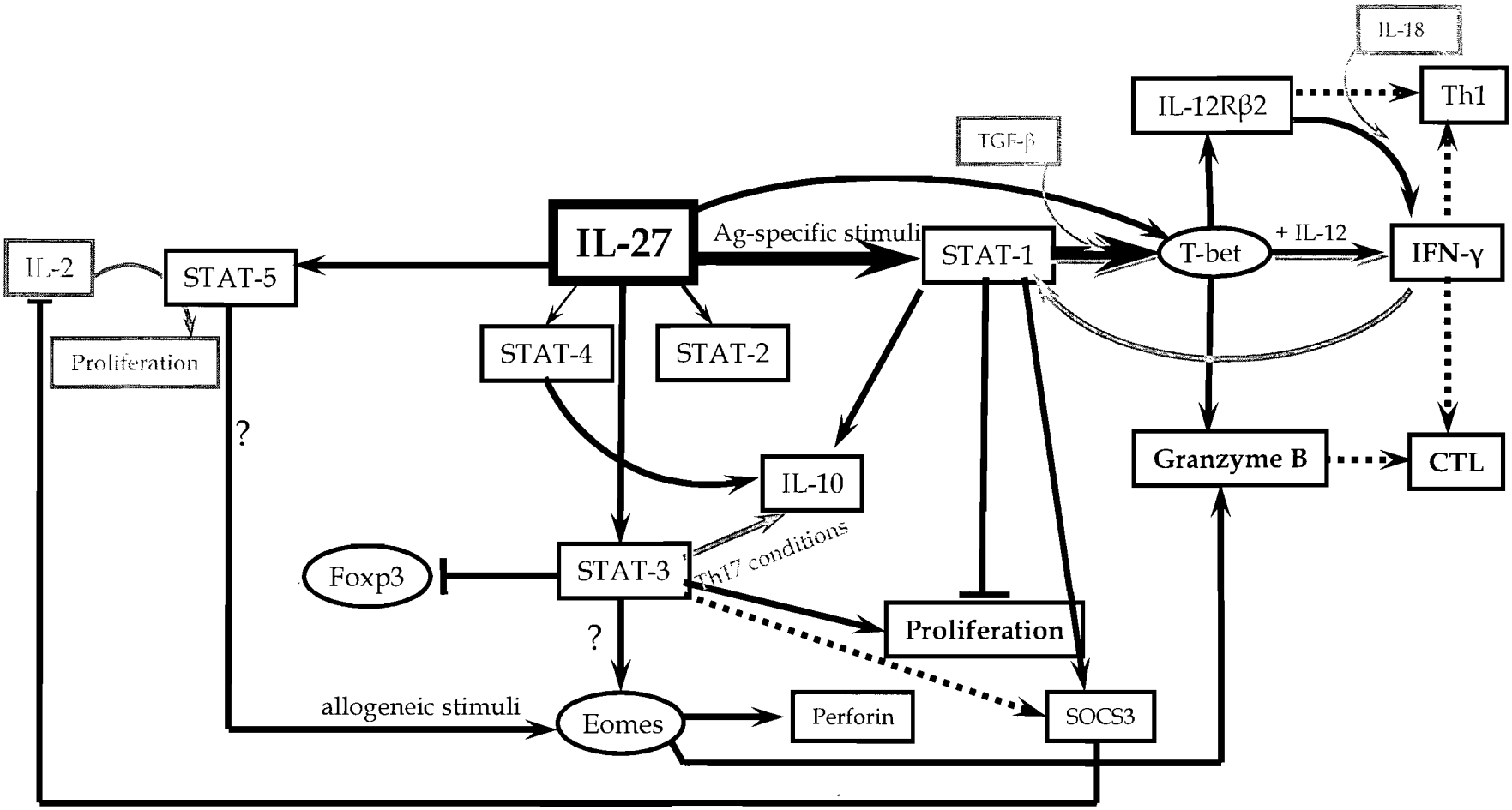


Figure 1: Effects of IL-27 signalling

IL-27 activates the JAK/STAT signalling pathway. The potential of IL-27 to activate many members of this pathway is responsible for its pleiotropic actions. Cytokines marked in grey can modulate IL-27 action.

3.6 Impact of IL-27 on monocytes, Mφ and dendritic cells

While IL-27 weakly activates STAT-1 and STAT-3 in murine Mφ, in human monocytes it induces moderate sustained STAT-1 and STAT-3 tyrosine phosphorylation, with predominant effect on STAT-1. IL-27 pre-treatment prior to TLR2 stimulation leads to increased TNF and IL-6 production (both on protein and mRNA levels), and IL-1β and IL-12p40 on mRNA levels by a STAT-1 dependent mechanism in human monocytes (Kalliolias and Ivashkiv, 2008). Such pre-treatment enhances also the production of cytokines, induced by TLR4 and TLR7/8 signalling in the same cells. Another group has also reported that IL-27 stimulation leads to STAT-1 and -3 phosphorylation in human monocytes and then upregulation of mRNA for TNF, IL-1β and IL-18 and IL-12p35 (last two only at 24 hours) (Pflanz et al., 2004). However, when IL-10 is added to IL-27-primed and TLR2-stimulated human monocytes, these cells secreted less TNF and IL-6. These results suggest the effects of IL-27 on human monocytes depend on the presence of other cytokines in the milieu, such as IL-10. For example, signalling through TLR4 strongly inhibits the downstream events of IL-27 ligation to its receptor in a p38-dependent manner through down-regulation of gp130 mRNA level (Kalliolias and Ivashkiv, 2008).

Studies performed using the pro-monocytic human cell line THP-1 have shown that IL-27 induces mRNA and surface expression of MHC class I and class II through IRF-1 (IFN-regulatory factor-1) (Feng et al., 2008). Furthermore, pro-inflammatory action of IL-27 is shown by the enhanced expression of co-stimulatory molecules CD80 and CD86 and adhesion molecule CD54, class II transactivator (CIITA) isoforms III and IV (but not I) transcripts in these cells (Feng et al., 2008).

3.7 Impact of IL-27 on B lymphocytes

IL-27R expression varies throughout the differentiation and after activation of human B cells; while naïve and memory B cells show constitutive expression of the complete receptor, germinal center (GC) B cells express barely detectable levels of TCCR which means that TCCR is down-regulated during differentiation of naïve B cells into GC B cells with a following up-regulation during the transition from GC to memory B cells (Larousserie et al., 2006). The IL-27R is up-regulated on all human B cell subsets upon in vitro stimulation through CD40 or surface IgG. And despite the similar expression levels of IL-27R on naïve and memory B cells, the former subset shows a stronger response to IL-27. Murine B cells express levels of TCCR comparative to those of naïve CD4 T cells (Yoshimoto et al., 2004; Gagro et al., 2006; Larousserie et al., 2006).

In naïve human B cells the signalling through IL-27R leads to phosphorylation of STAT-1 and STAT-3, whereas in the memory subset this leads to moderate activation of STAT-1 and low activation of STAT-3 (Larousserie et al., 2006). In line with this, a stronger T-bet activation is observed in naïve human B cells compared to the memory subset, regardless of the activation mode, corresponding to an increased proliferation in naïve but not in memory B cells (Larousserie et al., 2006). IL-27 has been also shown to increase the expression of IL-12β2 chain at both mRNA and protein levels, after anti-Ig (but not ati-CD40) stimulation in tonsillar human B cells (Larousserie et al., 2006). IL-27 enhances CD54 (ICAM-1), CD86 (B7.2) and CD95 (Fas) expression in a CD40-independent but BCR activation dependent manner (Larousserie et al., 2006). And while in T lymphocytes IL-27 induces IFN-γ production, no such effect is observed in the B cell population (Larousserie et al., 2006).

In murine B cells, IL-27 induces an IFN-γ independent, STAT-1 and T-bet – dependent IgG2a class antibody switching (Yoshimoto et al., 2004). It appears that this process of antibody switching is highly T-bet dependent when the stimuli are T-independent (e.g. LPS), and not that much T-bet dependent in the presence of T-dependent stimuli (e.g. CD40) (Morishima et al., 2005). However, in human B cells

different effects are observed for many reasons including the absence of the IgG2a isotype and the very low levels of TLR4 on human B cells, thus, human B cells respond very poorly to LPS stimulation (Larousserie et al., 2006).

3.8 Impact of IL-27 on other cell types

IL-27 has been shown to have impact on other immune cells. In mouse NK cells, IL-27 induces T-bet but that does not lead to IFN-γ production or activation of cytolytic activity according to one group (Lucas et al., 2003) but according another, IL-27 increases the IFN-γ production in NK cells in the presence of IL-2 and IL-12 (Pflanz et al., 2002). Human mast cells express TCCR and gp130 at the mRNA levels and incubation with IL-27 leads to phosphorylation of STAT3 (Pflanz et al., 2004). IL-27 also enhances inflammatory properties of these cells by enhancing the expression of IL-1α, IL-1β, IL-18, TNF, proliferation inducing ligand, B lymphocyte stimulator, and T cell-expressed activating specific receptor ligand without changing the expression levels of lymphotoxins α and β, CD40L and CD27L (Pflanz et al., 2004).

In human umbilical endothelial cells, IL-27 has been shown to up-regulate both MHC class I and MHC class II through IRF-1 and MHC class II transactivator (CIITA) isoforms III and IV with more prominent expression of isoform IV (Feng et al., 2007).

Murine osteoblasts (cells responsible for the bone formation through production of collagen type I and its mineralization) express both chains of IL-27R at the mRNA level. IL-27 has been shown to induce phosphorylation of STAT-1 and STAT-3 in these cells but the consequences of this phosphorylation are unclear. However, in the presence of exogenous IL-27 there is a reduced osteoclastogenesis but no change of osteoblast activity, as measured by alkaline phosphatase production, which is a marker of bone-formation (Kamiya et al., 2007).

3.9 Roles of IL-27 in mouse models of human diseases

Many studies attempting to decipher the roles of IL-27 during in vivo immune responses related to human diseases or conditions have compared the wild type mice to TCCR knockout ($TCCR^{-/-}$) mice. These mice ($TCCR^{-/-}$) do not display obvious defect of their immune system as assessed by flow cytometry phenotyping of major lymphoid organs (Chen et al., 2000) and do not spontaneously develop autoimmune diseases. Although TCCR has been described as the specific IL-27R chain, one cannot completely rule out the possibility that this chain could pair with other receptor chains than gp130 and respond to other cytokines. With this concern in mind, the $TCCR^{-/-}$ mice have been studied by many groups. T cells from $TCCR^{-/-}$ mice show enhanced proliferation, impaired IFN-γ and enhanced IL-4 production during the initial stages of activation both in vitro and in vivo (Hamano et al., 2003; Artis et al., 2004; Miyazaki et al., 2005). However, at later stages, the IFN-γ production is restored (Yoshida et al., 2001), probably through other pathways than IL-27 activation. Other groups have also either block IL-27 or injected IL-27 or used EBI3 knockout mice to elucidate the contribution of IL-27.

$TCCR^{-/-}$ mice are highly susceptible to different pathogens like *Leishmania major* (Yoshida et al., 2001; Artis et al., 2004), and *Trypanosoma cruzi* (Hamano et al., 2003), compared to wild type controls due to an impaired Th1 response (Chen et al., 2000; Yoshida et al., 2001). The absence of TCCR is also related to formation of poorly differentiated granulomas with dispersed accumulation of mononuclear cells, when bacillus Calmette-Guerin (BCG) infection is present (Yoshida et al., 2001).

In several Th2 mediated diseases, like asthma (Miyazaki et al., 2005), infection with *L. major* (Artis et al., 2004) and with *Trichuris muris* (Bancroft et al., 2004) similar observations have been made. Enhanced symptoms have been observed in $TCCR^{-/-}$ mice compared to wild type due to enhanced Th2 responses. In the murine asthma model increased hyperplasia and pulmonary eosinophil

infiltration and elevated serum IgE levels were observed in $TCCR^{-/-}$ deficient mice. This correlated with increased airway responsiveness after Ag sensitization and challenging, enhanced proliferation in the peribronchial lymph nodes, increased production of IL-4, -5 and -13 at both protein and mRNA levels, as well as increased production of IFN-γ but decreased expression of T-bet (Miyazaki et al., 2005).

mRNA coding for IL-27 (EBI3 and p28) and TCCR are expressed at low levels in normal mouse central nervous system (CNS) but during experimental autoimmune encephalomyelitis (EAE) (an animal model of multiple sclerosis,) such expression is dramatically up-regulated at the peak of the disease in CNS (microglia) and in lymph nodes but not in the spleen (Li et al., 2005; Fitzgerald et al., 2007a). IL-27 and TCCR expression is down-regulated during remission and again up-regulated during relapses (Li et al., 2005; Fitzgerald et al., 2007a). An anti-IL-27p28 antibody decreased multiple parameters: EAE clinical score, number of infiltrating parenchymal mononuclear cells in the spinal chord, Ag-specific proliferative response of T cells, IFN-γ production (both in terms of amount, production per cell, and number of cells producing this pro-inflammatory cytokine) (Goldberg et al., 2004). However, as Batten and Ghilardi highlighted, the polyclonal serum used in this study has not been shown to specifically block IL-27p28 and not the entire IL-27 molecule (Batten and Ghilardi, 2007). In contrast, using the same EAE model (MOG 35-55-immunized C57BL/6), $TCCR^{-/-}$ mice have been shown to be more susceptible to EAE induction suggesting that IL-27 signalling is protective by dampening the detrimental Th17 cell development (Batten et al., 2006). Additional publications (Fitzgerald et al., 2007b; Stumhofer et al., 2007) have shown that IL-27 mediates its suppressive effects on encephalitogenic T cells by inducing IL-10 production by T cells.

TCCR has been shown to play a critical role in the induction of experimental autoimmune uveitis (EAU) as in its absence massive infiltrates of lymphocytes, Mφ and neutrophils in the retina can be seen (Sonoda et al., 2007). Following

immunization with the specific Ag causing the autoimmune disease, CD4 T cells in $TCCR^{-/-}$ mice produce significantly lower levels of IFN-γ, CXCL10, RANTES and MCP-1, which normalize during the development of the disease. In addition, sub-retinal transplantation of immunized $TCCR^{-/-}$ T cells fail to transfer the disease as they do not accumulate in the eye and are suspected to have some additional functional changes (Sonoda et al., 2007).

A murine model of colon inflammation (dextran sulfate sodium (DSS) – induced colitis) was used to assess the role of IL-27 in inflammatory bowel diseases. $TCCR^{-/-}$ mice develop a less severe colitis than their wild type counterparts. These observations correlated with the reduced expression of T-bet in $TCCR^{-/-}$ mice, reduced IFN-γ production by lamina propria mononuclear cells and mesenteric lymph node cells, reduced IL-6 and TNF in serum (Honda et al., 2005). This study suggests that IL-27 contributes to boost the detrimental processes taking place in inflammatory bowel diseases.

Concanavalin A (Con A) injection is used as a mouse model of human viral or autoimmune hepatitis. Activated lymphocytes, especially NKT cells, and inflammatory cytokines (e.g. IFN-γ) play important role in this model. $TCCR^{-/-}$ mice demonstrated a more severe hepatitis than wild type mice, showing increased levels of alanine aminotransferase (as a marker of liver status) in serum, correlating with overproduction of IFN-γ and IL-4 by liver and spleen NKT cells (Yamanaka et al., 2004).

Depending on the type of immune responses involved in the detrimental inflammatory and/or autoimmune diseases, the absence of TCCR in the mouse model can abate or exacerbate the disease. Thus, in mouse models of Th1-mediated autoimmune disease, knocking out the receptor will make the disease less severe. This is due to the fact that IL-27 promotes Th1 immunity and in the absence of its signalling, the autoimmune disorder will be less severe.

Injection of IL-27 suppresses Th2 cell development and Th2 cytokines production from polarized Th2 cells in an animal model of Th2-mediated allergic

inflammation (Yoshimoto et al., 2007). IL-27 mitigates asthma through suppression of the highly bronchogenic IL-13 (Yoshimoto et al., 2007). IL-13 and IFN-γ have been shown to be implicated in fibrosis (IL-13 is a mediator, whereas IFN-γ is an antagonist). IL-27 has been show to also reduce IL-13 in schistosome granulomatous inflammation and thus decrease the fibrosis in this model (Shainheit et al., 2007).

In another murine model, in which experimental peritonitis is induced through cecal ligation and puncture (CLP), increased IL-27 protein levels in blood and increased IL-27p28 and EBI3 mRNA in lungs and spleen were detected (Wirtz et al., 2006). $EBI3^{-/-}$ mice have been shown to be less susceptible to both CLP peritonitis and to intraperitoneal *Escherichia coli* induced disease having lower bacterial loads in the blood (Wirtz et al., 2006). Moreover, administration of exogenous IL-27 in $EBI3^{-/-}$ mice has overturned the protection given the increased mortality observed. Also, in $EBI3^{-/-}$ mice granulocytes produce more reactive oxygen intermediates (ROI) as this process is controlled by IL-27 most probably upon TLR ligation (Wirtz et al., 2006). In the same model, reduced number of invariant NKT (iNKT) cells in liver and spleen has been reported. These cells share surface markers with both conventional T and NK cells and are important in the immune responses to tumours, infections and auto-Ag, in the induction of peripheral tolerance and hypersensitivity responses. However, the numbers of B and T cells were normal, as well as the ratios CD4 vs. CD8 and naïve vs. memory. $EBI3^{-/-}$ and $EBI3^{+/-}$ mice are almost completely protected from oxazolone-induced colitis, whereas wild type counterparts were not, suggesting that the threshold of susceptibility to tissue injury (Nieuwenhuis et al., 2002) is mediated by different levels of EBI3 or IL-27.

IL-27 has been shown to mediate growth inhibition and complete regression of murine neuroblastoma tumours through up-regulation of MHC class I molecules specific CTL activity and IFN-γ production (which is also implicated in up-regulation of MHC-I) (Salcedo et al., 2004). In IL-27-expressing tumours,

splenocytes have enhanced secretion of IFN-γ and cytolytic activity of CD8 T cells upon restimulation with parent cell lines, which correlate with immunological memory against tumour cells (Hisada et al., 2004; Chiyo et al., 2005). The enhanced cytotoxicity is STAT4 and IL-12p40-independent (Morishima et al., 2005), suggesting that IL-27-induced CTL activity is IL-12-independent. Furthermore, IL-27 suppresses tumour-induced neovascularisation by direct induction of antiangiogenic chemokines (IP-10 and MIG) by endothelial cells (Shimizu et al., 2006). And while $gp130^{-/-}$ mice develop gastric cancer (Tebbutt et al., 2002), it has to be further clarified whether it is due to p28, EBI3, TCCR (Kastelein et al., 2007) or other cytokines (including yet unidentified), signalling through this subunit.

3.10 Roles of IL-27 in human disorders

Few studies have addressed the potential roles of IL-27 in human diseases mainly looking first whether IL-27 is expressed during disorders such as viral infections, autoimmune diseases, inflammatory diseases and cancer.

IL-27 can be triggered in response to viral and bacterial infections (see table 3) (Veckman et al., 2004; Gafa et al., 2006; O'Dwyer et al., 2008) but the actual role of this cytokine in fighting these pathogens remains to be elucidated. In the context of HIV infection, IL-27 has been shown to inhibit both X4 (using the CXCR4 chemokine receptor) and R5 (using the CCR5 chemokine receptor) tropic HIV-1 replication through activation of anti-viral proteins like IFN-induced protein, IRF-1, IRF-8, myxovirus resistance protein and 2'-5'-oligoadenylate synthetase (Fakruddin et al., 2007). In human, severe sepsis has been correlated with higher mRNA levels of IL-27 than less severe (O'Dwyer et al., 2008). In animal models of sepsis, gene deletion studies have shown that IL-23 is protective while IL-27 impairs Mφ function, which could explain the observations in human (O'Dwyer et al., 2008).

Inflammatory bowel diseases encompass multiple diseases with share but also distinct characteristics. In Crohn's disease (CD, which a Th1-mediated disorder) elevated levels of IL-27p28 and EBI3 mRNA have been detected,

whereas no changes have been observed in ulcerative colitis (UC), and specific colitis (SC), which are Th2-mediated (Schmidt et al., 2005). IL-27p28 levels have been shown to normalize in CD patients after steroid treatment. Rheumatoid arthritis is another autoimmune disorder that has been long considered Th1-induced. Like in many other diseases, since the discovery of Th17 subset, it is now considered a Th17-mediated disease at least in part. IL-27 was detected by immunohistological staining on the synovial membranes of patients with rheumatoid arthritis (Niedbala et al., 2008).

EBI3 but not p28 expression has been detected in human EBV or Human T-cell Leukemia Virus type 1 (HTLV-1) transformed cells, both in situ and in culture supernatants (Larousserie et al., 2005) suggesting that EBI3 could play other roles in tumours.

3.11 Interleukin-35: EBI3 + IL-12p35

EBI3 has been described to form a heterodimer with IL-12p35 in the culture medium of COS7 and BJAB cells, co-transfected with both proteins (Devergne et al., 1997). This novel cytokine was later named IL-35 (Niedbala et al., 2007) and has been shown to have anti-inflammatory properties. IL-35 expanded regulatory T cells ($CD4^+CD25^+Foxp3^+IL\text{-}10^+$) and suppressed Th17 cell development (Niedbala et al., 2007). IL-35 preferentially polarized cells towards Th1 since it augmented the IFN-γ production by CD4+CD25- (effector T cells) (Niedbala et al., 2007) for still unresolved reason. IL-35 significantly suppressed Th17 development when added from the start to a Th17 conditioned murine culture (TGF-β, IL-1β, IL-23) (Niedbala et al., 2007). IL-35 has been shown to reduce the incidence of rheumatoid arthritis in a mouse model which is known to be Th17-mediated. This effect was due to a preferential increase of IL-10 production without changing the levels of IFN-γ, IL-6, IL-12, TNF and IL-1Rα (Niedbala et al., 2007). EBI3 was highly expressed at the mRNA levels in $CD4^+CD25^+$ regulatory T cells, as well in $Foxp3^+$ thymocytes. However, it was absent in naïve T_{eff} CD4 T cells and essentially absent in $Foxp3^-$ and $CD4^+CD8^+$ thymocytes. This suggests that EBI3 is

a downstream target of Foxp3 and most probably IL-35 is potentiated during active suppression of naïve T_{eff} cells. Furthermore, addition of recombinant IL-35 to α-CD3+α-CD28 stimulated naïve T_{eff} cells suppressed their proliferation while ectopic expression of this cytokine conferred regulatory functions to the same subset of cells (Collison et al., 2007). These recent publications place IL-35 in the group of inhibitory cytokines, produced by T_{reg} cells and required for maximal suppressive activity (Collison et al., 2007).

HYPOTHESES AND OBJECTIVES

HYPOTHESES

IL-27, a recently identified cytokine, has been shown to influence mouse CD4 and CD8 T cells by promoting type I immunity. It has been shown to enhance IFN-γ, perforin and granzyme B production. However, very few data have been published about its impact on human blood cells. Although the mouse model has been successfully exploited to understand the immune system, numerous differences have been reported between mouse and human immune responses. Thus, it is important to address the impact of this cytokine on human cells. In the light of the already acquired knowledge about the effects of IL-27 in the mouse system, we hypothesized:

1) IL-27 has pro-inflammatory effects on human T lymphocytes, leading to increased production of effector molecules such as IFN-γ and granzyme B.
2) The IL-27R is expressed at different levels among human blood cells and is modulated upon cell activation.

OBJECTIVES

In order to prove our hypotheses right or wrong, we had the following objectives:

1) To determine the levels of IL-27R expression on different cell types among human PBMC ex-vivo to be as close as possible to the in vivo situation.
2) To evaluate the surface expression of IL-27R by human T lymphocytes upon activation.
3) To assess the effects of IL-27 on human T cell proliferation and effector functions.
4) To determine whether T lymphocytes could be a source of IL-27.

MATERIALS AND METHODS

1.0 ISOLATION OF HUMAN BLOOD CELLS

1.1 Peripheral mononuclear cell isolation

Peripheral blood from consenting healthy donors was collected in 10 cc ethylenediaminetetraacetic acid (EDTA) treated tubes VACUTAINER® (Becton Dickinson, Oakville, ON, Canada). Peripheral blood mononuclear cells (PBMC) were isolated by density gradient using Ficoll-Paque Plus™ (Ficoll) (GE Healthcare Bio-Sciences AB, Uppsala, Sweden). Blood was mixed with an equivalent volume of Phosphate Buffered Saline (PBS) (137 mM NaCl (Wisent, Saint-Bruno, QC, Canada), 2.7 mM KCl (Wisent), 4.3 mM Na_2HPO_4 (Wisent) and 1.47 mM KH_2PO_4 (Sigma-Aldrich, Oakville, ON, Canada), containing 2 mM EDTA (Wisent) [PBS-EDTA] in order to avoid aggregation of cells. Diluted blood was put in 50 ml sterile tubes (Corning, distributed by Fisher Scientific Ltd., Nepean, ON, Canada) (35 ml/tube) and then Ficoll was under-layed at the bottom (14 ml/tube). Tubes were spun at 863 *x g* for 30 min with average acceleration but without brakes for deceleration. The most upper layer in the tube, which is the serum, was discarded and the layer of PBMC, just underneath was collected and transferred to new 50 ml tubes in order to perform washes since Ficoll is toxic for cells. All washes were performed in PBS-EDTA; the first wash was spun at 624 *x g* for 15 min. After the first wash, supernatants were discarded, cell pellets were resuspended and tubes were filled up again with PBS-EDTA to perform two additional washes at 423 *x g* for 10 min. The amount of PBMC obtained was evaluated by counting under a hemacytometer using Trypan Blue 0.16% (v/v in PBS) (Wisent) to exclude dead cells.

The formula used for cell number calculation was:

Total cell number = N x dilution factor x V x 10^4

N = Average number of cells (from at least two hematocytometer chambers)

V = total volume of cell suspension

PBMC were either resuspended in RPMI 1640 (Wisent) containing 10% (v/v) fetal bovine serum (FBS) (Wisent), Penicillin (100 IU/ml), Streptomycin (Wisent)

(100 μg/ml), and L-glutamine (Wisent) (2 mM) [complete RPMI] for culture or in MACS buffer for CD8 T cell subset isolation (see below).

1.2 Total CD8 T cell isolation

PBMC were resuspended in MACS buffer (PBS, 2 mM EDTA, 0.5% (v/v) FBS) (cold and freshly prepared), passed through a 70 um nylon mesh (BD) to remove cell aggregates, and were spun for 10 min at 216 *x g* to remove platelets which may clog the isolation column. Pellet was resuspended and cells were incubated with CD8 Microbeads (magnetic beads coated with anti-human CD8 antibodies (Abs))(Miltenyi Biotech Inc., Auburn, CA, USA) as for $100X10^6$ cells were put in 900 μl MACS buffer and incubated in the presence of 100 μl CD8 Microbeads for 15 min at 4°C. One wash in MACS buffer was then performed to remove unbound Microbeads at 423 *x g* for 10 min. Meanwhile, the LS column was washed with 3 ml MACS buffer. Cells were resuspended in 0.5 ml MACS buffer and put on the LS column. Cells not bound with CD8 Microbeads were removed by performing three consecutive washes with MACS buffer. Column was then removed from the magnetic holder and MACS buffer was added and pushed-through to collect the positive fraction (CD8 cells). In order to obtain a higher purity (>95%), the positive fraction was resuspended again in 0.5 ml MACS buffer and was passed through a new LS column via the same procedure. Purity was assessed for each cell isolation by flow cytometry (see below). Isolated CD8 T cells from the positive fraction were counted and resuspended in complete RPMI.

1.3 Naïve CD45RA$^+$ CD8 T cell isolation (negative selection)

PBMC were resuspended in MACS buffer so that the total volume of cells and MACS buffer was 400 μl for 100 $x10^6$ PBMC. Cells were then incubated in the presence of 100 μl Ab-cocktail from a CD8 T-cell isolation kit II (Miltenyi Biotech Inc) (cocktail of biotin-conjugated antibodies against CD4, CD14, CD16, CD19, CD36, CD56, CD123, TCR γ/δ, and CD235a), at 4°C for 10 min. MACS buffer (100 ul) and (200 μl) anti-biotin conjugated beads from the kit (CD8 T-cell isolation kit

II from Miltenyi Biotech Inc.) were added and cells incubated for another 10 min at 4°C. Then, 200 µl anti-CD45RO coated microbeads (Miltenyi Biotech Inc.) were added and incubated for 15 min at 4°C. To remove unbound Abs, cells were washed once with MACS buffer at 423 *x g* for 10 min. The pellet was resuspended in 1.2 ml MACS buffer and was passed through a pre-washed LS MACS column. The negative fraction was collected (the fraction that was not conjugated with beads). Purity was assessed each time cells were isolated by flow cytometry (see below) and in all cases purity was above 90% (contaminating cells were as follows: about 0.9% $CD14^+$; 2.9% $CD3^+CD8^-CD45RA^+$; 1.1% $CD3^-CD8^-CD45RA^+$ and 1.9% $CD3^-CD8^-CD45RA^-$). Cells were counted and then resuspended in RPMI for 5- (and 6-) carboxyfluorescein diacetate succinimidyl ester (CFSE) (from Molecular Probes, owned by Invitrogen, Burlington, ON, Canada) labelling. CFSE-labelled naïve CD8 T cells were finally resuspended into complete Iscove's medium [Iscove's medium (Gibco Invitrogen), 10% (v/v) FBS, 1 mM sodium pyruvate (Wisent), 100 µM non-essential Amino Acids (Wisent) and 50 µM 2-mercaptoethanol (Sigma-Aldrich)] and put in culture.

1.4 Memory $CD45RO^+$ CD8 T cell isolation

Two protocols were used in order to compare the purity of isolated cells. The first one was a negative selection - same as for naïve $CD8^+$ T cells, with the only difference that instead of anti-CD45RO Abs coated beads, anti-CD45RA Abs coated beads (Miltenyi Biotech Inc.) were used.

The second protocol consisted in one negative selection of $CD45RA^-$ cells and then a positive selection of CD8 T cells. PBMC ($100x10^6$) were put into 800 µl MACS buffer in the presence of 200 µl anti-CD45RA Microbeads (Miltenyi Biotech Inc.) and incubated for 15 min at 4°C and then washed with MACS buffer at 423 *x g* for 10 min. Pellet was resuspended in 1 ml MACS buffer and was passed through pre-washed LS column. The negative fraction was harvested, cells were pelleted at 423 *x g* for 10 min and then resuspended in MACS buffer (as for

$100x10^6$ cells -900 µl added) and then 100 µl anti-CD8 Microbeads were added for a 15 min incubation at 4°C. Cells were then washed with 20 ml MACS buffer as previously described and pellet was resuspended in 0.5 ml MACS buffer prior to be added to a pre-washed LS MACS column. Positive fraction was collected (the push-trough from the LS column). Cells were counted and spun down, then resuspended in complete RPMI to perform CFSE labelling (the use of this medium decreased cell death during CFSE labelling due to its protein content). Final cell fraction was resuspended in complete RPMI and was put into cell culture.

As for naïve CD8 T cells, each time cells were isolated, purity stain was performed and purity was about 75%. Contaminating cells were as follows: 10% $CD3^+CD8^-CD45RO^+CD45RA^-$ (supposedly CD4 memory T cells), less than 1% $CD8^+CD45RA^+$, 2.3% $CD14^+$ and 10% $CD3^-CD8^-CD45RA^-CD45RO^-$, which were also $CD19^-CD56^-CD14^-$.

2.0 FUNCTIONAL ASSAYS ON HUMAN T CELLS

2.1 CFSE labelling

PBMC were labelled with 1.25 µM CFSE for 10 min at 37°C in either RPMI or complete RPMI. Dye was quenched for few minutes with FBS (50% v/v) and cells were then washed twice with cold RPMI and spun down at 423 *x g* for 10 min, and finally resuspended in an appropriate complete medium. This green dye attaches non-specifically to proteins in the cytoplasm of the cells so at each round of cell division it is distributed evenly between daughter cells and thus its fluorescence diminishes by two, which allows following cell proliferation.

2.2 Cell stimulation

2.2.1 PBMC

To determine modulation of IL-27R (gp130 and TCCR) expression, PBMC were put in culture in complete RPMI ($2 X 10^6$ cells/ml) in 5 ml snap cap tubes (1 ml per tube) in a CO_2 (5%) incubator at 37°C in the absence or presence of anti-CD3 (α-CD3) (clone OKT3, grown and purified in house) Ab either at 17 and 340 ng/ml

(doses optimized at the beginning to provide about 50% or maximal proliferation of T cells respectively). Cells were harvested at day 2, 4, 6, and 8, spun down, resuspended in FACS buffer (PBS containing 1% (v/v) FBS and 0.1% (w/v) NaN_3) at 1 X 10^6 cells/50ul. Cells were stained as described below using the Abs listed in Table 5.

To determine effects of IL-27 on effector functions, CFSE- labelled PBMC were put into culture (1x10^6 cells/ml) in the presence or absence of α-CD3 antibody at different concentrations - from 8.5 to 17 ng/ml in presence or absence of recombinant human interleukin-27 (IL-27) (1 - 250 ng/ml) (R&D systems, distributed by Cedarlane Laboratories Ltd., Hornby, ON, Canada). The recombinant protein consists of EBI3 and p28 subunits, attached via a peptide linker. After 6 day-culture, supernatants were harvested and frozen for later ELISA tests and cells were transferred into 96-round bottomed plate and stimulated with Phorbol 12-myristate 13-acetate (PMA; 20 ng/ml) (Sigma-Aldrich), ionomycin (500 ng/ml) (Sigma-Aldrich) in the presence of brefeldin A from Penicillium Brefeldianum (BFA; 5 µg/ml) (Sigma-Aldrich), for 5 hours prior to surface and intracellular flow cytometry staining.

2.2.2 Naïve CD8 T cells (plate-bound)

Experiments with CFSE labelled naïve cells were performed on a flat bottomed 48-well plate, pre-incubated overnight (O/N) with α-CD3, diluted in PBS. Two concentrations were used: 3.4 µg/ml and 6.8 µg/ml (as determined by our optimisation assays for 50% proliferation). Before adding cells, the coating antibody was aspired to ensure complete removing. In each well 2x10^5 cells in 500 ul of complete Iscove's were put in the presence or absence of co-stimulating molecule anti-CD28 (1 ng/ml) and in the presence or absence of IL-27 (concentrations used: 10, 100, 250 and 500 ng/ml) and were incubated for 6 days. Supernatants were harvested and frozen for later ELISA tests and cells were transferred into 96-round bottomed plate and stimulated with the combination

PMA+ ionomycin + BFA as described above prior to be stained for flow cytometry analyses.

2.2.3 Memory CD8 T cells (plate-bound)

CFSE labelled memory cells were also incubated in 48-flat bottomed well plates, coated with OKT3 Ab, but at lower concentration of stimulating antibody (490 ng/ml), a ED50 dose previously optimized in the laboratory. Cells were incubated for five days in the presence or absence of IL-27 (concentrations used: 0.1, 1, 10, 100, 250 and 500 ng/ml). Cells were harvested and stained as described for naïve CD8 T cells.

2.3 Flow cytometry

Prior to all flow cytometry stainings, cells (PBMC or isolated $CD8^+$ T cell subsets) were blocked with normal mouse immunoglobulin (Caltag-Invitrogen) for at least 30 min at 4°C. Cells were then split into two wells of a V-bottom 96-well plate: one used for appropriate labelling and the second one used for isotype control Abs (put at the same concentration than labelling Abs). Cells were stained using the Abs specific for human Ag listed in Table 5. Surface staining was performed for at least 30 min on ice in FACS buffer (PBS containing 1% (v/v) FBS and 0.1% (w/v) NaN_3) in a total volume of 50µl. To remove unbound Abs, cells were washed with the same buffer 2 times. For intracellular staining, cells were permeabilized and fixed in paraformaldehyde (PFA) -saponin buffer [Hank's Balanced Salt Solution (HBSS) (Wisent), containing 4% (w/v) paraformaldehyde (Sigma-Aldrich), 1 mM HEPES (Wisent), and 0.1% (w/v) saponin from Quilla JA Bark (Sigma-Aldrich)] at room temperature for 10 min followed by two washes with saponin buffer (PBS containing 0.1% (v/v) saponin, 1% FBS and 0.1% NaN_3). Cells were stained for 30 min in saponin buffer, and unbound Abs were removed with 2 washes in saponin buffer. Cells were resuspended in staining buffer and were acquired on FACS LSR II (BD Biosciences). Controls used to set up the flow cytometer included: unstained cells, and cells stained with only one fluorochrome

at a time. Information regarding the laser used for exciting each flurochrome as well as the filters for photomultiplier tube is indicated in Table 4. Results were analyzed with software FlowJo (Treestar, San Carlos, CA). Percentages of cells expressing specific markers were determined for each analysis and when required intensity of signal was calculated using the median fluorescent intensity from which the isotype control was deducted (see formula below).

ΔMFI = Median intensity of staining - Median intensity of isotype control.

2.4 Ex-vivo expression of IL-27R (gp130 and TCCR)

Expression of both chains (gp130 and TCCR) of IL-27R was assessed on freshly isolated PBMC or activated PBMC using flow cytometry. To ensure efficient reaction between gp130, TCCR and their staining antibodies, cells were incubated for 60 min in the dark on ice with the Ab staining mix which contained also Abs specific for cell subset markers (CD8, CD14, and CD4). To reveal biotinylated-anti-TCCR-Ab, a second staining step consisting of Streptavidin conjugated with APC was consecutively performed for 30 min.

Table 4. List of fluorochroms used for flow cytometry analysis

Fluorochrome	Abbreviation	Laser used for excitation (nm)	Long Pass	Filters at the PMT
Fluorescein	FITC	488	505	530/30
Phycoerythrin	PE	488	550	575/26
Pacific Blue™	PB	405		440/40
Allophycocyanin	APCy	633		660/20
Allophycocyanin-Cy™7	APC-Cy7	633	735	780/60
Phycoerythrin-Cy™7	PE-Cy7	488	735	780/60
Alexa Fluor®700	A700	633	710	730/45
Alexa Fluor®647	A647	633		660/20

Table 5: List of antibodies used for flow cytometry analysis

Antibody (Ab)	µg per stain	Isotype	Distributor of Ab and isotype
CD3-A700	0.1	Mouse IgG1- A700	BD Biosciences
CD4-PB	0.04	Mouse IgG1-PB	BD Biosciences
CD8-APC-Cy7	0.024	Mouse IgG1-APC-Cy7	BD Biosciences
CD14-FITC	0.1	Mouse IgG2a-FITC	BD Biosciences
CD45RA-FITC	0.1	Mouse IgG 2b-FITC	BD Biosciences
CD45RO-PE	0.05	Mouse IgG2a-PE	BD Biosciences
gp130-PE	0.25	Mouse IgG1-PE	R&D Systems
TCCR-biotin	0.25	Normal goat-biotin	R&D Systems
Streptavidin-APCy	0.1		BD Biosciences
IFN-γ - A700	0.04	Mouse IgG1-A700	BD Biosciences
GranzymeB-A647	0.002	Mouse IgG1-A647	BD Biosciences

2.5 ELISA for IFN-γ

Flat bottom immunoplate Maxisorp Nunc plates (96 wells) were coated at 4°C over night with capturing anti-human IFN-γ monoclonal Ab (0.4 µg/ml) (Pierce Biotechnology, Rockford, IL, USA) in PBS. Plates were washed with PBS-Tween buffer [0.5% (v/v) Tween 20 (Fisher, Nepean, ON, Canada) in PBS] and then blocked for at least 30 min at room temperature with 5% (v/v) FBS in PBS. After one wash, samples or standards were added to plates concomitantly with detecting biotinylated antibody anti-IFN-γ (0.2 µg/ml) (Pierce Biotechnology), diluted in PBS/FBS 5%, and incubated for 90 min at room temperature in the dark. Three washes were performed and streptavidin-horse radish peroxidase (0.25 µg/ml) (Poly HRP20-streptavidin) (Fitzgerald Industries International Inc., distributed by Cedarlane) was added for 30 - 45 min at room temperature in the dark. After 3 washes, Enhanced K-blue 3, 3', 5, 5' tetramethylbenzidine (TMB) substrate (Neogen, distributed by Cedarlane) was added and after 5-10 min, the

reaction was stopped by adding 2N H_2SO_4. Results were read on an ELISA reader EL800 from Bio-Tek at wave length 450 nm.

3.0 DETERMINATION OF IL-27 mRNA EXPRESSION BY REAL-TIME PCR

3.1 RNA extraction

Cellular RNA was extracted using the RNeasy Mini Kit (Qiagen, Mississauga, ON, Canada) according to the manufacturer's instructions. Harvested cells were disrupted by addition of RLT buffer (lysing buffer containing guanidinium thiocyanate, exact composition undisclosed) (350 µl for less than $5x10^6$ cells and 600 µl for $5x10^6$ - $10x10^6$cells). The lysate was passed 5 - 6 times through a 1 ml RNase-free syringe (BD Biosciences) with a 20-gaugle needle (0.9 mm diameter). Then to the suspension was added the same volume (as the RLT buffer) of 70% ethanol and the total volume was pipetted up and down several times to homogenize. Samples (including all precipitates) were transferred in RNase-free spin columns with a collection tube (provided by the kit) and were spun down for 15 s at 8000 *x g*. If the volume was more than 700 µl, this step was repeated twice. RW1 buffer (700 µl) was added to each spin column to wash its membrane, and columns were spun again for 15 s at the same rate. The flow-through was discarded. The same procedure was performed with 500 µl of buffer RPE and the flow-through was again discarded. A second wash with RPE buffer followed, and columns were spun for 2 min at 8000 *x g*. To ensure complete removal of washing buffers, the spin columns were transferred to new collection tubes and were spun again for 1 min at full speed. Then spin columns were transferred into 1.5 ml RNase-free Eppendorf tubes. To each spin column membrane were added 35-40 µl RNase-free water and columns were spun for 1 min at 8000 *x g* to elute RNA.

RNA concentration and purity were determined on a spectrophotometer Ultrospec 2100 pro at 260 nm. Purity of RNA as determined by ratio of the optical density at 260 over 280 nm was 1.7 - 2.0. RNA was either stored at -80°C or was subjected to a cDNA transcription.

3.2 Complementary DNA (cDNA)

Complementary DNA was obtained using the Quantitect Reverse Transcription Kit (Qiagen) according to manufacturer's instructions. In order to get rid of any potential contaminating genomic DNA, total RNA (1 ug) was incubated with gDNA Wipeout Buffer (exact composition undisclosed) in RNase-free water for 2 min at 42°C. RNA was then transcribed into cDNA in the presence of Omniscript® and Sensicript® Reverse Transcriptases (RT), RNase inhibitor, dNTPs, oligo-dT and random primers for 30 min at 42°C, and then for 3 min at 95°C to inactivate RT.

3.3 Real time-Polymerase Chain Reaction.

The real-time PCR approach used employs the 5′ nuclease activity of the AmpliTaq Gold® DNA Polymerase to cleave a TaqMan probe during PCR. PCR amplification was performed in the presence of 5′ end and 3′end specific primers and an internal primer used as a probe labelled with a reporter dye and a quencher dye. At the annealing step the internal probe binds to the specific sequence and then during the elongation step as the DNA polymerase approaches the internal probe it cleaves it leading to the separation of the reporter and quencher dyes thus allowing the reporter dye to emit detectable fluorescence. This fluorescence intensity is proportional to the number of internal probes cleaved at each cycle thus provides a direct readout of the accumulation of PCR products. Real-time PCR was performed for three genes: ribosomal 18S RNA (as an internal amplification control), EBI3, and p28 (all from Applied Biosystems, Foster City, CA, U.S.A.). The amplification was performed in the presence of AmpliTaq Gold DNA Polymerase, AmpErase UNG, dNTPs with dUTP, Passive Reference (ROX), and optimized buffer components according to manufacturer's instructions. For ribosomal 18S amplification 200 nM of internal probe conjugated to VIC™ dye and 50 nM of the forward and reverse primers were added whereas for EBI3 or p28 12.5 nM FAM™ dye-labeled TaqMan® MGB probe and 45 nM of the forward and reverse primers were added (Applied Biosystems). TaqMan Ribosomal RNA

Control 18S generated an amplicon of 187 bp in length, whereas a 75 bp amplicon for p28 (amplifying exon 3-4 boundary) and a 94 bp amplicon for EBI3 (amplifying exon 4-5 boundary). Transcribed cDNAs from LPS-treated dendritic cells (DC) (iDC) with duration of treatment 6 hours and 24 hours were used as positive controls for p28 and EBI3 respectively. These positive controls were prepared by a colleague. Two dilutions of positive controls were used - with a dilution factor 10 and a dilution factor 100. RNase-free water was used as a negative control. Each sample was run in a 25 µl volume and in duplicate in a 96-PCR plate. Plate was spun at 624 *x g* for several seconds to ensure the proper position of the sample on the bottom of the well. All bubbles were removed for proper read-out. Plate was incubated in 7900 Fast Real Time PCR System (Applied Biosystems) for 2 min at 50°C to activate the AmpErase UNG enzyme (ensures depletion of potential contamination from previous PCR) with a subsequent UNG deactivation at 95°C for 10 min. This is followed by 40 cycles of PCR, each consisting of two phases: denaturation of cDNA at 95°C for 15 sec and subsequent synthesis of a new brand DNA on the denaturated sample at 60°C for 1 min. C_T, which is the PCR cycle at which the detected signal was significantly above background signal, was automatically determined by the Sequence Detection System (SDS) software. Ribosomal 18S gene was used as a reference gene. Serial 10-fold dilutions of cDNA from LPS activated monocyte-derived human DC were used as a calibrator to create a relative standard curve, since these cells were reported to express high levels of both p28 and EBI3 (Pflanz et al., 2002; Coulomb-L'hermine et al., 2007; Molle et al., 2007). The relative expression of each gene (p28 and EBI3) was compared to ribosomal 18S and calculated as ΔC_T which is described as the difference in threshold cycles for target (p28 or EBI2) and reference (18S) using this formula:

$$\Delta C_T = C_{T\,(target)} - C_{T\,(reference)}$$

The efficiency of the both target and reference amplification were approximately equal as the obtained ΔC_T did not vary much in different cDNA dilutions of the same sample.

The final step was to calculate $\Delta\Delta C_T$ which represents the difference between the ΔC_T of the target gene in each sample, and the average ΔC_T of the duplicates of the calibrator (the positive control for the same gene).

q = any sample

cb = calibrator

$\Delta\Delta C_T = \Delta C_{T,q} - \Delta C_{T,cb}$

The amount of target, normalized to an endogenous reference and compared to a calibrator, was calculated as being: $2^{-\Delta\Delta C_T}$, a value of 1 meant that the target gene (p28 or EBI3) was expressed at very similar level than in the LPS-activated iDC, whereas value below one represented lower expression of such genes compared to our control.

4. 0 STATISTICAL ANALYSIS

Data were analysed using GraphPad Prism software (San Diego, CA, USA). Results are shown as mean and standard error of the mean (SEM) and statistical analyses included paired students't test.

RESULTS

The activation paradigm selected to evaluate the functions of human T cells *in vitro* consisted of a polyclonal activation using α-CD3 monoclonal antibodies (Ab) causing the activation of T lymphocytes through the T cell receptor (TCR)-CD3 complex, a well accepted model of TCR-induced activation. When using PBMC, α-CD3 Ab were added directly to cell suspension as APC could present these activating Ab via their Fc receptors and thus induce the cross-linking of the TCR-CD3 complex on T cells. Conversely, activation of isolated CD8 T lymphocyte population was achieved by pre-coating the α-CD3 Ab on plastic wells thus, forcing the cross-linking of the TCR-CD3 complex as the T cells are touching the bottom of the plate by gravity.

1.0 OPTIMIZATION OF ANTI-CD3 ACTIVATION OF HUMAN PBMC

1.1 Proliferation of human T cells in response to α-CD3 Ab

At first, α-CD3 antibody (Ab) was added to PBMC in various concentrations to establish the effective dose 50 (ED_{50}) - e.g. concentrations at which 50% of maximal cell proliferation was observed. Most dose-dependent reactions can be represented by an S-curve where at low doses of the reagent its effect is not detectable, then at increasing doses, increasing effects take place whereas at even higher doses, the maximal effects are achieved and any increase in doses does not augment its effects (plateau). The ED_{50} doses experimentally determined for 4 donors were used in order to allow the detection of any modulation of the response by IL-27 addition be an increased or a decreased cell proliferation. The concentrations of α-CD3 Ab tested were: 340, 136, 68, 34, 17, 8.5 and 3.4 ng/ml (Fig. 2).

CFSE labelled PBMC ($1x10^6$ cells/tube) were put in culture in 5 ml snap-cap tubes with α-CD3 Ab and after 5 days, cells were stained with Abs specific for human CD4 and CD8. Samples were acquired by flow cytometry and analyzed to determine proliferation levels in the CD4 and the CD8 compartments. Fig. 2 illustrates pooled data obtained from two independent experiments each

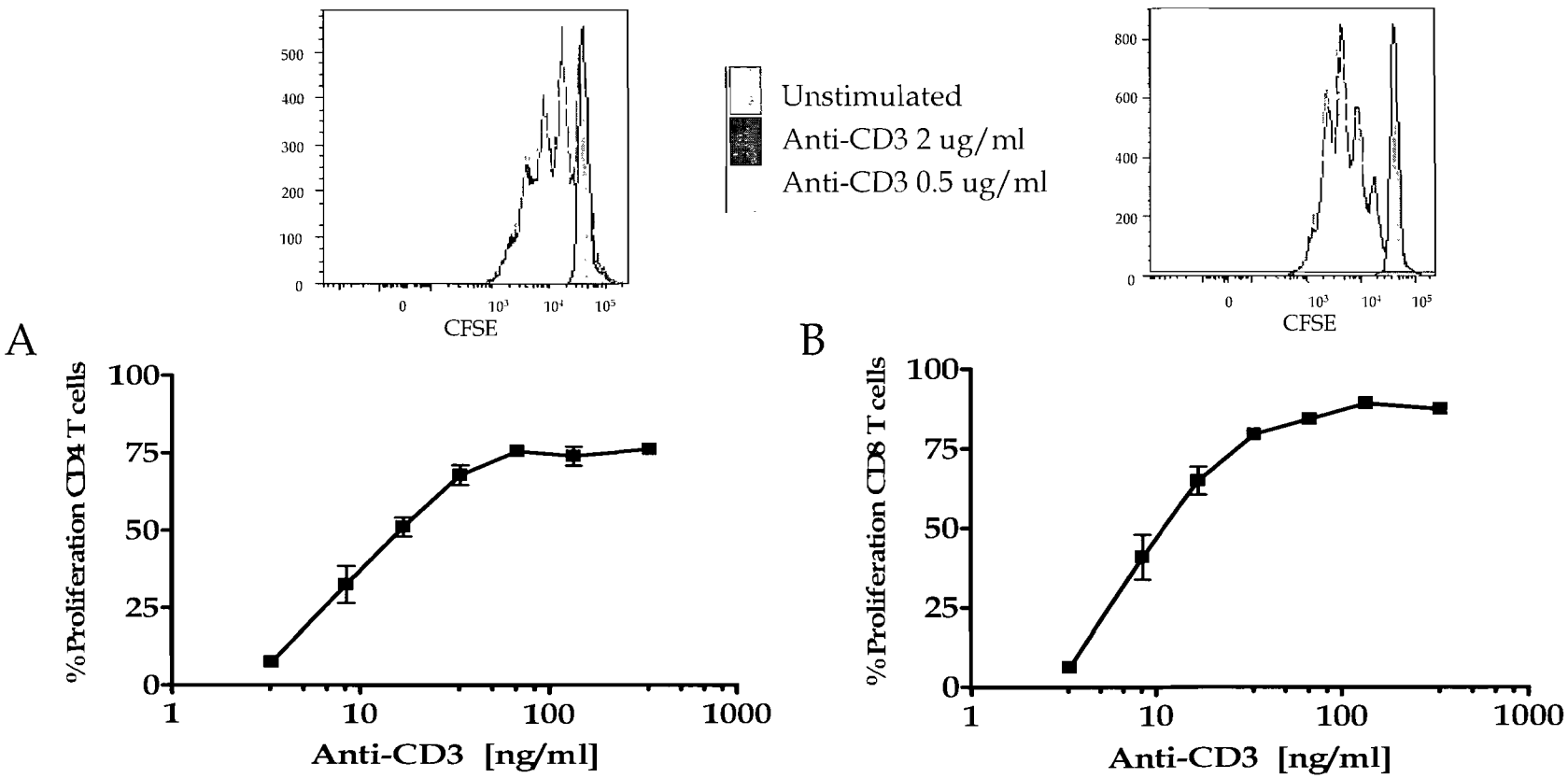


Figure 2: Anti-CD3 Ab dose response by PBMC as assessed by proliferation

CFSE labelled PBMC ($1x10^6$ cells/ml) were incubated in 5 ml snap-cap tubes in the presence of different concentrations of anti-CD3 Ab for 5 days. On the fifth day cells were stained for CD4 (panel A) and CD8 (panel B) markers to determine proliferation levels in both T cell compartments. Samples were acquired on a Becton Dickinson LSRII flow cytometer. For each cell type a profile for three different conditions (unstimulated, α-CD3 [0.5 ug/ml] and α-CD3 [2 ug/ml]) is shown above the corresponding dose-response curve. Data from 4 donors done in duplicates are presented as mean + SEM.

experiment performed with PBMC from two healthy donors in duplicates. The α-CD3 Ab concentration is represented on the X-axis on a $\log_{10}$ scale whereas the percentage of either gated CD4 (panel A) or CD8 (panel B) T lymphocytes that have proliferated in response to the corresponding concentration of α-CD3 is presented on the Y-axis. Few (6 - 7%) CD8 and CD4 T cells proliferated when stimulated with the lowest α-CD3 dose used (3.4 ng/ml). But 30-50% of CD8 and 25-40% of CD4 T cells proliferated when stimulated with 8.4 ng/ml and 60-70% of CD8 and 48-55% of CD4 T cells proliferated in the presence of 17 ng/ml of α-CD3 Ab. At higher doses (34 ng/ml and 68 ng/ml) 60-83% of CD8 and 60–70% of CD4 T cells proliferated reaching a plateau of the α-CD3 Ab doses. According to the obtained results, the doses of 8.5 and 17 ng/ml were chosen as ED_{50}.

Although we did not specifically test cell death (via 7aminoactinomycin D or Live/Dead® fixable dead cell stain) our FSC/SSC flow cytometry data are very similar for all cells activated with α-CD3, regardless of the addition of IL-27. These observations suggest thet IL-27 did not affect survival. According to our data, activated cells would undergo about five divisions. This is where we observed differences in the presence of IL-27. As it can be observed on Fig. 5, the cytokine increased the number of cells entering divisions 4 and 5, and sometimes affected the number of cells entering division 3. This effect is most prominent at high doses of IL-27. This suggests that IL-27 plays a role for increasing number of cell divisions.

2.0 EXPRESSION OF IL-27 SIGNALLING RECEPTOR (IL-27R) BY HUMAN PBMC

To evaluate which cell types could potentially be targeted by IL-27, the presence of both chains necessary for a functional signalling IL-27R: gp130 and TCCR was evaluated on human PBMC.

2.1 Ex vivo expression of IL-27R by human T lymphocytes and monocytes

PBMC from 10 healthy donors were stained ex-vivo for CD14 (monocytes), CD3 (lymphocytes), CD4 and CD8 and both chains of the IL-27R (TCCR and gp130) and then acquired by flow cytometry. Obtained data were analysed for: *i)* percentage of cells (%), expressing both chains constituting the signalling receptor for IL-27 and *ii)* intensity of labelling detectable on expressing cell (represented by median fluorescence intensity (MFI)) for both TCCR and gp130. Data from all donors are represented on Fig. 3. On the upper two panels are represented the percentage of cells expressing the complete IL-27R (panel A) or the gp130-chain only (panel B), whereas on the lower two panels are represented the Δ Median Fluorescence Intensity for TCCR (panel C) and gp130 (panel D). Each dot represents one donor.

The majority of monocytes (75% $\pm$ 2.6%) expressed IL-27R. In the T cell compartment, a greater proportion of CD8 T (mean: 6.6 $\pm$ 0.1%) cells expressed the complete IL-27R, compared to CD4 T cells (mean 0.9 $\pm$ 1.0%). In terms of number of TCCR chains as assessed by the ΔMFI, again highest levels were observed on the monocytes, whereas the expression was comparable between positive CD4 and CD8 T cells. Concerning gp130 expression, the levels were comparable between monocytes and T lymphocytes, slightly lower in the CD8 T cell compartment. On the contrary, few monocytes (mean: 3.8 $\pm$ 1.2%) expressed only gp130 in absence of TCCR whereas a great proportion of CD4 T cells (mean: 63.8 $\pm$ 3.7%) and an important, though lower proportion of CD8 T cells (mean: 29.9 $\pm$ 4.4%) did express gp130 in absence of TCCR, suggesting that these cells can respond to other cytokines using the gp130 as a co-receptor. Some cells expressed TCCR in absence of gp130 (less than 5% for both T cells and monocytes). As an ex-vivo staining was repeated for some of the donors, it appeared that the levels of expression of both IL-27R chains are stable over time for each subject (data not shown). The greater number of ex-vivo CD8 T cells expressing IL-27R suggests that CD8 T cells could be more susceptible to IL-27 effects than CD4 T cells.

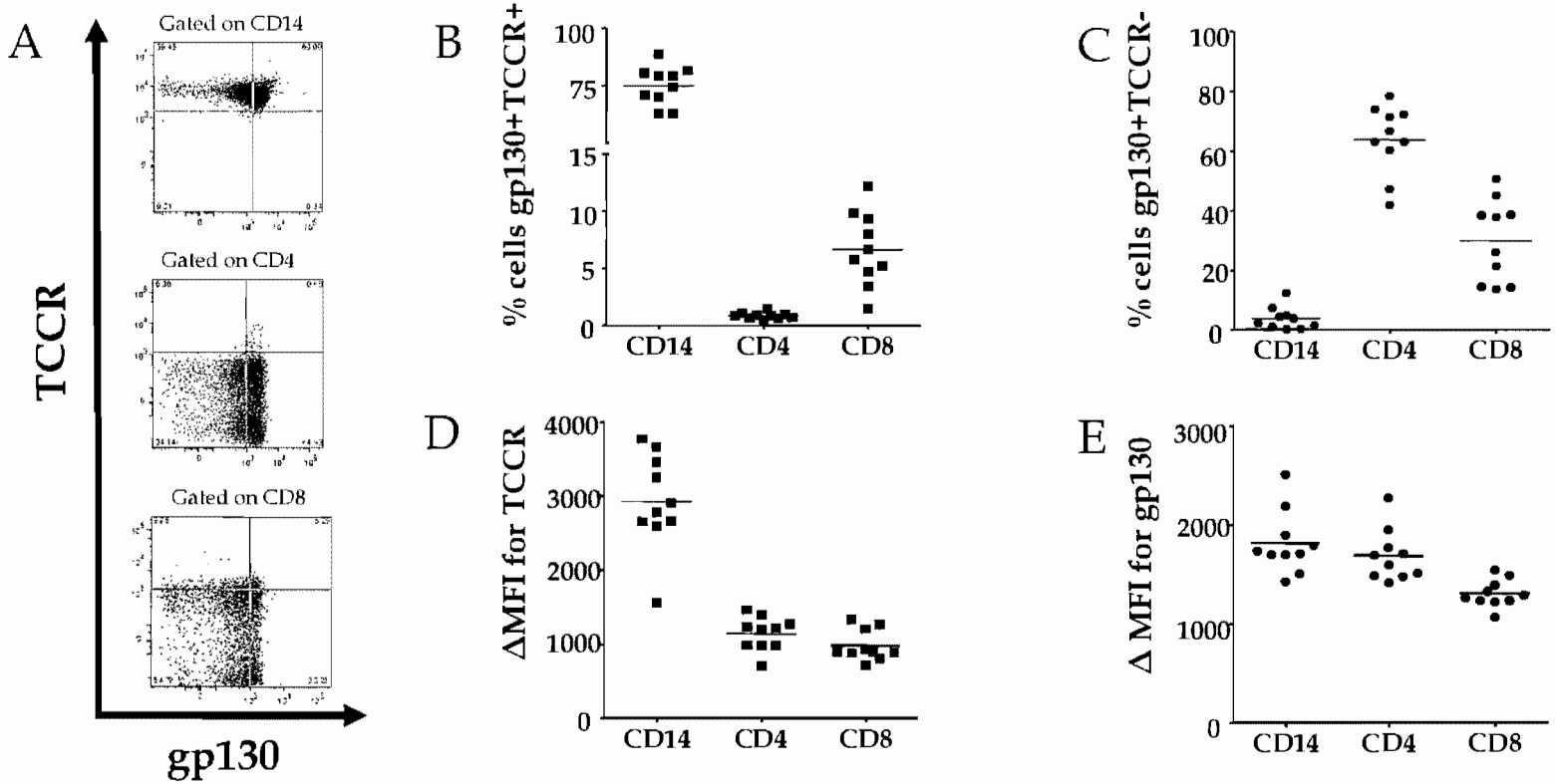


Figure 3: Expression of IL-27 receptor chains on ex-vivo monocytes (CD14), CD4 and CD8 T cells

PBMC ($1x10^6$ per sample) were stained ex-vivo for both chains of the IL-27 receptor (TCCR and gp130) and analysed by flow cytometry. One representative donor is depicted (panel A). Graphs illustrate the percentage of cells, expressing both chains (panel B), gp130-chain only (panel C), and the Δ median fluorescence intensity (MFI) for TCCR-chain (panel D) and gp130-chain (panel E) obtained for 10 healthy donors. Each dot represents one donor. The middle line illustrates the mean.

2.2 IL-27R expression upon activation

To investigate whether T cell activation has an impact on the expression level of both chains of IL-27R, such expression was monitored following in vitro T cell activation (Fig. 4). Anti-CD3 Ab was used at two different concentrations: 17 ng/ml, previously identified as ED_{50} for T cell proliferation in bulk PBMC culture, and 340 ng/ml, used to achieve maximal effect (see Fig. 2). Two (D2), four (D4) and six (D6) days after stimulation, surface staining for CD4, CD8, gp130 and TCCR was performed on 5 distinct donors. The percentage of CD4 (panel A) and CD8 (panel B) T cells expressing both IL-27R chains are represented on Fig. 4. On the X-axis three conditions were compared for each time point D2, D4 or D6: non-stimulated cells (white bars); cells incubated with α-CD3 Ab (17 ng/ml) (black bars), and cells incubated with α-CD3 Ab (340 ng/ml) (bricked bars). The ex-vivo percentages are presented at the extreme left-hand side (hatched bars). A significant up-regulation of both chains was already detectable at D2 and more prominent in the CD8 T cell compartment than in the CD4 T cell one (to facilitate comparison, both graphs are plotted on the same scale). The up-regulation of IL-27R was the strongest at D2 and over days decreased. These data show that similarly to the ex-vivo observations, upon polyclonal activation, more CD8 T cells than CD4 T cells expressed IL-27R.

Even at low doses of α-CD3 Ab the upregulation of both chains was almost maximal (14.6 $\pm$ 4.4% CD8 T cells up-regulated the complete IL-27R at α-CD3 [17 ng/ml] vs. 18.3 $\pm$ 3.3% CD8 T cells at α-CD3 [340 ng/ml]) already at D2. At D4 and D6 the effects of both doses of α-CD3 were comparable (D4: 12.4 $\pm$ 4.5% CD8 T cells with α-CD3 [17 ng/ml] vs. 12.6 $\pm$ 3.5% - α-CD3 [340 ng/ml], D6: 13.1 $\pm$ 4.2% [17 ng/ml] vs. 16.0 $\pm$ 5.4% [340 ng/ml]). This suggests that even a non-maximal signal in conditions of inflammation is sufficient for CD8 T cells to up-regulate their IL-27R during the early phases and to influence their effector functions.

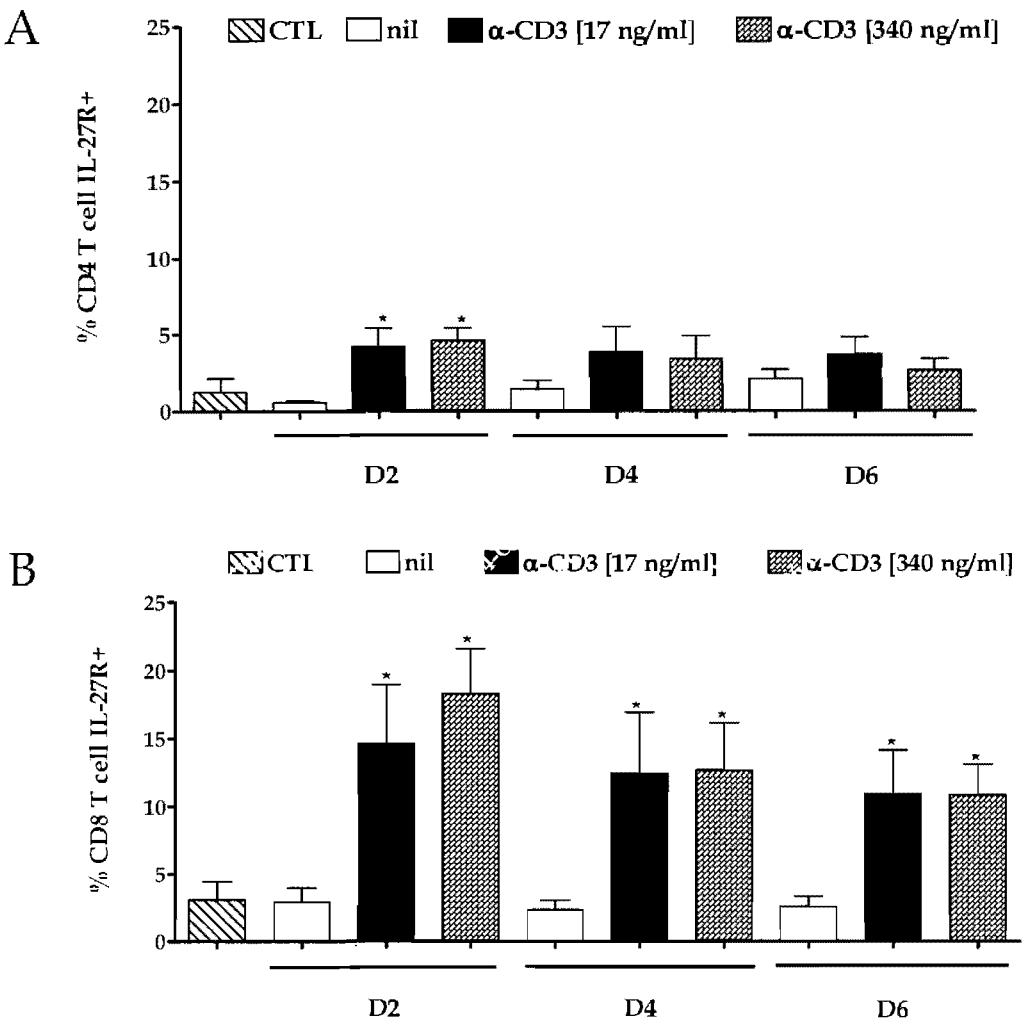


Figure 4: Expression of IL-27R on T cells upon activation

PBMC ($1x10^6$) were incubated in the presence or absence of α-CD3 Ab (2 different concentrations tested: 17 ng/ml and 340 ng/ml) for several days. On the second day (D2), fourth day (D4) and the sixth day (D6), cells were harvested and stained for CD4, CD8 and both chains of the IL-27 receptor (TCCR and gp130). Data were analysed by flow cytometry. Graphs illustrate the percentage of CD4 (panel A), and CD8 (panel B) T cells, expressing both chains of IL-27R at different days. Data are presented as mean ± SEM for 5 healthy donors, * means $p<0.05$, when compared to non-stimulated cells on the same day.

3.0 EFFECTS OF IL-27 ON T CELL FUNCTIONS.

3.1 PBMC

Other groups have shown that IL-27 increases the production of IFN-γ by human CD4 T cells (Matsui et al., 2004) but no data were available on human CD8 T cells. The effects of IL-27 on both CD4 and the CD8 T cells were compared for distinct effector functions: proliferation, IFN-γ production (as a prototypic pro-inflammatory cytokine), and granzyme B production (as a prototypic lytic enzyme).

CFSE-labelled PBMC were stimulated in the presence of two ED_{50} α-CD3 Ab doses (8.5 and 17 ng/ml) in the presence or absence of IL-27 (0-250ng/ml). After a 6-day culture, cells were shortly activated in the presence of PMA, ionomycin and BFA and then stained for surface (CD4, CD8) and intracellular proteins (IFN-γ and granzyme B). Although T cells have started to divide after three days, the number of divisions as well as the total percentage of cell proliferation were small. Thus longer time-points were optimal for read-out of both proliferation and effector molecule production. Our choice of effector molecules was based on publications in the mouse system. We also tried to detect IL-4 in human stimulated PBMC but since the results were negative (data not shown), we excluded the mentioned staining from the panel. Cells were acquired on a flow cytometer and analyzed for proliferation (CFSE low cells), granzyme B and IFN-γ content for each T cell subsets: CD4 and CD8. Dot-plots obtained from one representative donor are illustrated on Fig. 5. CD8 T cell-gated events are shown for CFSE fluorescence (X-axis) and either IFN-γ (panel A) or granzyme B (panel B) on the Y-axis. Cells that did not proliferate had the strongest CFSE fluorescence and are depicted in the lower-right and upper-right quadrants, whereas cells that did undergo proliferation are either in the upper-left quadrant (being also positive for either IFN-γ or granzyme B) or in the lower-left quadrant (being negative for either IFN-γ or granzyme B). Some non-activated and some non-proliferating cells stained positive for IFN-γ due to the stimulation with PMA

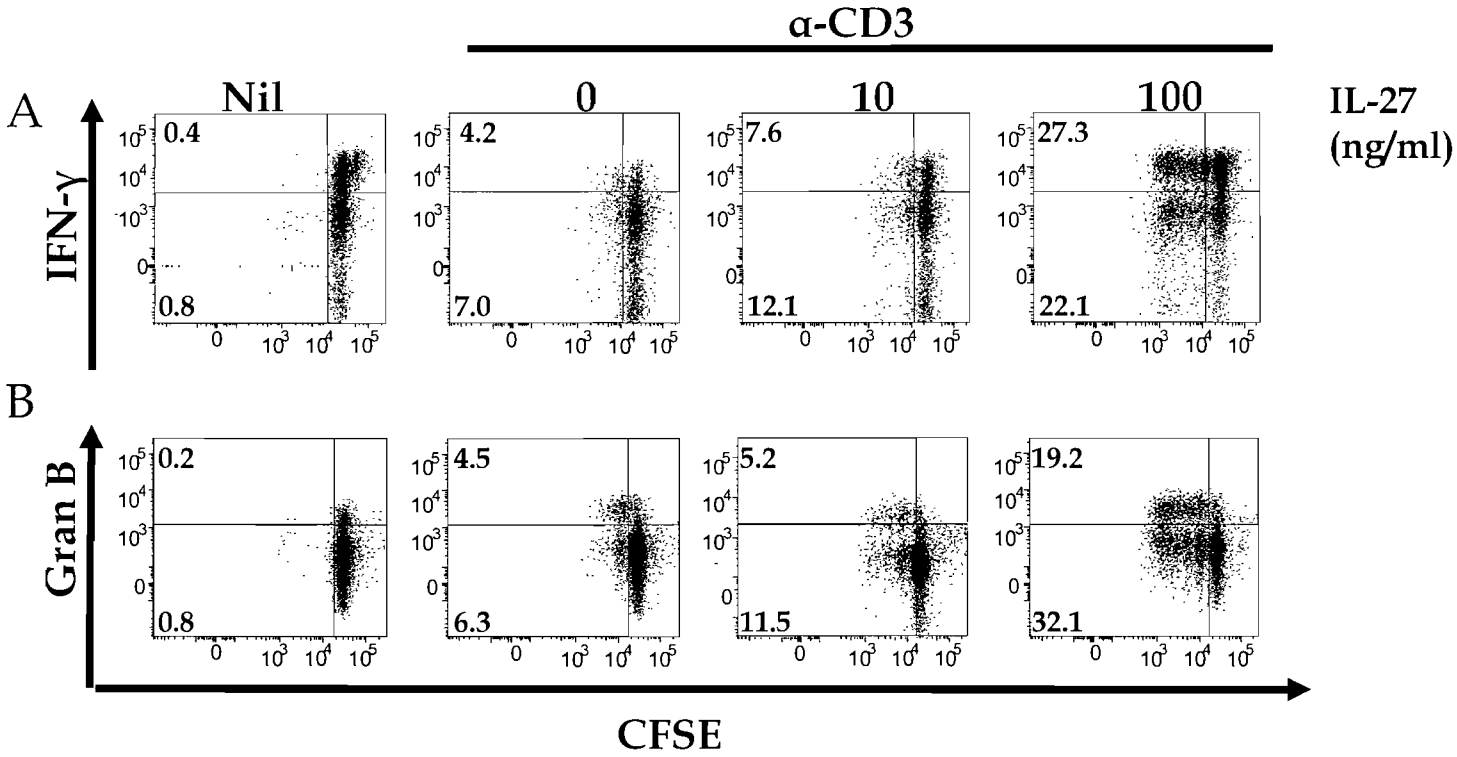


Figure 5: Illustration of IL-27 effects on the proliferation of, and IFN-γ and granzyme B production by human CD8 T cells

CFSE labelled-PBMC were cultured in the absence (Nil) or presence of anti-CD3 (15 ng/ml) with or without recombinant human IL-27 (0, 10 or 100 ng/ml) for 6 days, and then shortly stimulated with PMA and ionomycin in the presence of BFA. Surface (CD8) and intracellular (IFN-γ (panel A) and granzyme B (panel B)) staining was performed. One representative donor out of 10 is depicted; dot plots from flow cytometry results illustrate gated events for CD8 T cells. Percentage of cells that proliferated (CFSE low) is indicated in appropriate quadrants either IFN-γ+ or granzyme B+ (upper left quadrant), or IFN-γ- or GranzymeB- (lower left quadrant).

(activates protein kinase C) and ionomycin (boosts Ca^{2+} influx). This phenomenon is typically observed in human cells upon such activation (Arbour et al., 2003).

The impact of IL-27 on these T cell effector functions was tested on healthy donors and pooled data are illustrated on Fig.6-7-8-9. The first parameter analyzed was the total proliferation (Fig.6). T cells minimally proliferated when put in culture in absence of stimuli (< 5%) whereas α-CD3 Ab doses induced significant proliferation of both CD4 (panel A) and CD8 (panel B) T cells although CD8 T cells proliferation was more important than CD4 T cells. The addition of IL-27 had a minimal impact on CD4 T cell proliferation at the higher α-CD3 Ab dose tested, but at the lower dose of α-CD3 an IL-27 dose-response is visible and the maximal enhancement mediated by IL-27 was observed at 10 ng/ml (mean 42.1 $\pm$ 5.2%), reaching significance compared to α-CD3 Ab in absence of this cytokine (mean 33.4 $\pm$ 4.7%).

In the CD8 T cell compartment a proliferative dose-response to IL-27 was observed for both doses of α-CD3 used for stimulation. For the higher dose of stimulating antibody, maximal cell proliferation was observed in the presence of IL-27 at 100 ng/ml (mean 65.2 $\pm$ 3.4%) with a high statistical significance ($p<0.005$) when compared to stimulation with α-CD3 only (mean 56.7 $\pm$ 3.9%). For the lower dose of α-CD3, IL-27 exerts a maximal effect at 10 ng/ml (mean 53.7 $\pm$ 4.7%) again with a high statistical significance, when compared to stimulation in the absence of IL-27 (40.2 $\pm$ 5.8%).

The difference IL-27's impact on the T-cells (more obvious on CD8 T cells) could be due to the already strong proliferative signal provided by of the higher α-CD3 dose [17 ng/ml] such that detection of IL-27-enhancing effects was hindered. It should be also mentioned that these results are obtained from bulk PBMC through gating on CD4 and CD8 T cells. Therefore, the observations made were a consequence of the action of IL-27 on all types of cells present in the culture, and the action of all types of cytokines, produced by these cells. These experiments were also representative of the in vivo situation while all cell types would be present when IL-27 will be released in the vicinity.

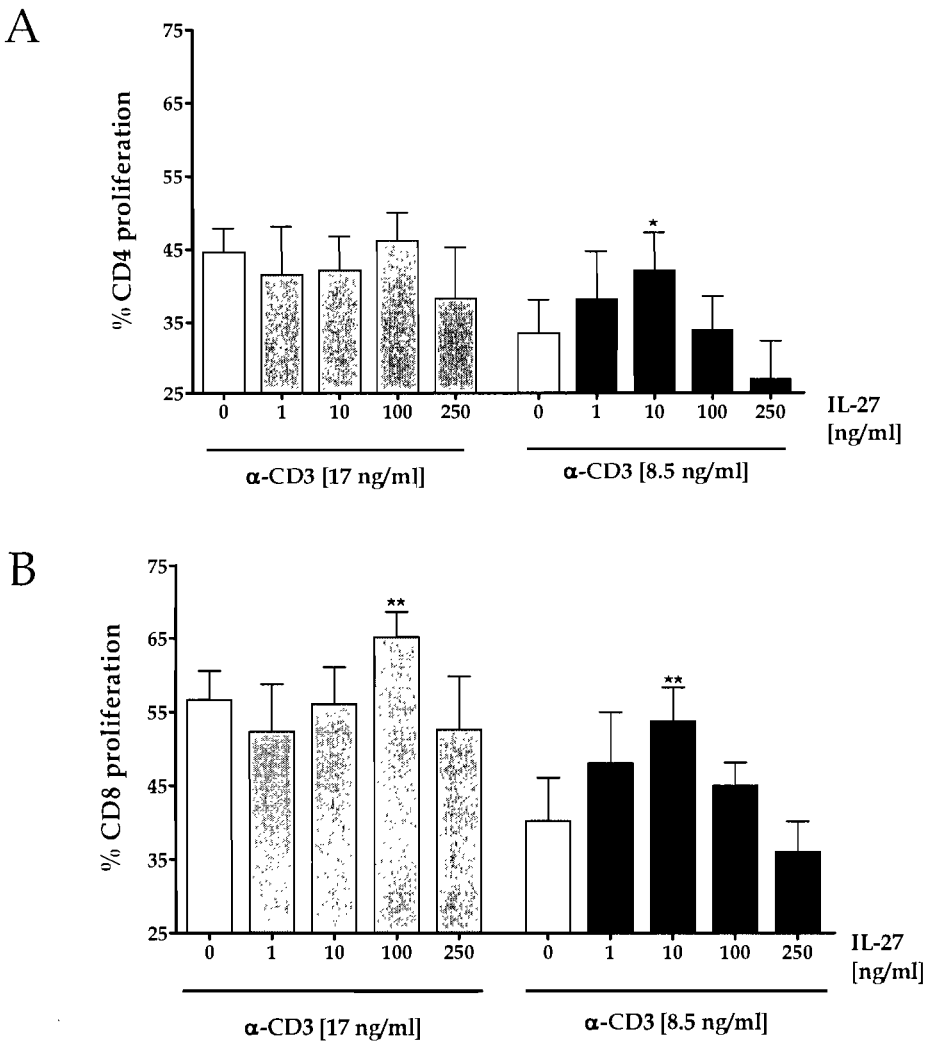


Figure 6: IL-27 boosts the proliferation of T cells

CFSE labelled-PBMC were cultured with α-CD3 (17 or 8.5 ng/ml) in the absence or presence of IL-27 (1, 10, 100 or 250 ng/ml) as indicated on the X-axis for 6 days. Supernatants were then collected and analyzed by ELISA for IFN-γ and cells were shortly stimulated with PMA and ionomycin in the presence of BFA prior to surface and intracellular staining for flow cytometry analysis. Percentage of CD4 (panel A) and CD8 (panel B) T cells that have proliferated are illustrated for 8 healthy donors. Controls (cells alone) for all donors minimally proliferated < 5%. Data are presented as mean ± SEM; * means $p<0.05$, ** means <0.005 when compared to α-CD3 in absence of IL-27.

The second response measured was the percentage of T cell that proliferated and could produce IFN-γ (Fig. 7). Graphs illustrating CD4 (panel A) and CD8 (panel B) T cell responses are put on the same scale to facilitate comparison. A similar trend to boosted levels of cells that have proliferated and produced IFN-γ in the presence of IL-27 was observed in both subsets of T cells (CD4 and CD8). Consistent with the other observed effector functions, IL-27 had slightly stronger effect on CD8 T cells than on CD4 T cells; in the presence of IL-27 at 10 ng/ml 15% of CD8 T cells (mean 15.5 $\pm$ 3.5%) proliferated and produced IFN-γ vs. only 7% of CD4 T cells (mean 7.2 $\pm$ 1.9%) in the same conditions. Although the trend was very obvious for the CD8 T cell compartment, the results were not statistically significant, and the only conditions in which statistical significance was observed in the CD4 T cell compartment in the presence of α-CD3 [8.5 ng/ml] + IL-27 [10 ng/ml]. Overall, the ΔMFI of IFN-γ did not differ in the presence or absence of IL-27. This suggests that IL-27 effect is due to increased number of proliferated cells that are able to produce IFN-γ but not to cytokine production on per cell basis.

The effects of IL-27 on lytic enzyme content of human CD8 T cells was tested since this cytokine has been shown to boost these enzymes in murine CD8 T cells (Morishima et al., 2005). As shown on Fig. 8 (panel B), IL-27 increased in a dose-dependent manner granzyme B content of human CD8 T cells. The different increasing doses of IL-27 evoked responses, representing a bell-shaped curve with a maximal response in the presence of IL-27 at [100 ng/ml] for the higher dose of α-CD3, and [10 ng/ml] of IL-27 for the lower dose of stimulating Ab. Statistical significance in the presence of IL-27 was reached at doses of [1 and 10 ng/ml] when stimulating with α-CD3 [17 ng/ml] and at [10 ng/ml] IL-27 when stimulating with the lower dose of α-CD3. However, the impact of the lowest dose

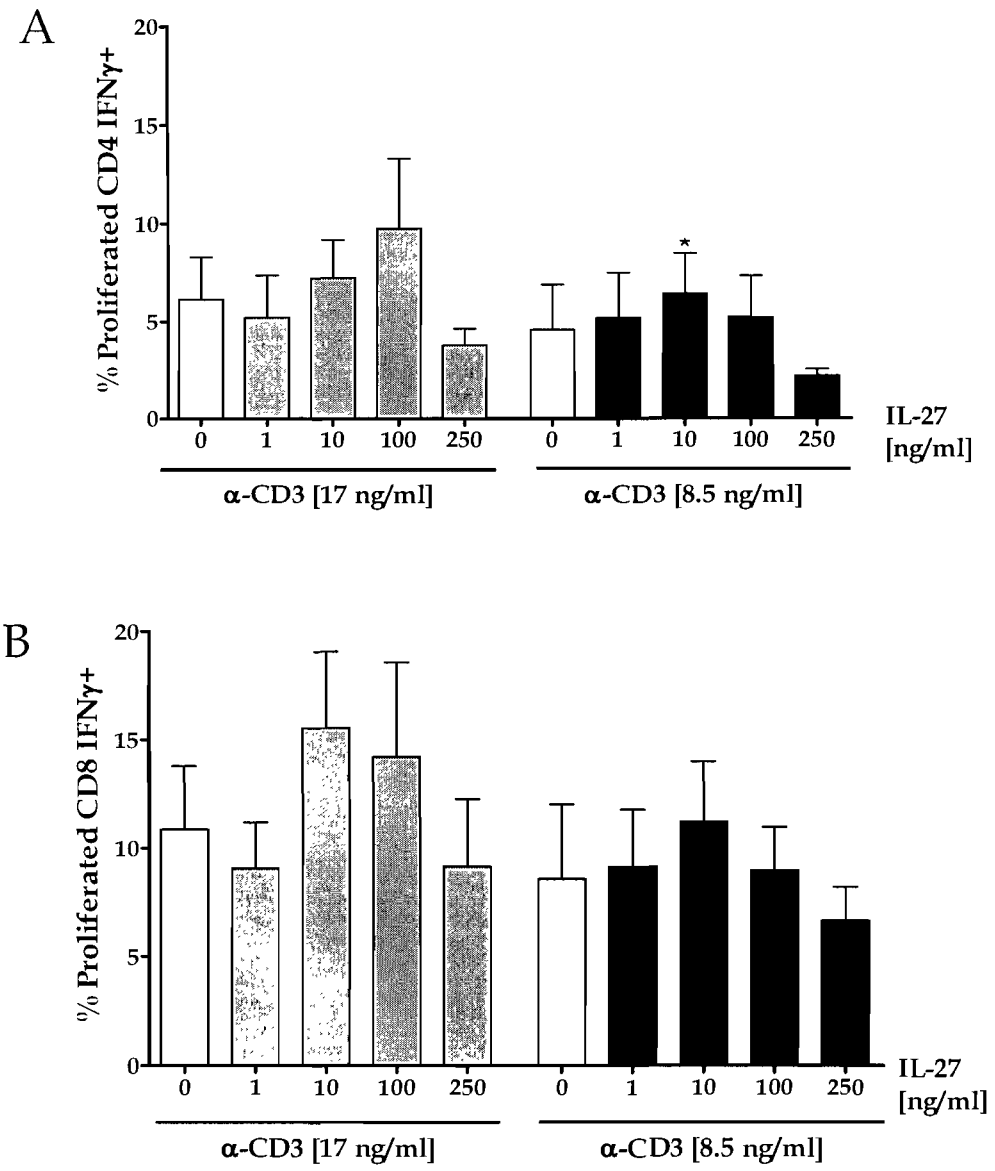


Figure 7: IL-27 augments proportion of IFN-γ producing T cells

CFSE labelled-PBMC were cultured with α-CD3 (17 or 8.5 ng/ml) in the presence or absence of IL-27 (0, 1, 10, 100 or 250 ng/ml) for 6 days Percentage of CD4 T cells (panel A) or CD8 T cells (panel B) that proliferated and produced IFN-γ was determined by intracellular staining after short stimulation with PMA and ionomycin in the presence of BFA. Controls (cells only) minimally proliferated (< 2%). Data are presented as mean ± SEM for 7 healthy donors, * means $p<0.05$ when compared to α-CD3 in absence of IL-27.

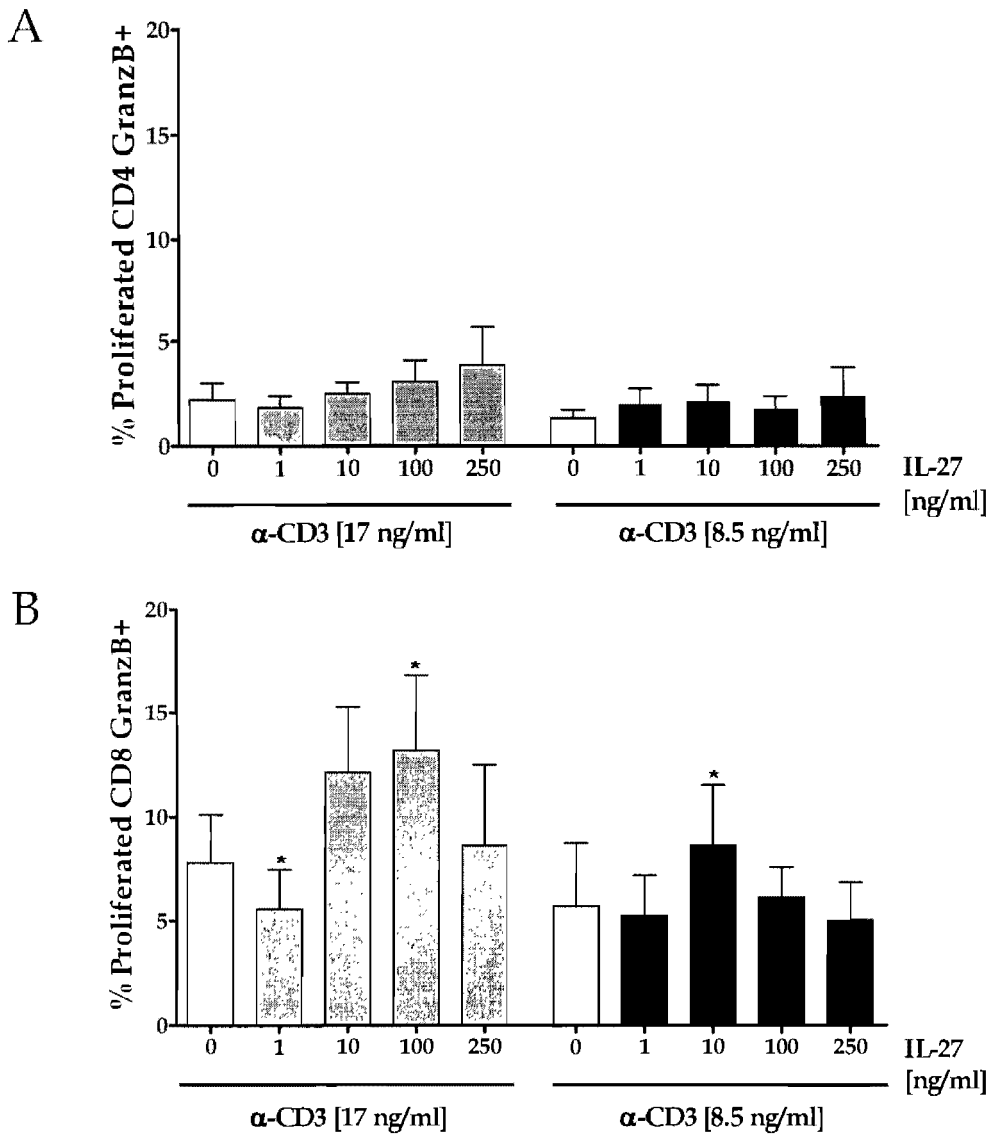


Figure 8: IL-27 increases Granzyme B production by CD8 T cells

CFSE labelled-PBMC were cultured with α-CD3 (17 or 8.5 ng/ml) in the presence or absence of IL-27 (0, 1, 10, 100 or 250 ng/ml) for 6 days and then stained for surface markers (CD4, CD8) and intracellular granzyme B. Percentages of proliferated CD4 (panel A) or CD8 (panel B) T cells containing granzyme B were determined by flow cytometry analysis. Controls (cells alone) minimally proliferated and produced granzyme B (< 2%). Data are presented as mean ± SEM for 7 healthy donors; * means $p<0.05$ when compared to α-CD3 in absence of IL-27.

of IL-27 [1 ng/ml] reduced the α-CD3-induced granzyme B production. A similar analysis was performed on human CD4 T cells, but a smaller proportion of these cells produced granzyme B in comparison to CD8 T cells, as expected and IL-27 had minimal impact on such expression (Fig. 8 panel A). Remarkably, the curves of the dose-response for IFN-γ and for granzyme B for CD8 T cells were very similar in magnitude and shape whereas this was not the case for CD4 T cells for the reasons mentioned above.

Finally, we investigated by ELISA the total IFN-γ production in the cell culture medium of stimulated PBMC (Fig. 9). While the intracellular staining represents the actual capacity of T cells to produce cytokines, the ELISA assay represents the equilibrium between the production and consumption by all types of cells present in the culture. Addition of IL-27 increased IFN-γ secretion in a dose-dependent manner for both concentrations of stimulating α-CD3 Ab used, reaching statistical significance for α-CD3 [17 ng/ml] + IL-27 [100 ng/ml] and α-CD3 [8.5 ng/ml] + IL-27 [10 ng/ml] when compared to stimulation only with α-CD3 Ab. IL-27 effects were not observed at the same dose for the distinct α-CD3 Ab doses used; when cells were stimulated in the presence of 17 ng/ml α-CD3 the 100 ng/ml IL-27 led to the greatest IFN-γ secretion (600 pg/ml), whereas the highest concentration of IFN-γ was detected (400 pg/ml) in the presence of 10 ng/ml IL-27 for the lower dose of α-CD3.

The IL-27 dose-response curve as measured by the amount of IFN-γ in the culture medium corresponded to the dose-response curve observed for the CD4 T cells proliferation+ IFN-γ^+ cells as assessed by flow cytometry (Fig. 7 panel A) with maximal response to stimuli α-CD3 [17 ng/ml] + IL-27 [100 ng/ml]. This might indicate that while in the presence of α-CD3 [17 ng/ml] + IL-27 [100 ng/ml] there is an equilibrium of production and consumption of IFN-γ, the higher production of this cytokine seen in the CD8 T cell compartment in the presence of α-CD3 [17 ng/ml] + IL-27 [10 ng/ml] can be masked by the cytokine consumption by CD4 T cells and monocytes, thus leading to lower concentrations of IFN-γ in the milieu. Moreover, CD4 T cells are twice as numerous as CD8 T cells amongst

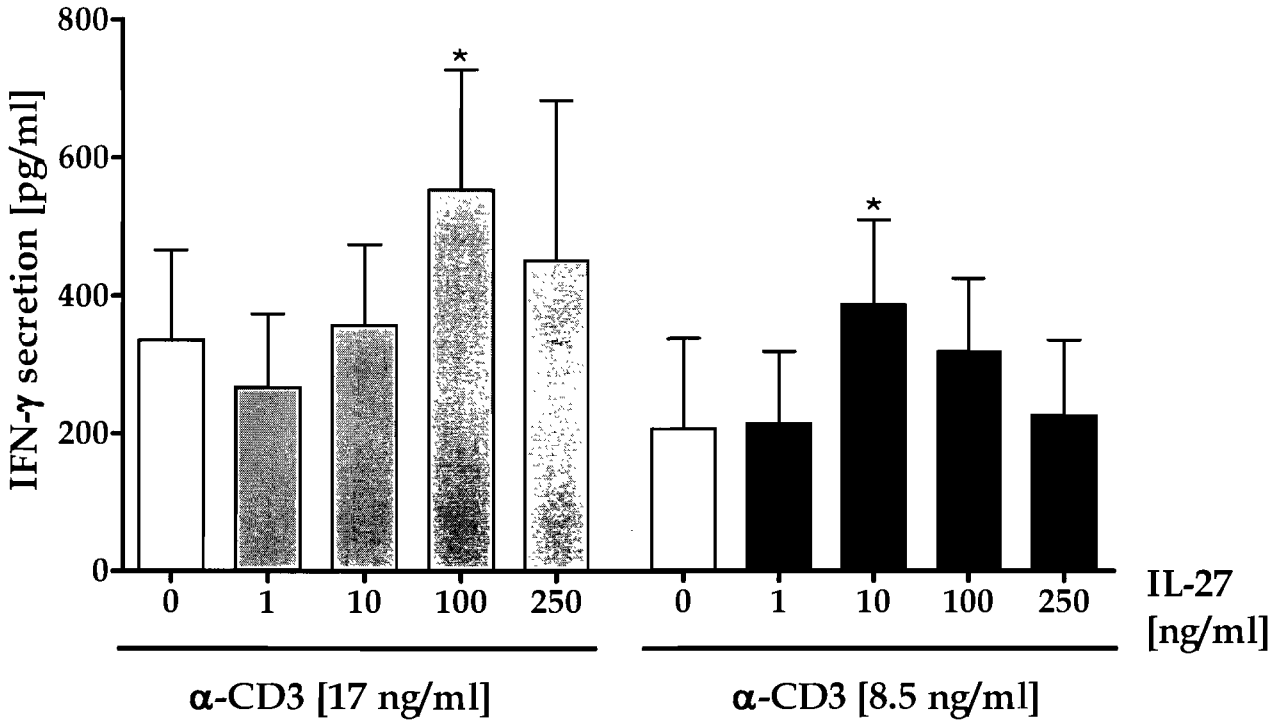


Figure 9: IL-27 augments IFN-γ production by anti-CD3 stimulated PBMC

CFSE labelled-PBMC were cultured with anti-CD3 (17 or 8.5 ng/ml) in the presence or absence of IL-27 (0, 1, 10, 100 or 250 ng/ml) for 6 days. Supernatants were then collected and analyzed by ELISA for IFN-γ. Controls (cells alone) for all donors minimally secreted IFN-γ (< 80 pg/ml). Data represents pooled results from 8 healthy donors and are presented as mean ± SEM, * means $p<0.05$ when compared to anti-CD3 in absence of IL-27.

PBMC, thus, CD4 T cell contribution to overall IFN-γ secretion could be twice the CD8 T cell contribution.

3.2 Naïve CD8 T cells

To dissect the effects of IL-27 on CD8 T cells according to their differentiation status, naïve and memory cells were purified from healthy donors and similar experiments were performed. The stimulation paradigm was slightly modified and naïve CD8 T cells were stimulated using plate-bound α-CD3 Ab in the presence or absence of anti-CD28 Ab. IL-27 (10, 100, 250 or 500 ng/ml) was added to assess the impact of this cytokine on the effector functions of naïve CD8 T cells being activated in vitro. The results obtained from healthy donors are illustrated in Fig. 10 (proliferation) and Fig. 11 (IFN-γ and granzyme B).

CFSE labelled purified CD8 CD45RA+ T cells ($2x10^5$ cells/well) were incubated for 6 days in the presence of α-CD3 Ab (3.4 and 6.8 μg/ml) and anti-CD28 (1 ng/ml) with or without IL-27; these doses were chosen as ED_{50} according to a dose-response performed by a colleague similarly to what was shown for PBMC (Fig. 2). Naïve CD8 T cells grown on plate bound α-CD3 Ab only did not significantly proliferate in comparison to cells left alone in adjacent wells as expected since naïve T cells necessitate two signals (activation (via TCR) and co-stimulation) for their activation. Regarding naïve CD8 T cell proliferation (Fig. 10), when cells were stimulated only with α-CD3 in the absence of co-stimulation, IL-27 increased in a dose-dependent manner from 10 to 500 ng/ml. Despite this continuous enhancing effect of IL-27, doses higher than 500 ng/ml were considered non-physiological and were not tested. The effect of all IL-27 doses used reached statistical significance when compared to stimulation with α-CD3 Ab only. In conditions of co-stimulation, the proliferation of naïve CD8 T cells was significantly greater than the α-CD3 Ab stimulation (Fig. 10). IL-27 significantly increased the proliferation of naïve CD8 T cells induced by the α-CD3+anti-CD28 combination from 100 ng/ml and continued to increase in a dose-dependent manner the proliferation of these cells up the 500 ng/ml tested. To our surprise,

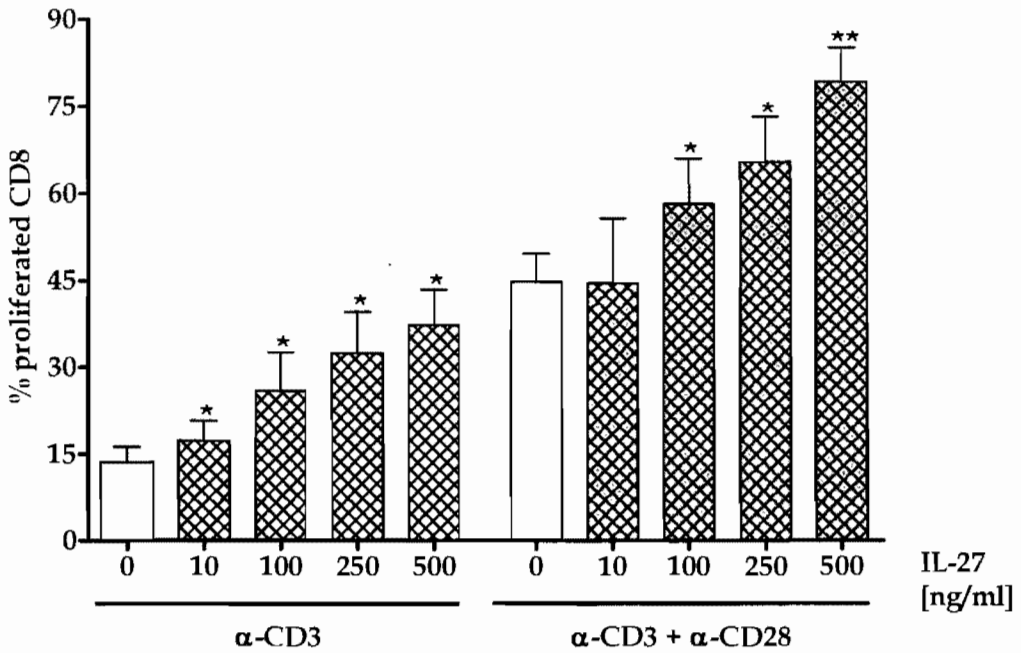


Figure 10: IL-27 enhances naïve CD8 T cell proliferation upon anti-CD3 stimulation in the presence or absence of co-stimulation

Purified CFSE labelled CD45RA+ CD45RO- CD8 T cells were incubated on plate bound anti-CD3 Ab (3.4 µg/ml and 6.8 µg/ml) with or without soluble anti-CD28 in the presence or absence of IL-27 (10, 100, 250 or 500 ng/ml) for 6 days. On the sixth day, cells were shortly stimulated with PMA and ionomycin in the presence of BFA prior to surface (for CD8) and intracellular (for IFN-γ and Granzyme B) staining for flow cytometry analysis. Pooled percentage of CD8 T cells proliferation for 6 healthy donors are illustrated. Data are presented as mean ± SEM. * means $p<0.05$, ** means $p<0.005$ when compared to the same treatment (anti-CD3 +/- anti-CD28) in absence of IL-27. Controls (cells alone) minimally proliferated for all donors (< 10%).

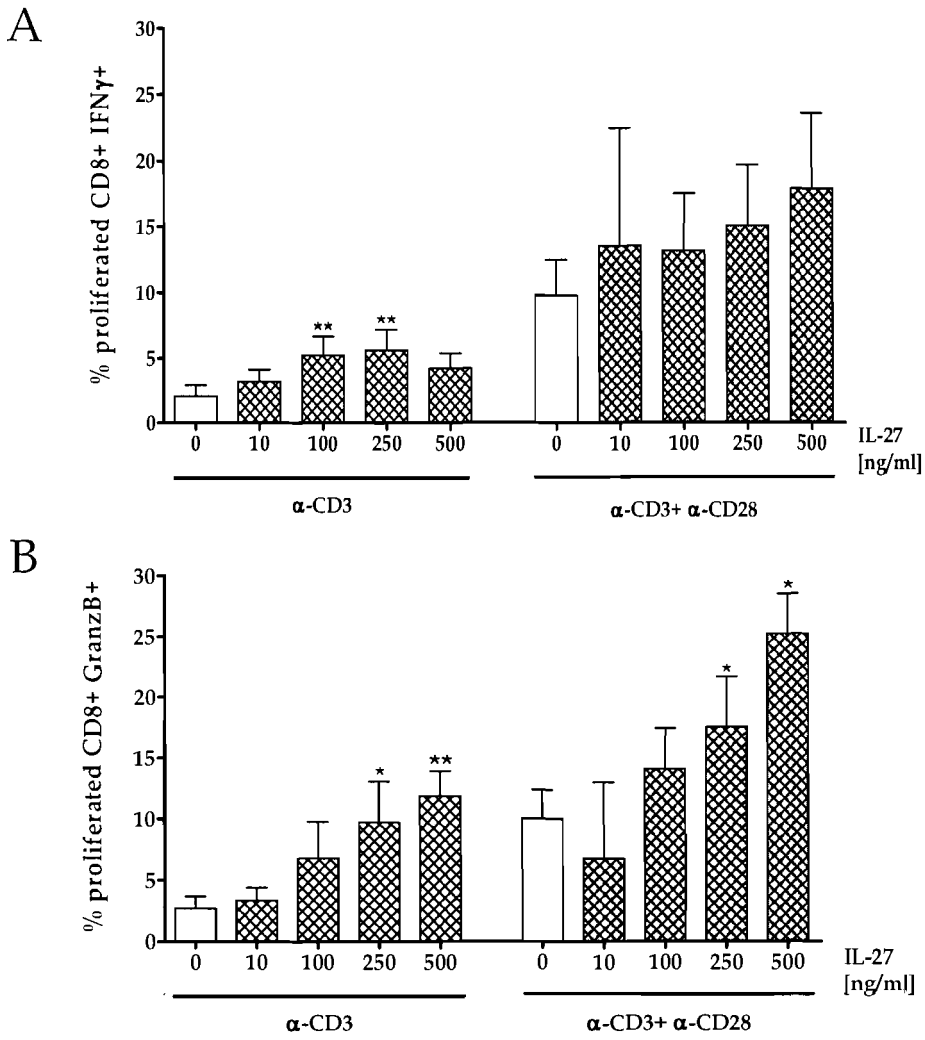


Figure 11: Effects of IL-27 on effector functions of naïve CD8 T cells

Purified CFSE labelled CD45RA+ CD45RO- CD8 T cells were incubated on plate-bound α-CD3 Ab (3.4 μg/ml and 6.8 μg/ml) with or without soluble α-CD28 in the presence or absence of IL-27 (10, 100, 250 or 500 ng/ml) for 6 days. Cells were then shortly stimulated with PMA and ionomycin in the presence of BFA prior to surface (for CD8) and intracellular (for IFN-γ (panel A) and granzyme B (panel B)) staining for flow cytometry analysis. Pooled percentages of proliferation+ effector+ (IFN-γ or granzyme B) CD8 T cells for 6 healthy donors are illustrated. Controls (cells alone) for all donors minimally proliferated (< 3%). Data are presented as mean ± SEM. * corresponds to $p<0.05$, ** means $p<0.005$ when compared to the same treatment in the absence of IL-27.

we could observe a certain level (up to 40% with [500 ng/ml] of IL-27) of proliferation in the absence of co-stimulation but in the presence of IL-27. This could be due to the presence of contaminating cells able to provide co-stimulation, but in our purified naïve CD8 T cells only 0.9% were monocytes and less than 2% were non-T cells non-monocytes. Another reason could be the IL-27 signalling pathway as it has been already suggested that the cytokine can synergize with TCR signal in murine T cells (Brender et al., 2007) and thus IL-27 could probably mimic the co-stimulatory α-CD28 signal.

Similarly to PBMC experiments, IFN-γ (Fig. 11, panel A) and granzyme B production (Fig. 11, panel B) were investigated. Graphs represent naïve CD8 T cells that have proliferated and produced either IFN-γ or granzyme B (indicated on the Y-axes). The doses of IL-27 used are shown on the X-axes. Although the effect of IL-27 on IFN-γ production by human naïve CD8 T cells was not strong in the absence of co-stimulation with α-CD28 Ab, it was still dose dependent and statistically significant for the 100 ng/ml (mean 5.2 $\pm$ 1.4%) and 250 ng/ml (5.6 $\pm$ 1.6%) doses when compared with stimulation with α-CD3 only (mean 2.7 $\pm$ 0.99%). The response in conditions of co-stimulation (α-CD3 + α-CD28) demonstrated a trend towards an increased of the percentage of proliferated and IFN-γ producing cells by IL-27 but it did not reach statistical significance.

IL-27 had significant effects also on granzyme B (Fig. 11, panel B) content by activated human naïve CD8 T cells. IL-27 exerted a boosting effect at low doses, [100 ng/ml] and continuously increased in a dose-dependent manner the percentage of granzyme B expressing cells till reaching statistical significance at [250ng/ml] (mean 9.7 $\pm$ 3.3%) and [500 ng/ml] (mean 11.9 $\pm$ 2.0%) when compared to treatment with α-CD3 only (mean 2.7 $\pm$ 1.0%). A similar trend was observed in the presence of co-stimulation with α-CD3+α-CD28 leading to enhanced percentage of granzyme B expressing cells amongst the proliferated ones a continuously increasing dose-response, reaching a statistical significance at doses of IL-27 [250 ng/ml] (mean 17.5 $\pm$ 4.1%) and 500 ng/ml (mean 25.2 $\pm$ 3.3%) when compared to stimulation with α-CD3 only (mean 10.0 $\pm$ 2.3%).

Of note, whereas 500 ng/ml of IL-27 added to α-CD3 Ab stimulation decreased IFN-γ production, at the same concentrations it did boost the proportion of granzyme B expressing cells, suggesting that IL-27 distinctly modulates different effector functions of CTLs (cytokine vs. lytic enzyme). In contrast, IL-27 added in condition of stimulation + co-stimulation increased in a dose-dependent manner both effector functions: cytokine and lytic enzyme production.

3.3 Memory CD8 T cells

We further proceeded with the same experiments on CD8 memory T cells. Purified CFSE labelled $CD8^+$ $CD45RO^+$ T cells were incubated for 5 days ($2x10^5$ cell/well) in the presence or absence of stimulating α-CD3 Ab, and presence or absence of IL-27 (1, 10, 100, 250 and 500 ng/ml). Although memory cells are known to produce effector molecules very fast (within hours), we left them in conditions of activation for 5 days to assess both proliferation and effector molecule production at the same time. In an attempt to circumvent the difficulty of obtaining pure memory human CD8 T cells, two different protocols of $CD8^+$ $CD45RO^+$ isolation were used (described in chapter "Materials and Methods"). The contaminating cells were $CD3^+CD4^-CD8^-CD56^-$, $CD19^+$, very few $CD14^+$, $CD3^-CD56^+$, $CD3^-CD4^-CD8^-CD56^-$, some $CD8^-CD45RO^+$, but no naïve CD8 T cells ($CD45RA^+CD8^+$) were detected. Thus, all results presented are gated on CD8 T cell compartment.

The effect of IL-27 on the proliferation (Fig. 12), production of IFN-γ (Fig. 13 panel A) and granzyme B content (Fig. 13 panel B) of memory CD8 T cells isolated from 7 healthy donors are illustrated. On the X-axis is presented the concentration of IL-27. Memory CD8 T cells proliferated very well in the presence of α-CD3 Ab alone and such proliferation was boosted by the addition IL-27 with maximal effects for doses [10 ng/ml] (mean 35.2 $\pm$ 9.1%) and [100 ng/ml] (mean 36.6 $\pm$ 7.2%) with a statistical significance only in the presence of IL-27 at [1

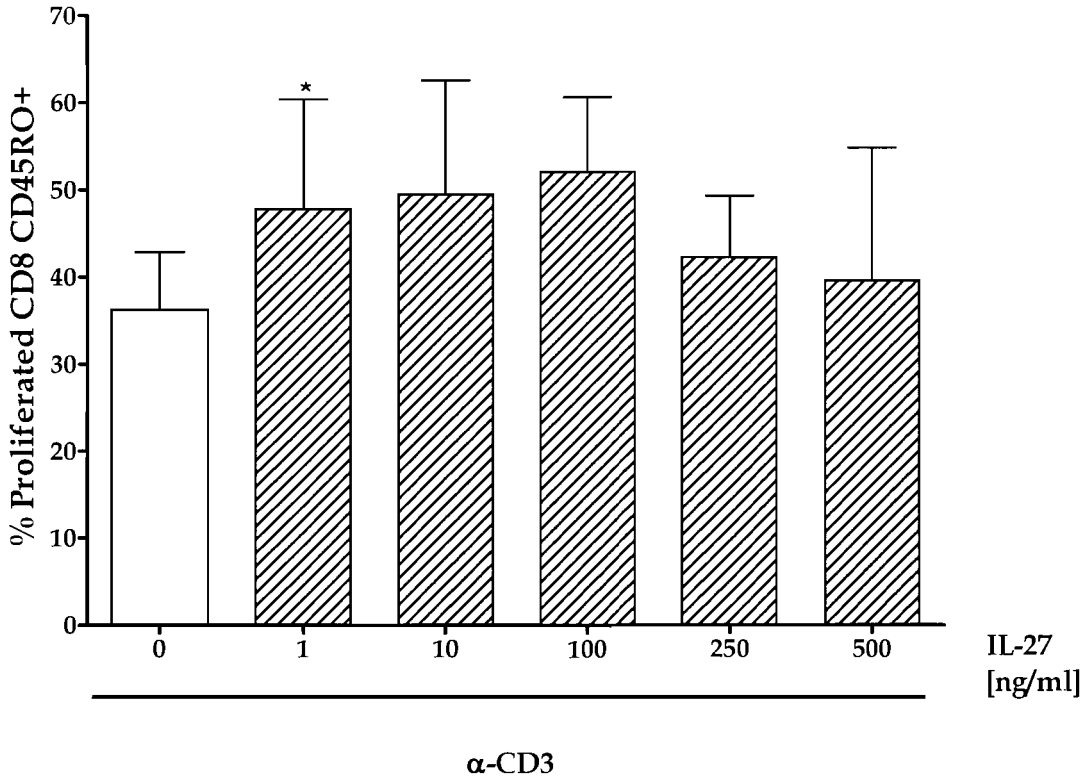


Figure 12: IL-27 enhances memory CD8 T cell proliferation upon anti-CD3 stimulation

Purified CFSE labelled CD45RO+ CD8 T cells were incubated on plate bound anti-CD3 Ab in the presence or absence of IL-27 (1, 10, 100, 250 or 500 ng/ml) for 5 days. Cells were then shortly stimulated with PMA and ionomycin in the presence of BFA prior to surface (for CD8) and intracellular (for IFN-γ and Granzyme B) staining for flow cytometry analysis. Percentages of memory CD8 T cells that proliferated are illustrated for 7 pooled healthy donors. Data are presented as mean + SEM, * means $p<0.05$

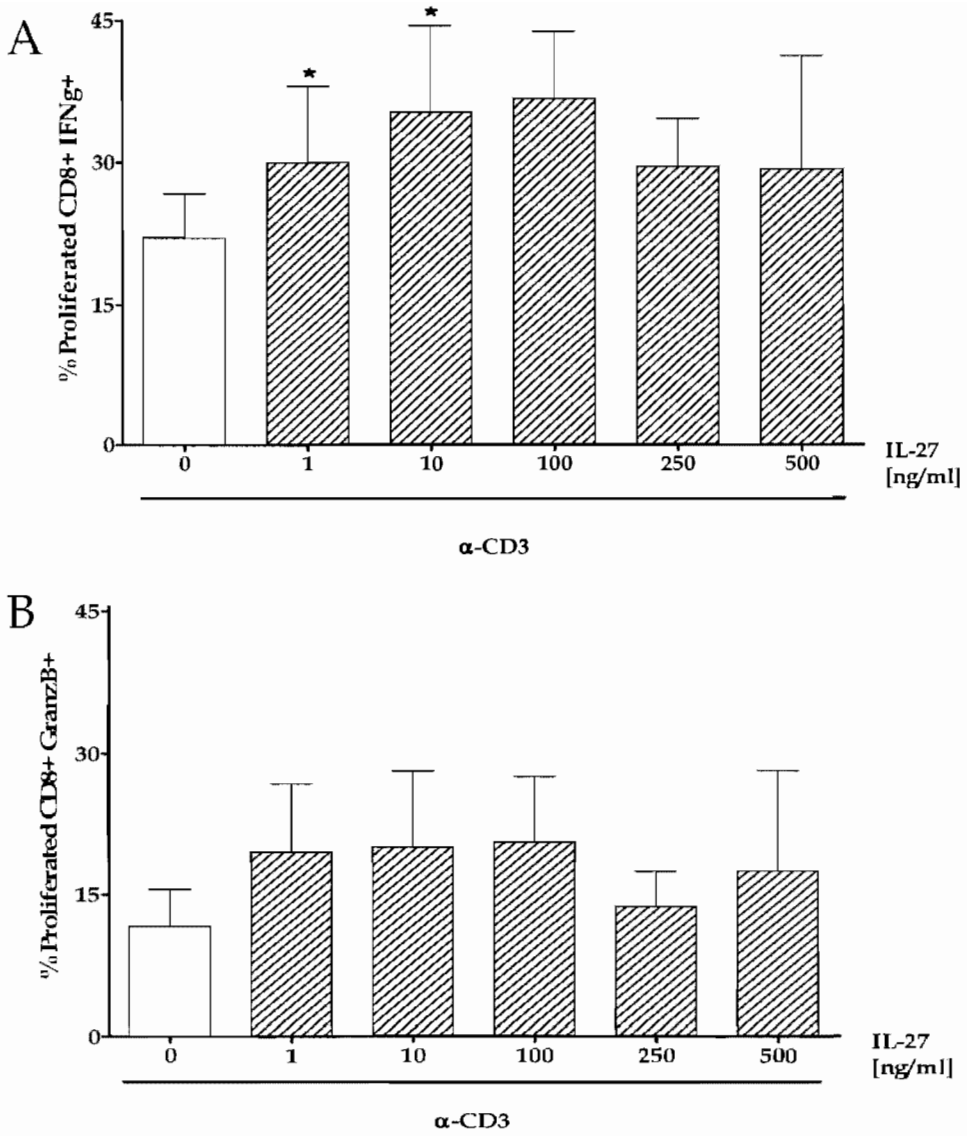


Figure 13: Effects of IL-27 on effector functions of memory CD8 T cells

Purified CFSE labelled CD45RO+ CD8 T cells were incubated on plate-bound α-CD3 [485.7 ng/ml] Ab for 5 days and then shortly stimulated with PMA and ionomycin in the presence of BFA prior to surface (for CD8) and intracellular (for IFN-γ (panel A) and granzyme B (panel B)) staining for flow cytometry analysis. Graphs illustrate the percentage of memory CD8 T cells that proliferated and expressed IFN-γ (panel A), or granzyme B (panel B) for 7 healthy donors. Data are presented as mean ± SEM, * means $p<0.05$ when compared to α-CD3 in absence of IL-27.

ng/ml]. Higher doses of IL-27 had less effect on human memory CD8 T cells proliferation. IL-27 addition during the α-CD3 Ab stimulation of the memory CD8 T cells led to a bell-shaped dose-response curve as assessed by the increased percentage of IFN-γ producing cells and granzyme B producing cells. IL-27 effects were more prominent on the production of IFN-γ than on granzyme B content, reaching statistical significance during stimulation with α-CD3 + IL-27 [1 and 10 ng/ml] for IFN-γ production. It is not possible to rule out the contribution of the contaminating cells on the memory CD8 T cell responses. Given the higher levels of granzyme B positive cells in α-CD3 Ab stimulated memory CD8 T cells than in the naïve CD8 T cells, it is possible that addition of IL-27 could not increase these levels further.

4.0 CD8 T CELLS AS A SOURCE OF IL-27

A recent publication suggested that murine CD8 T cells can be a source of IL-27 (Brender et al., 2007). To evaluate whether human CD8 T cells also produce this cytokine, these cells were purified, activated and then total RNA was extracted for real-time PCR analysis. Given the absence of commercially available ELISA kit for human IL-27 at the time of these experiments, only mRNA levels were evaluated. Purified CD8 T cells (purity > 98%) were incubated for 6 or 24 hours in presence or absence of stimulating α-CD3 [3.4 μg/ml] Ab and co-stimulating α-CD28 Ab (5 μg/ml). Total RNA was extracted, transcribed into cDNA, and used for real-time PCR amplification for both IL-27 subunits. Expression levels of both subunits of IL-27 (EBI3 and p28) were compared to the internal control 18S RNA (Fig. 14). As a positive control were used DC stimulated with LPS for 6 hours (for p28 expression) and for 24h (for EBI3 expression). The choice was based on already published data on the kinetics of expression of both subunits of IL-27 (Pflanz et al., 2002). EBI3 was clearly up-regulated after 24h of stimulation in the presence of both α-CD3 and α-CD28, whereas p28 was not detected even after 40 PCR cycles. This suggests that human CD8 T cells can not be a source of IL-27.

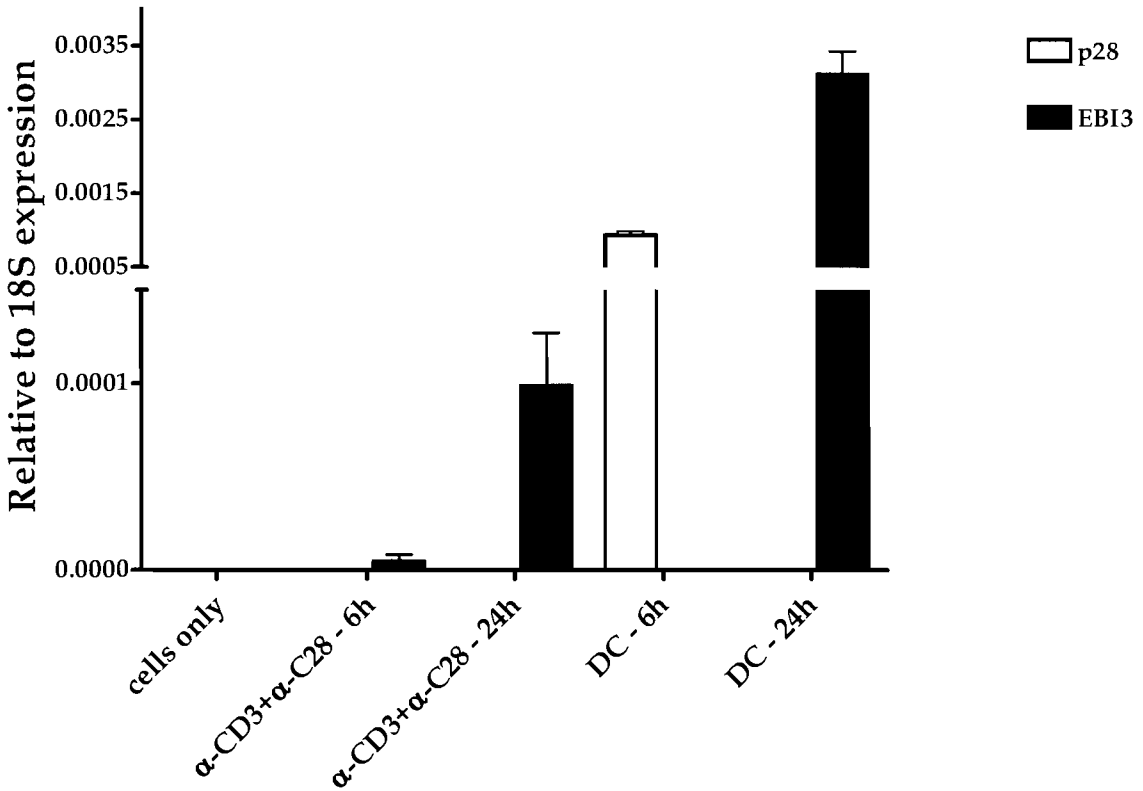


Figure 14: IL-27 is not produced by human CD8 T cells upon stimulation

Purified CD8 T cells ($5x10^6$ cells/1.5 ml/well) were incubated in the presence or absence of anti-CD3 antibody (3.4 µg/ml) and anti-CD28 (5 µg/ml) for 6 or 24 hours. After indicated time, cells were collected and disrupted for RNA extraction, which was consecutively converted in cDNA. The latter one was mixed with specific primers and was subjected to real time- PCR. Purity of CD8+T cells > 98%. Data are presented as mean ± SEM of 3 healthy donors.

DISCUSSION

The immune protection of the organism depends on the orchestral action of all the components of the immune system. The innate arm and the adaptive arm, of the immune system communicate through cytokines and direct contact, where the point of interaction between two cells is called "immunological synapse". Because of their capacity to spread in their environment and thus to influence more cells, while the physical space for direct cell-cell interaction is limited, cytokines have a crucial role in the immune responses. These secreted mediators can direct the "immune symphony" by skewing the T cells towards pro-inflammatory Th1, anti-inflammatory humoral Th2, and/or inflammatory/autoimmune Th17 type responses.

A recently discovered member of IL-6/IL-12 family, IL-27, has been shown to signal through two chains: TCCR and gp130. Like all cytokines (IL-6, cardiotrophin 1 (CT-1), leukemia inhibitory factor (LIF), ciliary neurotrophic factor (CNTF), oncostatin M (OSM), and IL-11) signalling through the gp130 chain (with IL-6 being the most studied among them), IL-27 has also this paradoxical characteristic of exerting both pro-and anti-inflammatory actions (Villarino et al., 2004). A substantial amount of data has been published recently on IL-27 effects on different immune cell subsets mainly in the mouse system, but very little is known about its effects on their human counterparts.

1.0 EXPRESSION AND MODULATION OF IL-27R

1.1. Ex-vivo IL-27R expression

The literature suggests that both human and mouse CD4 T cells and cells of the monocyte - Mφ lineage express both chains of the IL-27R (TCCR and gp130) but most of these published data have reported mRNA levels (Pflanz et al., 2004; Li et al., 2005). Very few quantitative data exist concerning surface protein expression and most of them have been performed on mouse cells (Villarino et al., 2005). Among human mononuclear cells (which includes mainly T and B lymphocytes, monocytes, NK cells, and DC), in healthy conditions, about 10% have been shown to express TCCR, and about 40% express this receptor chain in conditions of acute

myeloid leukemia (Pradhan et al., 2007). In human, tonsillar B cells have been shown to express barely detectable levels of both chains (no percentage of cells have been reported, neither the mean fluorescence intensity) (Larousserie et al., 2006). More precise information has been published about human naïve B cells from cord blood, where 30% of these cells are $TCCR^+$ and 26% are $gp130^+$, though it lacks information regarding cells expressing both chains: $TCCR^+gp130^+$. Splenic B cells also express distinct levels of TCCR and gp130 whereas the proportion of naïve B cells expressing each chain is 39% for $TCCR^+$ and 40% for $gp130^+$, the portion of memory cells expressing TCCR is 56% and $gp130^+$ 58% (Boumendjel et al., 2006). Moreover, expression levels between T cell and monocyte - Mφ subsets have not been compared (Pflanz et al., 2002; Larousserie et al., 2004; Pflanz et al., 2004; Coulomb-L'hermine et al., 2007).

Our study has assessed surface expression of the complete IL-27R (TCCR and gp130 chains) on monocytes and CD4 and CD8 T cell subsets (Fig. 3A). We also compared gp130 expression in absence of TCCR, as it is used by other cytokines (IL-6, IL-11, OSM, and LIF) (Fig. 3B). We analyzed both percentage of positive cells as well as the proportional number of chains per cell (respectively Fig. 3C for TCCR and Fig. 3D for gp130). The ten healthy subjects studied for the surface expression of the complete IL-27R showed great homogeneity of chain expression among all cell subpopulations tested (Fig. 3), which was stable over time for the same donor (data not shown). Monocytes showed the greatest percentage (75% $\pm$ 2.6%) as well as the highest expression of the complete IL-27R among the tested subsets. In the T cell compartment, a greater proportion of CD8 T cells (mean: 6.6 $\pm$ 0.1%) than CD4 T cells (mean 0.9 $\pm$ 1.0%) expressed the complete IL-27R. It will be of great interest to further distinguish what type of CD8 T cells exactly express the IL-27R: what type of memory markers they express and to which type of Tc cells they belong (Tc1/Tc2/Tc17). Similarly, in the mouse model not all T cells express the IL-27R. Villarino and colleagues have published data on the percentage of naïve mouse CD4 (21%) and CD8 (22%) T cells expressing TCCR

and that the percentage of activated or memory T cells expressing such receptor was increased to 42-51% in both compartments (Villarino et al., 2005). Our data suggests lower number of T cells expressing IL-27R in humans than in mice. The high level of IL-27R expression among APC could be explained as a mechanism for a negative feed-back loop and secretion control of this cytokine. APCs have been shown to both express the receptors for and secrete IL-6, IL-12 and IL-27 (Rival et al., 2006) This suggests that these cells can affect the availability of the mentioned cytokines in their environment by secretion and uptake. Although APC express the receptors for other cytokines such as IFN-γ, which is produced by T cells, they do not produce the cytokine itself. However, DC have also been shown to secrete IFN-γ upon IL-4 stimulation (Suto et al., 2005)).

A greater proportion of CD4 T cells expressed gp130 (65%) (Fig. 3, panel B) than CD8 T cells (30%), showing lower levels of expression of this receptor chain. This is consistent with other publications, showing that 77% of CD4 T cells in the peripheral blood express gp130 vs. 43% of CD8 being $gp130^{+}$ (Oberg et al., 2006), whereas 27% of Langherhan's cells (which belong to the monocyte -Mφ lineage) express gp130 (Larregina et al., 1996). As CD4 T cells play important roles for the generation of long-term memory CD8 T cells, and that gp130 is also used by IL-6, which confers anti-apoptotic and proliferative signals (Kamimura et al., 2003), one can speculate that the higher gp130 levels on CD4 T cells compared to CD8 T cells ensure a greater sensitivity to IL-6 survival effects on CD4 T cells than CD8 T cells.

While this thesis was in process, the TCCR-Ab producing company (R&D System™) sent new information regarding the antibody's specifications mentioning that their antibody was not suitable for flow cytometry analysis but only for Western blot analysis. To confirm the validity of the results obtained, CD14, CD4 and CD8 cells were purified by MACS sorting, cells were lysed and proteins were subjected to Western blot analysis. The band observed had the expected size (74 kDa) (see appendix I). The levels observed on T cells were quite high (see appendix) and similarly to the FACS data obtained, Western blot analysis confirmed that human CD8 T cells express higher levels of TCCR protein

than CD4 T cells. However, CD14 cells expressed much lower levels of TCCR than T cells by Western blot. These new data suggest that the flow cytometric data and the Western blot data are different. Since a goat polyclonal serum was used, it is possible that some Ab attached to a still unknown molecule different than TCCR on cell surface, especially on monocytes. Non-specific attachment to the surface of monocytes could be ruled out since cells were pre-blocked with normal mouse immunoglobulin and that the staining obtained was different than that of the isotype control (normal goat serum). Flow cytometry and Western blot techniques do not detect the same kind of information. As total cellular proteins are analyzed by Western blot, this raises the question whether the protein of interest is in the intracellular compartment or on the surface. Flow cytometry, on the other hand, assesses surface expression unless cells are permeabilized. It is thus possible that both results are accurate. Some receptors have been shown to be retained in the Golgi apparatus or in the endosomes after synthesis, and not to be fully expressed on the surface (e.g. leptin receptor and CD95 (Fas)) (Haynes et al., 2002; Belouzard et al., 2004). By contrast, some receptors have shown more efficiently expressed on the surface in the presence of JAKs in the intracellular milieu (Haan et al., 2006). Moreover, differences observed between mRNA and protein levels for mouse cells (Pflanz et al., 2002; Villarino et al., 2003; Bancroft et al., 2004; Pflanz et al., 2004; Li et al., 2005; Villarino et al., 2005; Kamiya et al., 2007) suggest that the more appropriate method to determine TCCR expression should assess protein rather than mRNA expression. Additional experiments using multiple antibodies and methods (flow cytometry, immunocytochemistry) should determine the relative expression of IL-27R among monocytes, CD4 and CD8 T cells, since IL-27R surface rather than intracellular presence will dictate the susceptibility to IL-27.

1.2 IL-27R modulation upon activation

Using the flow cytometry approach with the potential caveat mentioned above, we observed that both chains of the IL-27R were up-regulated early after stimulation (already at D2) and their expression decreased over time (Fig 3). This up-regulation was observed in multiple donors (n=5) and was more important in the CD8 than in the CD4 T cell compartment. Although gp130 is known to be down-regulated upon activation in mouse T cells (Betz and Muller, 1998), we observed an up-regulation of this chain on the surface of human T cells early upon activation. Furthermore, in the mouse system, very little is known about gp130 surface expression. Published data on mouse thymocytes suggested 10-30% of the CD8 and 40-50% of the CD4 expressed gp130 (Betz and Muller, 1998), and these percentages are close to our observations on human T cells. On the other hand, TCCR is up-regulated upon activation in murine cells (Villarino et al., 2005) similarly to what we observed on their human counterparts. New data should be generated to assess the expression of TCCR on CD4 and CD8 T cells upon activation comparing multiple techniques (see previous section) and multiple antibodies.

Activation of both mouse CD4 and CD8 T cells via the TCR regardless of the skewing cytokines present led to a rapid induction of TCCR (Villarino et al., 2005). The authors also discussed that although TCR-signalling and proliferation are necessary for up-regulation of TCCR, continuous stimulation and cellular division leads to the opposite effect probably due to the inhibitory effects of IL-2, also induced upon TCR activation, on TCCR expression (Villarino et al., 2005). It will be necessary to assess TCCR expression on human T cells under different conditions to determine whether such differences between naïve and activated human T cells follow the same principles as observed in mouse system. Differences in expression of IL-27R on these cells would permit to asses their susceptibility to the action of IL-27 and to determine whether this cytokine exerts the same pro- and anti-inflammatory effects on human T cells.

2.0 EFFECTS OF IL-27 ON T CELL FUNCTIONS.

In order to mimic inflammation in the body, stimulation of bulk PBMC was performed to assess the impact of IL-27. All kinds of cytokines as well as cells are present in this setup and can contribute to the overall observations and this situation is most representative of the real "in vivo" situation. Since α-CD3 antibodies (e.g. OKT3) specifically stimulate T cells, at first only these cells will be activated. However, after stimulation, they up-regulate co-stimulatory molecules such as CD137 (OX40) and CD40L. Cognate ligand or receptor of these molecules is expressed by APC. For example, activated CD4 T cells up-regulate CD40 ligand and can activate DC through CD40 (Lane, 2000). As CD40 is also present on Mφ and B cells, most probably a similar process can be observed in these subsets. Moreover, since APC present in the bulk culture (B cells and monocytes) have been shown to bear IL-27R on their surface, they could also respond to IL-27 when we added it to our in vitro culture.

We used the advantage of CFSE labeling that allowed us to follow all cell divisions occurring during the incubation period over other methods (e.g. BrdU incorporation), which assess divisions only taking place during the last few hours. In addition, combining CFSE assay with intracellular staining for granzyme B and IFN-γ permitted the analysis of the acquired phenotype of cells that have proliferated.

Addition of IL-27 to α-CD3 stimulation enhanced proliferation (Fig. 6), IFN-γ (Fig. 7), and granzyme B production (Fig. 8) in a dose-dependent manner for both CD4 and CD8 T cells in bulk PBMC cultures. These phenomena were more prominent for CD8 T cells which correlate with the higher levels of expression of the two chains of IL-27R on CD8 than on CD4 T cells. Our results are in line with published data in the mouse system showing that IL-27 induces granzyme B in activated CD8 T cells (Morishima et al., 2005) and IFN-γ production in CD4 T cells and NK cells (Pflanz et al., 2002; Lucas et al., 2003; Takeda et al., 2003). Differences observed between the results obtained via intracellular staining for IFN-γ (Fig. 7) and the ones obtained by ELISA (Fig. 9) of bulk PBMC are probably due to the

ongoing dynamics of production and consumption in the cell culture. Whereas according to the intracellular staining, IL-27 has maximal effect at [10 ng/ml] for α-CD3 [17 ng/ml], our ELISA results showed a maximal IFN-γ production at [100 ng/ml] of IL-27, which suggests that at lower doses of IL-27 more IFN-γ is consumed by different cells like NK, Mφ and T cells. In addition, we did not detect significant differences in the Δ MFI for IFN-γ in our different cultures suggesting that the amount of IFN-γ produced on a per cell basis was not different. Interestingly, the results obtained with the lower dose of stimulating Ab are very similar for both intracellular staining and ELISA (Fig. 9). Remarkably, the bell-shaped curve of IFN-γ production was preserved in all cultures tested: bulk PBMC (Fig. 7), naïve (Fig. 11A) and memory (Fig. 13A) CD8 T cells, with the exception of the curve of α-CD3+α-CD28 stimulation of naïve CD8 T cells. But while for bulk PBMC and memory CD8 T cells the maximal effect of IL-27 on IFN-γ production was at [10 and 100 ng/ml], for naïve T cells it was at [10 and 250 ng/ml] when stimulating only with α-CD3 and at [500 ng/ml] in the presence of co-stimulation. The resemblance between the former two cultures might be due to the presence of contaminating APC in the memory subset isolated whereas for the naïve CD8 T cell culture, the very few contaminating cells could not have had an impact on the overall outcome. This problem, with contamination of the memory subset, should be further resolved in future experiments by isolating very pure $CD8^+CD45RO^+$ subpopulation via FACS-sorting.

The different behavior of naïve (Fig. 10) and memory (Fig. 12) T cells upon stimulation in the presence of IL-27 probably represents the pro- and anti-inflammatory effects of IL-27. While with increasing concentration of IL-27, the proliferation of naïve CD8 T cells augmented, in the memory CD8 T cell subset this process started to abate after [100 ng/ml] IL-27. This might be due to differential expression of SOCS between naïve and memory T cells leading to the observed impact of the activation status of T cells on their responses to IL-27. Suppressors of cytokine signalling (SOCS) are a family of proteins, induced by cytokine stimulation that can inhibit the same cascade initiating their production

(a classical negative feedback mechanism) (Larsen and Ropke, 2002). It has been suggested that the action of IL-27 in the absence of SOCS-3 mimics the situation in naïve T cells (Brender et al., 2007). SOCS-3 also regulates co-stimulatory signals from CD28 whereas SOCS-1 modulates the γ_c cytokines (Brender et al., 2007) (including IL-2, -4, -7, -9, -15 and -21) (Alves et al., 2007). The latter can be induced by a variety of cytokines (like IL-2, IL-6, IFN-α,β,γ etc.), including those signalling through gp130 (Davey et al., 2006). The absence of SOCS-3 leads to hyperproliferation, resulting from hypersensitivity to gp130 cytokines, and prolonged phosporylation of STAT-1, -3 and -5 (Brender et al., 2007). Concerning SOCS-1, one study on mouse CD4 T cells (Villarino et al., 2006) showed that IL-27 did not induce SOCS-1 mRNA. However, nothing has been published on CD8 T cells, and especially on human ones.

Through STAT-3, IL-27 enhances cell proliferation on one hand and on the other STAT-3 activation induces SOCS3. However, after α-CD3+α-CD28 co-stimulation of mouse naïve CD4 T cells, IL-27 induces SOCS3 in a STAT1 dependent manner, leading to abrogation of IL-2 production (and its proliferative effect through STAT-5) (Owaki et al., 2006b). Studies on mouse naïve CD4 T cells (Owaki et al., 2006b) suggest that in the presence of α-CD3 + α-CD28, increasing doses of IL-27 decreases proliferation, but this is not what we observed in human naïve CD8 T cells (Fig. 10). This might be due to differences between the mouse and human, or might represent a difference between the effects of IL-27 on naïve CD4 vs. naïve CD8 T cells, as our results pertained to CD8 T cells. Alternatively, activated naïve T cells can differentiate into activated cells during the incubation period used in our experiment although the time used may have been insufficient for a complete naïve-memory transition but rather a naïve- activated transition. Of note, mouse memory T cells have been shown to express higher levels of TCCR than their naïve counterparts (Villarino et al., 2005). We observed high levels on activated CD4 and CD8 T cells, which could lead to the speculation that on memory T cells, IL-27 can provide a different effect because of the higher TCCR levels while gp130 is down-regulated upon activation (Betz and Muller, 1998).

However, gp130 is up-regulated by IRF-1 (Erickson et al., 2004) and IL-27 has been shown to induce this factor (Fakruddin et al., 2007) raising the question on how these two signals: TCR and IL-27 will dictate the final gp130 expression.

According to observations made in the mouse model (Owaki et al., 2006b), IL-27 induces SOCS3 in the presence of co-stimulation, which further suppresses IFN-γ and IL-2 production and thus proliferation. On one hand, IL-2 can be produced by CD4 and CD8 T cells, as well as by some APC (DC have been shown to produce small amounts which are considered essential for T cell proliferation) (Gaffen and Liu, 2004). On the other hand, monocytes can be activated by cytokines such as IFN-γ. We cannot rule out that the increased IFN-γ production (Fig. 7) observed in our culture may have affected monocytes. Furthermore, given that α-CD3 is a T-specific stimulation, the decrease of proliferation with increase of IL-27 concentration in bulk PBMC may be due to exhaustion of IL-2 sources under these conditions, thus IL-2 being a limiting factor. It has been already described that by secreting IL-2, CD4 T cells enhance the proliferation of CD8 T cells (Lanzavecchia, 1998; Cheng et al., 2002) so this could explain why in the same conditions CD8 T cells proliferate more than CD4 T cells. Furthermore, while at different concentrations of α-CD3, both CD4 and CD8 T cell subsets had similar proliferation profiles (Fig. 2), IL-27 more prominently boosted the proliferation of CD8 T cells (Fig. 6) whereas at higher α-CD3 dose [17 ng/ml] detection of IL-27-enhancing effects was hindered due to the already strong proliferative signal. Another hypothesis could be that the combination of both signals through TCR, induced by α-CD3, and the one delivered by IL-27 might activate different transcription factors, which have been shown for cells in different activation state, and thus lead to different results. It has already been suggested that IL-27 can synergize with TCR for signal transduction in mouse cells (Brender et al., 2007).

Interestingly, in bulk PBMC and in memory CD8 T cell culture, the IFN-γ and granzyme B (Fig. 7B, 8B, 11 and 13) production in response to IL-27 followed the same trend, whereas naïve CD8 T cells showed a different profile. The IFN-γ and granzyme B gene have been shown to be regulated by transcription factors NFAT

(Nuclear factor of activated T cells) and AP-1 (Activator protein-1), which work in tandem. Other genes regulated by the tandem NFAT:AP-1 are the genes of IL-2, GM-CSF, IL-4, -5, -13, CD40L, CD95 (FAS), CD25 (which is the α-chain of the IL-2 receptor) and MIP-1α. To make the story more complicated, IFN-γ and granzyme B can be driven also by Eomes, which is expressed in NK and CD8 T cells (but not by NKT cells), which probably compensates in the absence of T-bet (Glimcher et al., 2004). But while T-bet binds directly to the granzyme B promoter, the mechanism of action of Eomes is still unknown (Glimcher et al., 2004). The expression of granzymes can be induced by different cytokines such as IL-2, IL-12 and IL-15, and IL-2 can further synergize with IL-12 to boost granzyme B levels (Glimcher et al., 2004). As SOCS3 has been shown to inhibit NFAT (Banerjee et al., 2002) and IL-2 (Owaki et al., 2006b), it can potentially decrease granzyme B expression. One can speculate that our observations with human naïve CD8 T cells (increase of granzyme B expression with increasing dose of IL-27) is due to the absence of SOCS3. However, activation through TCR and CD28 induces AP-1 as well as NFAT and NF-κB transcription factors (Ward, 1996). AP-1 is regulated at the transcriptional and posttranslational levels of jun and fos genes (Angel and Karin, 1991). C-fos gene is induced by ERK1/2 kinases, whereas c-jun is induced by JNK-1/2 and p38 kinases. As IL-27 can activate both p38 MAPK and ERK 1/2 signalling pathways with ERK 1/2 activation, leading to T-bet expression (Owaki et al., 2006a), this suggests that IL-27 can potentially induce AP-1 as well. This could probably explain why in the absence of α-CD28 but presence of α-CD3 IL-27 boosted granzyme B, whereas in the presence of CD28 signals (Fig. 11, panel B) IL-27 synergized to increase granzyme B production, as previously suggested by others (Brender et al., 2007). This could explain the increased IFN-γ production by naïve CD8 T cells in the presence of co-stimulation and IL-27 (Fig. 11, panel A). As ERK 1/2 induce T-bet, but also AP-1, these two factors probably compensate or synergize with each other, so in the absence of co-stimulation, increasing doses of IL-27 decreased IFN-γ production, while in the presence of α-CD28 and IL-27, there seems to be a continuous enhanced production of this cytokine. This could

also be the reason (lack of synergy with a stronger stimulus like CD28) for the bell-shaped curve of response of both IFN-γ and granzyme B production by memory CD8 T cells (Fig. 13).

Another hypothesis could be related to the suggestion that when the stimuli are allogenic, IL-27 induces the expression of Eomes, which further enhances the expression of IFN-γ, granzyme B and perforin (Pearce et al., 2003), whereas with Ag-specific stimuli, IL-27 induces T-bet. The in vitro stimulation paradigm we used may be closer to the former situation as increasing doses of IL-27 have prominent enhancing effects on granzyme B production in naïve CD8 T cells, whereas it is only a trend in memory CD8 T cells. As memory T cells differentiate into effector cells faster than naïve T cells, it is possible that the plateau of the lytic enzyme production was reached faster, with very small doses of IL-27. To answer the question, concerning different effects of IL-27 via different transcription factors (T-bet and Eomes), further experiments should be done with allogeneic, Ag specific and polyclonal activation. Allogeneic stimulation (Mixed Leukocyte Reaction (MLR)) can be performed in the presence or absence of IL-27, in which purified T cells from one donor are mixed with APC from another donor. To mimic the Ag specific stimulation in the body, whole PBMC can be labeled with CFSE and can be incubated with viral Ag such as Influenza (as most people were infected at least once in their life) or Tetanus (as most people have been vaccinated for this Ag). However, the last experimental set-up would allow only analysis of memory cells as frequency of naïve T cells is too low to be studied in vitro. Polyclonal T lymphocyte stimulation can be induced with i) α-CD3 antibody as it cross-links the T cell receptor and induces signalling; ii) phytohemagglutinin (PHA), which glues together all surface molecules.

The question that remains to be answered is what determines whether IL-6 and IL-27 (both from the same family of cytokines) exert pro- or anti-inflammatory action. It would be of great interest to determine how these cytokines are balanced and under which hierarchy: which one gets silenced first and how it is determined when IL-27 and IL-6 will have either pro- or anti-inflammatory action. This

phenomenon might be dependent on several variables. The first variable might be the type of receptors and ligands recruited to the immunological synapse and the sum of all these distinct signals determining the final outcome. The second could be the spatial characteristics of IL-27 secretion; whether it is released or not in the immunological synapse, together with IL-12 and IFN-γ it could affect the overall activation. This would further pose the question whether cytokines secreted together in the immunological contact could have interactions affecting each other's accessibility to their cognate receptor. Additional complexity is added to the problem as cytokines such as IL-6 and IL-27 can compete for a common chain for signalling. What favours one cytokine's attachment to this receptor over the other cytokine? Is it a matter of stronger affinity to the receptor or concentration of cytokine or both factors? The third variable concerns the type of APC and its cytokine production profile, as the combination of cytokines may result in different outcomes. The balance between cytokines is a very intriguing question in the light of the suggested redundancy between IL-27 and IFN-γ on one hand, and IL-27 and IL-12 on the other. The other open question pertains to the threshold determining the effects of IL-27 toward pro or anti-inflammatory properties, if there is a threshold or rather combination with other cytokines dictating such outcome.

In the light of the observations that IL-27 synergizes with IL-12 for inducing IFN-γ production in a T-bet dependent manner (Pflanz et al., 2002; Takeda et al., 2003), while IL-27 alone does not induce much IFN-γ production (Lucas et al., 2003) the results concerning IFN-γ production by purified subsets of CD8 T cells can be interpreted in two ways. One possibility is that in human, IL-27 does not need to synergize with IL-12 for IFN-γ production or, even their small quantity, contaminating cells could contribute to the observed phenomena. Further experiments are needed to clarify the question of synergized action of IL-12 and IL-27 on human cells by addition of blocking Ab for IL-12 in the experimental set up. If the results obtained by blocking IL-12 differ significantly from the ones described here, this would mean that both cytokines synergize.

IL-27 and IFN-γ have been suggested to have a redundant action on human monocytes though via different regulation mechanims (tighter regulation of IL-27 signalling (Kalliolias and Ivashkiv, 2008)) to tune Mφ responses to the environment. This means, that given the different sources of these two cytokines (IL-27 is produced mainly by APC, while IFN-γ is produced by NK, NKT and activated T cells (Kalliolias and Ivashkiv, 2008)) it would be easier for Mφ to switch off their own source of signalling than controlling a signal coming from another source and could bring a "different message".

We have not looked at the signalling pathway induced upon IL-27 addition in our in vitro cultures. IL-27 has been reported to activate STAT-1, -2, -3, -5, and to a lesser extent STAT-4 in mouse naïve CD4 and CD8 T cells (Hibbert et al., 2003; Lucas et al., 2003; Takeda et al., 2003; Kamiya et al., 2004; Morishima et al., 2005). For cytokines with dual pro- and anti-inflammatory action, like IL-6, the final effect has been shown to be dependent on the relative balance of STAT-1 and STAT-3 activation. Since STAT-1 and -3 are the best studied transcription factors induced by IL-27, only they will be discussed here-in. Though STAT-1 activation has been associated so far with promoting inflammation, inhibitory effect of this transcription factor has also emerged as type I and II IFNs can inhibit through this pathway IFN-γ production and proliferation of T cells (Lee et al., 2000; Nguyen et al., 2000). STAT-3 phosphorylation is also related to inhibitory events (Levy and Darnell, 2002; O'Shea et al., 2002). While STAT-1 mediates inflammatory, proapoptotic and antiproliferative effects, STAT-3 has the opposite action (Kalliolias and Ivashkiv, 2008) in Mφ. Furthermore, IL-27 has been shown to suppress IL-2 production through induction of suppressor of cytokine signalling 3 (SOCS3) (Owaki et al., 2006b). SOCS3 induction could probably explain the bell-shaped curve of proliferation (as IL-2 is involved in this process) and in IFN-γ production, as SOCS3 is a negative feed-back regulator of IL-2, which is also influenced in the same way by SOCS1. While SOCS1 is low in naïve T cells and is up-regulated upon Ag-stimulation, SOCS-3 is highly expressed in resting T cells and is down-regulated in newly activated T cells (Brender et al., 2007). Of note,

more is known about SOCS expression in mouse than in human. However, some of the data in the mouse system have been confirmed in man as well. In mouse, SOCS-1 and 3 can be induced by different stimuli like granulocyte colony stimulating factor (G-CSF), granulocyte-macrophage colony stimulating factor (GM-CSF), macrophage-colony-stimulating factor (M-CSF), IFN-γ, TNF-α, IL-2, IL-6, IL-11, IL-12, IL-27, oncostatin M (OSM) (Inoue and Kubo, 2004; Davey et al., 2006) and most of these SOCS inducers become their targets. This far, in human cells, IL-2, IL-11 and type I IFNs have been confirmed to induce SOCS proteins (Brender et al., 2005; Dimitriadis et al., 2006; Zitzmann et al., 2007) though further quantitative comparison is needed between the two systems as the quantity of SOCS supposedly is important for the level of silencing of the cytokine signalling.

3.0 CD8 AS A SOURCE OF IL-27

As CD8 T cells have been proposed to be a source of IL-27 (Brender et al., 2007), human CD8 T cells were purified, shortly stimulated in vitro, and then subjected to real time PCR analysis for both subunit of IL-27 (EBI3 and p28) (Fig. 14). Highly pure (>98%) human CD8 T cells did not show IL-27 expression at the mRNA level and thus were not considered a source of this cytokine. The difference between these results and the publication may be due to several reasons: i) the group has worked with purified T-cells but there might have been contaminating APC which have led to false positive results; ii) the published results were obtained from murine cells and the principle of secretion of IL-27 in human and mouse might be different. However, both subunits of IL-27 in human can be secreted by different cell types and due to the lack of disulfide bond between the two subunits, theoretically the cytokine can be formed in the extracellular milieu, although not proven in physiological conditions (Batten and Ghilardi, 2007). Thus, human CD8 T cells can contribute to the increase of the extracellular concentration of EBI3 (see Fig. 14), but not of p28.

4.0 FUTURE DIRECTIONS

Apart from APC and T cells, IL-27 has effects on different other cell types. It been shown to induce IFN-γ and IL-10 production in NK cells (Pflanz et al., 2002; Grant et al., 2008). In mast cells, IL-27 induces the production of pro-inflammatory cytokines like IL-1α, IL-1β, IL-18, TNF-α (Pflanz et al., 2004). It would be interesting to understand the molecular mechanisms of switching between these pro- and anti-inflammatory effects of IL-27. In addition, it would be important to investigate the protein levels of this cytokine in serum of subjects with different health disorders and the effect of the cytokine on adhesion molecules, different than ICAM-1 and homing receptors. Such effects on up- and down-regulation of adhesion molecules could elucidate the contribution of IL-27 in modulating cell trafficking in different organs like brain, pancreas, intestine, skin etc. This would help to further understand the mechanism of action of this pleiotropic cytokine in autoimmune diseases like Multiple sclerosis, diabetes, Crohn's disease and colitis, rheumatoid arthritis, lupus erythematosus and others. Th1/Tc1 responses have been implicated for many years in many autoimmune diseases (i.e. lupus erythematosus, rheumatoid arthritis, multiple sclerosis (Masutani et al., 2001; Yamada et al., 2007; Hedegaard et al., 2008)). IL-27 promotes type 1 immunity responses and upregulation of adhesion molecules on T cells. Thus, it will be highly relevant to compare the capacity of both CD4 and CD8 T cells at migrating across endothelial layers following IL-27 treatment. In adition, cytokines from IL-6 family, namely IL-6 and LIF, are involved in the recruitment of Mφ at the site of peripheral nerve injury (Tofaris et al., 2002) as LIF has been shown to attract Mφ and IL-6 to boot LIF production be denervated Schwann cells (Tofaris et al., 2002). This creates a possibility for IL-27, which belongs to the same family and is produced by APC, to be involved in Mφ recruitment in the nervous system.

Given that many cytokines are involved in immune responses, and IL-27 has been shown to synergize with TGF-β, IL-6, IL-10 and IL-12 in IL-10 production (Awasthi et al., 2007; Fitzgerald et al., 2007b; Stumhofer et al., 2007) and that IL-27 synergizes with IL-12 in IFN-γ production (Lucas et al., 2003) it would be of great

benefit to further reveal different partners of IL-27, as well as cytokines that get silenced in the presence of IL-27, so immune responses could be manipulated by changing the balance between the different cytokines.

Despite there are many unresolved questions about the biology of IL-27, our work contributes to enrich the knowledge about this cytokine. This study shows for the first time levels of expression of both chains of IL-27R on human CD4 and CD8 T cells and modulation of this receptor upon cell activation. As more CD8 T lymphocytes showed IL-27R expression, they were more susceptible to the action of this cytokine. Being a cytokine, produced by APC in the early stages of an infection (Pflanz et al., 2002), IL-27 plays a role in the skewing of the immune responses towards type 1 immunity very early on. This has been shown not only in vitro, but also in many mouse models of human diseases. T cells from $TCCR^{-/-}$ mice have shown impaired IFN-γ and enhanced IL-4 production during the initial stages of activation (Hamano et al., 2003; Artis et al., 2004; Miyazaki et al., 2005), significantly reduced production of different chemokines like CXCL10, CCL2 and CCL5 in an Ag-specific response (Sonoda et al., 2007). Thus, human IL-27 could modulate the outcome of diseases and play a central role in the orchestraton of detrimental vs. beneficial immune responses. It should not be forgotten that every process in an organism is a result of the joint action of many factors and thus is a result of their balance. However, further knowledge would allow us to modulate this action and thus to bring back the balance in cases of health disorders. Because the purpose of science is to give answers to questions and the most important one is the question of physical and mental health.

BIBLIOGRAPHY

Alves NL, Arosa FA, van Lier RA (2007) Common gamma chain cytokines: dissidence in the details. Immunol Lett 108:113-120.

Angel P, Karin M (1991) The role of Jun, Fos and the AP-1 complex in cell-proliferation and transformation. Biochim Biophys Acta 1072:129-157.

Artis D, Villarino A, Silverman M, He W, Thornton EM, Mu S, Summer S, Covey TM, Huang E, Yoshida H, Koretzky G, Goldschmidt M, Wu GD, de Sauvage F, Miller HR, Saris CJ, Scott P, Hunter CA (2004) The IL-27 receptor (WSX-1) is an inhibitor of innate and adaptive elements of type 2 immunity. J Immunol 173:5626-5634.

Awasthi A, Carrier Y, Peron JP, Bettelli E, Kamanaka M, Flavell RA, Kuchroo VK, Oukka M, Weiner HL (2007) A dominant function for interleukin 27 in generating interleukin 10-producing anti-inflammatory T cells. Nat Immunol 8:1380-1389.

Bancroft AJ, Humphreys NE, Worthington JJ, Yoshida H, Grencis RK (2004) WSX-1: a key role in induction of chronic intestinal nematode infection. J Immunol 172:7635-7641.

Banerjee A, Banks AS, Nawijn MC, Chen XP, Rothman PB (2002) Cutting edge: Suppressor of cytokine signalling 3 inhibits activation of NFATp. J Immunol 168:4277-4281.

Batten M, Ghilardi N (2007) The biology and therapeutic potential of interleukin 27. J Mol Med 85:661-672.

Belouzard S, Delcroix D, Rouille Y (2004) Low levels of expression of leptin receptor at the cell surface result from constitutive endocytosis and intracellular retention in the biosynthetic pathway. J Biol Chem 279:28499-28508.

Betz UA, Muller W (1998) Regulated expression of gp130 and IL-6 receptor alpha chain in T cell maturation and activation. Int Immunol 10:1175-1184.

Boumendjel A, Tawk L, Malefijt RD, Boulay V, Yssel H, Pene J (2006) IL-27 induces the production of IgG1 by human B cells. Eur Cytokine Netw 17:281-289.

Brender C, Lovato P, Sommer VH, Woetmann A, Mathiesen AM, Geisler C, Wasik M, Odum N (2005) Constitutive SOCS-3 expression protects T-cell lymphoma against growth inhibition by IFN-alpha. Leukemia 19:209-213.

Brender C, Tannahill GM, Jenkins BJ, Fletcher J, Columbus R, Saris CJ, Ernst M, Nicola NA, Hilton DJ, Alexander WS, Starr R (2007) SOCS3 regulates CD8 T cell proliferation by inhibition of IL-6 and IL-27. Blood.

Cheng LE, Ohlen C, Nelson BH, Greenberg PD (2002) Enhanced signalling through the IL-2 receptor in CD8+ T cells regulated by Ag recognition results in preferential proliferation and expansion of responding CD8+ T cells rather than promotion of cell death. Proc Natl Acad Sci U S A 99:3001-3006.

Coulomb-L'hermine A, Larousserie F, Pflanz S, Bardel E, Kastelein RA, Devergne O (2007) Expression of Interleukin-27 by Human Trophoblast Cells. Placenta.

Davey GM, Heath WR, Starr R (2006) SOCS1: a potent and multifaceted regulator of cytokines and cell-mediated inflammation. Tissue Ag 67:1-9.

Dimitriadis E, Stoikos C, Tan YL, Salamonsen LA (2006) Interleukin 11 signalling components signal transducer and activator of transcription 3 (STAT3) and suppressor of cytokine signalling 3 (SOCS3) regulate human endometrial stromal cell differentiation. Endocrinology 147:3809-3817.

Erickson L, Crews G, Pan F, Fisniku O, Jang MS, Wynn C, Kobayashi M, Jiang H (2004) Unique gene expression profiles of heart allograft rejection in the interferon regulatory factor-1-deficient mouse. Transpl Immunol 13:169-175.

Fakruddin JM, Lempicki RA, Gorelick RJ, Yang J, Adelsberger JW, Garcia-Pineres AJ, Pinto LA, Lane HC, Imamichi T (2007) Noninfectious papilloma virus-like particles inhibit HIV-1 replication: implications for immune control of HIV-1 infection by IL-27. Blood 109:1841-1849.

Fitzgerald DC, Zhang GX, El-Behi M, Fonseca-Kelly Z, Li H, Yu S, Saris CJ, Gran B, Ciric B, Rostami A (2007) Suppression of autoimmune inflammation of the central nervous system by interleukin 10 secreted by interleukin 27-stimulated T cells. Nat Immunol 8:1372-1379.

Gaffen SL, Liu KD (2004) Overview of interleukin-2 function, production and clinical applications. Cytokine 28:109-123.

Glimcher LH, Townsend MJ, Sullivan BM, Lord GM (2004) Recent developments in the transcriptional regulation of cytolytic effector cells. Nat Rev Immunol 4:900-911.

Grant LR, Yao ZJ, Hedrich CM, Wang F, Moorthy A, Wilson K, Ranatunga D, Bream JH (2008) Stat4-dependent, T-bet-independent regulation of IL-10 in NK cells. Genes Immun.

Hamano S, Himeno K, Miyazaki Y, Ishii K, Yamanaka A, Takeda A, Zhang M, Hisaeda H, Mak TW, Yoshimura A, Yoshida H (2003) WSX-1 is required for resistance to Trypanosoma cruzi infection by regulation of proinflammatory cytokine production. Immunity 19:657-667.

Haynes AP, Daniels I, Abhulayha AM, Carter GI, Metheringham R, Gregory CD, Thomson BJ (2002) CD95 (Fas) expression is regulated by sequestration in the Golgi complex in B-cell lymphoma. Br J Haematol 118:488-494.

Hedegaard CJ, Krakauer M, Bendtzen K, Lund H, Sellebjerg F, Nielsen CH (2008) T helper cell type 1 (Th1), Th2 and Th17 responses to myelin basic protein and disease activity in multiple sclerosis. Immunology.

Inoue H, Kubo M (2004) SOCS proteins in T helper cell differentiation: implications for allergic disorders? Expert Rev Mol Med 6:1-11.

Kalliolias GD, Ivashkiv LB (2008) IL-27 Activates Human Monocytes via STAT1 and Suppresses IL-10 Production but the Inflammatory Functions of IL-27 Are Abrogated by TLRs and p38. J Immunol 180:6325-6333.

Kamimura D, Ishihara K, Hirano T (2003) IL-6 signal transduction and its physiological roles: the signal orchestration model. Rev Physiol Biochem Pharmacol 149:1-38.

Kamiya S, Nakamura C, Fukawa T, Ono K, Ohwaki T, Yoshimoto T, Wada S (2007) Effects of IL-23 and IL-27 on osteoblasts and osteoclasts: inhibitory effects on osteoclast differentiation. J Bone Miner Metab 25:277-285.

Lane P (2000) Role of OX40 signals in coordinating CD4 T cell selection, migration, and cytokine differentiation in T helper (Th)1 and Th2 cells. J Exp Med 191:201-206.

Lanzavecchia A (1998) Immunology. Licence to kill. Nature 393:413-414.

Larousserie F, Pflanz S, Coulomb-L'Hermine A, Brousse N, Kastelein R, Devergne O (2004) Expression of IL-27 in human Th1-associated granulomatous diseases. J Pathol 202:164-171.

Larousserie F, Charlot P, Bardel E, Froger J, Kastelein RA, Devergne O (2006) Differential effects of IL-27 on human B cell subsets. J Immunol 176:5890-5897.

Larregina A, Morelli A, Kolkowski E, Fainboim L (1996) Flow cytometric analysis of cytokine receptors on human Langerhans' cells. Changes observed after short-term culture. Immunology 87:317-325.

Larsen L, Ropke C (2002) Suppressors of cytokine signalling: SOCS. APMIS 110:833-844.

Lee CK, Smith E, Gimeno R, Gertner R, Levy DE (2000) STAT1 affects lymphocyte survival and proliferation partially independent of its role downstream of IFN-gamma. J Immunol 164:1286-1292.

Levy DE, Darnell JE, Jr. (2002) Stats: transcriptional control and biological impact. Nat Rev Mol Cell Biol 3:651-662.

Li J, Gran B, Zhang GX, Rostami A, Kamoun M (2005) IL-27 subunits and its receptor (WSX-1) mRNAs are markedly up-regulated in inflammatory cells in the CNS during experimental autoimmune encephalomyelitis. J Neurol Sci 232:3-9.

Lucas S, Ghilardi N, Li J, de Sauvage FJ (2003) IL-27 regulates IL-12 responsiveness of naïve CD4+ T cells through Stat1-dependent and -independent mechanisms. Proc Natl Acad Sci U S A 100:15047-15052.

Masutani K, Akahoshi M, Tsuruya K, Tokumoto M, Ninomiya T, Kohsaka T, Fukuda K, Kanai H, Nakashima H, Otsuka T, Hirakata H (2001) Predominance of Th1 immune response in diffuse proliferative lupus nephritis. Arthritis Rheum 44:2097-2106.

Miyazaki Y, Inoue H, Matsumura M, Matsumoto K, Nakano T, Tsuda M, Hamano S, Yoshimura A, Yoshida H (2005) Exacerbation of experimental allergic asthma by augmented Th2 responses in WSX-1-deficient mice. J Immunol 175:2401-2407.

Morishima N, Owaki T, Asakawa M, Kamiya S, Mizuguchi J, Yoshimoto T (2005) Augmentation of effector CD8+ T cell generation with enhanced granzyme B expression by IL-27. J Immunol 175:1686-1693.

Nguyen KB, Cousens LP, Doughty LA, Pien GC, Durbin JE, Biron CA (2000) Interferon alpha/beta-mediated inhibition and promotion of interferon gamma: STAT1 resolves a paradox. Nat Immunol 1:70-76.

O'Shea JJ, Gadina M, Schreiber RD (2002) Cytokine signalling in 2002: new surprises in the Jak/Stat pathway. Cell 109 Suppl:S121-131.

Oberg HH, Wesch D, Grussel S, Rose-John S, Kabelitz D (2006) Differential expression of CD126 and CD130 mediates different STAT-3 phosphorylation in CD4+CD25- and CD25high regulatory T cells. Int Immunol 18:555-563.

Owaki T, Asakawa M, Fukai F, Mizuguchi J, Yoshimoto T (2006a) IL-27 induces Th1 differentiation via p38 MAPK/T-bet- and intercellular adhesion molecule-1/LFA-1/ERK1/2-dependent pathways. J Immunol 177:7579-7587.

Owaki T, Asakawa M, Kamiya S, Takeda K, Fukai F, Mizuguchi J, Yoshimoto T (2006b) IL-27 suppresses CD28-mediated [correction of medicated] IL-2 production through suppressor of cytokine signalling 3. J Immunol 176:2773-2780.

Pearce EL, Mullen AC, Martins GA, Krawczyk CM, Hutchins AS, Zediak VP, Banica M, DiCioccio CB, Gross DA, Mao CA, Shen H, Cereb N, Yang SY, Lindsten T, Rossant J, Hunter CA, Reiner SL (2003) Control of effector CD8+ T cell function by the transcription factor Eomesodermin. Science 302:1041-1043.

Pflanz S, Hibbert L, Mattson J, Rosales R, Vaisberg E, Bazan JF, Phillips JH, McClanahan TK, de Waal Malefyt R, Kastelein RA (2004) WSX-1 and glycoprotein 130 constitute a signal-transducing receptor for IL-27. J Immunol 172:2225-2231.

Pflanz S et al. (2002) IL-27, a heterodimeric cytokine composed of EBI3 and p28 protein, induces proliferation of naïve CD4(+) T cells. Immunity 16:779-790.

Pradhan A, Lambert QT, Reuther GW (2007) Transformation of hematopoietic cells and activation of JAK2-V617F by IL-27R, a component of a heterodimeric type I cytokine receptor. Proc Natl Acad Sci U S A 104:18502-18507.

Rival C, Theas MS, Guazzone VA, Lustig L (2006) Interleukin-6 and IL-6 receptor cell expression in testis of rats with autoimmune orchitis. J Reprod Immunol 70:43-58.

Sonoda KH, Yoshimura T, Takeda A, Ishibashi T, Hamano S, Yoshida H (2007) WSX-1 plays a significant role for the initiation of experimental autoimmune uveitis. Int Immunol 19:93-98.

Stumhofer JS, Silver JS, Laurence A, Porrett PM, Harris TH, Turka LA, Ernst M, Saris CJ, O'Shea JJ, Hunter CA (2007) Interleukins 27 and 6 induce STAT3-mediated T cell production of interleukin 10. Nat Immunol 8:1363-1371.

Suto A, Nakajima H, Tokumasa N, Takatori H, Kagami S, Suzuki K, Iwamoto I (2005) Murine plasmacytoid dendritic cells produce IFN-gamma upon IL-4 stimulation. J Immunol 175:5681-5689.

Takeda A, Hamano S, Yamanaka A, Hanada T, Ishibashi T, Mak TW, Yoshimura A, Yoshida H (2003) Cutting edge: role of IL-27/WSX-1 signalling for induction of T-bet through activation of STAT1 during initial Th1 commitment. J Immunol 170:4886-4890.

Villarino A, Hibbert L, Lieberman L, Wilson E, Mak T, Yoshida H, Kastelein RA, Saris C, Hunter CA (2003) The IL-27R (WSX-1) is required to suppress T cell hyperactivity during infection. Immunity 19:645-655.

Villarino AV, Huang E, Hunter CA (2004) Understanding the pro- and anti-inflammatory properties of IL-27. J Immunol 173:715-720.

Villarino AV, Stumhofer JS, Saris CJ, Kastelein RA, de Sauvage FJ, Hunter CA (2006) IL-27 limits IL-2 production during Th1 differentiation. J Immunol 176:237-247.

Villarino AV, Larkin J, 3rd, Saris CJ, Caton AJ, Lucas S, Wong T, de Sauvage FJ, Hunter CA (2005) Positive and negative regulation of the IL-27 receptor during lymphoid cell activation. J Immunol 174:7684-7691.

Ward SG (1996) CD28: a signalling perspective. Biochem J 318 (Pt 2):361-377.

Yamada H, Nakashima Y, Okazaki K, Mawatari T, Fukushi JI, Kaibara N, Hori A, Iwamoto Y, Yoshikai Y (2007) Th1 but not Th17 cells predominate in the joints of patients with rheumatoid arthritis. Ann Rheum Dis.

Zitzmann K, Brand S, De Toni EN, Baehs S, Goke B, Meinecke J, Spottl G, Meyer HH, Auernhammer CJ (2007) SOCS1 silencing enhances antitumour activity of type I IFNs by regulating apoptosis in neuroendocrine tumour cells. Cancer Res 67:5025-5032.

Brender C, Tannahill GM, Jenkins BJ, Fletcher J, Columbus R, Saris CJ, Ernst M, Nicola NA, Hilton DJ, Alexander WS, Starr R (2007) SOCS3 regulates CD8 T cell proliferation by inhibition of IL-6 and IL-27. Blood.

Matsui M, Moriya O, Belladonna ML, Kamiya S, Lemonnier FA, Yoshimoto T, Akatsuka T (2004) Adjuvant activities of novel cytokines, interleukin-23 (IL-23) and IL-27, for induction of hepatitis C virus-specific cytotoxic T lymphocytes in HLA-A*0201 transgenic mice. Journal of virology 78:9093-9104.

Morishima N, Owaki T, Asakawa M, Kamiya S, Mizuguchi J, Yoshimoto T (2005) Augmentation of effector CD8+ T cell generation with enhanced granzyme B expression by IL-27. J Immunol 175:1686-1693.

Acosta-Rodriguez EV, Napolitani G, Lanzavecchia A, Sallusto F (2007a) Interleukins 1beta and 6 but not transforming growth factor-beta are essential for the differentiation of interleukin 17-producing human T helper cells. Nat Immunol 8:942-949.

Acosta-Rodriguez EV, Rivino L, Geginat J, Jarrossay D, Gattorno M, Lanzavecchia A, Sallusto F, Napolitani G (2007b) Surface phenotype and antigenic specificity

of human interleukin 17-producing T helper memory cells. Nat Immunol 8:639-646.

Afzali B, Lombardi G, Lechler RI, Lord GM (2007) The role of T helper 17 (Th17) and regulatory T cells (Treg) in human organ transplantation and autoimmune disease. Clin Exp Immunol 148:32-46.

Agarwal A, Nayak BP, Rao KV (1998) B cell responses to a peptide epitope. VII. Antigen-dependent modulation of the germinal center reaction. J Immunol 161:5832-5841.

Alves NL, Arosa FA, van Lier RA (2007) Common gamma chain cytokines: dissidence in the details. Immunol Lett 108:113-120.

Alves NL, Hooibrink B, Arosa FA, van Lier RA (2003) IL-15 induces antigen-independent expansion and differentiation of human naive CD8+ T cells in vitro. Blood 102:2541-2546.

Amadi-Obi A, Yu CR, Liu X, Mahdi RM, Clarke GL, Nussenblatt RB, Gery I, Lee YS, Egwuagu CE (2007) T(H)17 cells contribute to uveitis and scleritis and are expanded by IL-2 and inhibited by IL-27/STAT1. Nat Med 13:711-718.

Angel P, Karin M (1991) The role of Jun, Fos and the AP-1 complex in cell-proliferation and transformation. Biochim Biophys Acta 1072:129-157.

Annunziato F, Cosmi L, Santarlasci V, Maggi L, Liotta F, Mazzinghi B, Parente E, Fili L, Ferri S, Frosali F, Giudici F, Romagnani P, Parronchi P, Tonelli F, Maggi E, Romagnani S (2007) Phenotypic and functional features of human Th17 cells. J Exp Med 204:1849-1861.

Arbour N, Holz A, Sipe JC, Naniche D, Romine JS, Zyroff J, Oldstone MB (2003) A new approach for evaluating antigen-specific T cell responses to myelin antigens during the course of multiple sclerosis. J Neuroimmunol 137:197-209.

Artis D, Villarino A, Silverman M, He W, Thornton EM, Mu S, Summer S, Covey TM, Huang E, Yoshida H, Koretzky G, Goldschmidt M, Wu GD, de Sauvage F, Miller HR, Saris CJ, Scott P, Hunter CA (2004) The IL-27 receptor (WSX-1) is an inhibitor of innate and adaptive elements of type 2 immunity. J Immunol 173:5626-5634.

Aujla SJ, Dubin PJ, Kolls JK (2007) Interleukin-17 in pulmonary host defense. Exp Lung Res 33:507-518.

Awasthi A, Carrier Y, Peron JP, Bettelli E, Kamanaka M, Flavell RA, Kuchroo VK, Oukka M, Weiner HL (2007) A dominant function for interleukin 27 in generating interleukin 10-producing anti-inflammatory T cells. Nat Immunol 8:1380-1389.

Badovinac VP, Harty JT (2006) Programming, demarcating, and manipulating CD8+ T-cell memory. Immunol Rev 211:67-80.

Banchereau J, Steinman RM (1998) Dendritic cells and the control of immunity. Nature 392:245-252.

Bancroft AJ, Humphreys NE, Worthington JJ, Yoshida H, Grencis RK (2004) WSX-1: a key role in induction of chronic intestinal nematode infection. J Immunol 172:7635-7641.

Banerjee A, Banks AS, Nawijn MC, Chen XP, Rothman PB (2002) Cutting edge: Suppressor of cytokine signaling 3 inhibits activation of NFATp. J Immunol 168:4277-4281.

Barry M, Bleackley RC (2002) Cytotoxic T lymphocytes: all roads lead to death. Nat Rev Immunol 2:401-409.

Batten M, Ghilardi N (2007) The biology and therapeutic potential of interleukin 27. J Mol Med 85:661-672.

Batten M, Li J, Yi S, Kljavin NM, Danilenko DM, Lucas S, Lee J, de Sauvage FJ, Ghilardi N (2006) Interleukin 27 limits autoimmune encephalomyelitis by suppressing the development of interleukin 17-producing T cells. Nat Immunol 7:929-936.

Becker C, Wirtz S, Neurath MF (2005) Stepwise regulation of TH1 responses in autoimmunity: IL-12-related cytokines and their receptors. Inflamm Bowel Dis 11:755-764.

Belouzard S, Delcroix D, Rouille Y (2004) Low levels of expression of leptin receptor at the cell surface result from constitutive endocytosis and

intracellular retention in the biosynthetic pathway. J Biol Chem 279:28499-28508.

Berard M, Brandt K, Bulfone-Paus S, Tough DF (2003) IL-15 promotes the survival of naive and memory phenotype CD8+ T cells. J Immunol 170:5018-5026.

Bettelli E, Carrier Y, Gao W, Korn T, Strom TB, Oukka M, Weiner HL, Kuchroo VK (2006) Reciprocal developmental pathways for the generation of pathogenic effector TH17 and regulatory T cells. Nature 441:235-238.

Betz UA, Muller W (1998) Regulated expression of gp130 and IL-6 receptor alpha chain in T cell maturation and activation. Int Immunol 10:1175-1184.

Boumendjel A, Tawk L, Malefijt RD, Boulay V, Yssel H, Pene J (2006) IL-27 induces the production of IgG1 by human B cells. Eur Cytokine Netw 17:281-289.

Boyman O, Purton JF, Surh CD, Sprent J (2007) Cytokines and T-cell homeostasis. Curr Opin Immunol 19:320-326.

Brender C, Lovato P, Sommer VH, Woetmann A, Mathiesen AM, Geisler C, Wasik M, Odum N (2005) Constitutive SOCS-3 expression protects T-cell lymphoma against growth inhibition by IFNalpha. Leukemia 19:209-213.

Brender C, Tannahill GM, Jenkins BJ, Fletcher J, Columbus R, Saris CJ, Ernst M, Nicola NA, Hilton DJ, Alexander WS, Starr R (2007) SOCS3 regulates CD8 T cell proliferation by inhibition of IL-6 and IL-27. Blood.

Bullens DM, Van Den Keybus C, Dilissen E, Kasran A, Ceuppens JL (2004) Allergen-specific T cells from birch-pollen-allergic patients and healthy controls differ in T helper 2 cytokine and in interleukin-10 production. Clin Exp Allergy 34:879-887.

Calo V, Migliavacca M, Bazan V, Macaluso M, Buscemi M, Gebbia N, Russo A (2003) STAT proteins: from normal control of cellular events to tumorigenesis. J Cell Physiol 197:157-168.

Chae SC, Li CS, Kim KM, Yang JY, Zhang Q, Lee YC, Yang YS, Chung HT (2007) Identification of polymorphisms in human interleukin-27 and their association with asthma in a Korean population. J Hum Genet.

Chen Q, Ghilardi N, Wang H, Baker T, Xie MH, Gurney A, Grewal IS, de Sauvage FJ (2000) Development of Th1-type immune responses requires the type I cytokine receptor TCCR. Nature 407:916-920.

Cheng LE, Ohlen C, Nelson BH, Greenberg PD (2002) Enhanced signaling through the IL-2 receptor in CD8+ T cells regulated by antigen recognition results in preferential proliferation and expansion of responding CD8+ T cells rather than promotion of cell death. Proc Natl Acad Sci U S A 99:3001-3006.

Chiyo M, Shimozato O, Yu L, Kawamura K, Iizasa T, Fujisawa T, Tagawa M (2005) Expression of IL-27 in murine carcinoma cells produces antitumor effects and induces protective immunity in inoculated host animals. Int J Cancer 115:437-442.

Collison LW, Workman CJ, Kuo TT, Boyd K, Wang Y, Vignali KM, Cross R, Sehy D, Blumberg RS, Vignali DA (2007) The inhibitory cytokine IL-35 contributes to regulatory T-cell function. Nature 450:566-569.

Coulomb-L'hermine A, Larousserie F, Pflanz S, Bardel E, Kastelein RA, Devergne O (2007) Expression of Interleukin-27 by Human Trophoblast Cells. Placenta.

Daniel G. Remick JSF, ed (1997) Cytokines In Health and Disease. New York - Basel: Marcel Dekker, Inc.

Davey GM, Heath WR, Starr R (2006) SOCS1: a potent and multifaceted regulator of cytokines and cell-mediated inflammation. Tissue Antigens 67:1-9.

Devergne O, Birkenbach M, Kieff E (1997) Epstein-Barr virus-induced gene 3 and the p35 subunit of interleukin 12 form a novel heterodimeric hematopoietin. Proc Natl Acad Sci U S A 94:12041-12046.

Devergne O, Hummel M, Koeppen H, Le Beau MM, Nathanson EC, Kieff E, Birkenbach M (1996) A novel interleukin-12 p40-related protein induced by latent Epstein-Barr virus infection in B lymphocytes. J Virol 70:1143-1153.

Dimitriadis E, Stoikos C, Tan YL, Salamonsen LA (2006) Interleukin 11 signaling components signal transducer and activator of transcription 3 (STAT3) and suppressor of cytokine signaling 3 (SOCS3) regulate human endometrial stromal cell differentiation. Endocrinology 147:3809-3817.

Dong C (2008) TH17 cells in development: an updated view of their molecular identity and genetic programming. Nat Rev Immunol 8:337-348.

Dorner BG, Scheffold A, Rolph MS, Huser MB, Kaufmann SH, Radbruch A, Flesch IE, Kroczek RA (2002) MIP-1alpha, MIP-1beta, RANTES, and ATAC/lymphotactin function together with IFN-gamma as type 1 cytokines. Proc Natl Acad Sci U S A 99:6181-6186.

Erickson L, Crews G, Pan F, Fisniku O, Jang MS, Wynn C, Kobayashi M, Jiang H (2004) Unique gene expression profiles of heart allograft rejection in the interferon regulatory factor-1-deficient mouse. Transpl Immunol 13:169-175.

Evans DE, Munks MW, Purkerson JM, Parker DC (2000) Resting B lymphocytes as APC for naive T lymphocytes: dependence on CD40 ligand/CD40. J Immunol 164:688-697.

Faassen AE, Dalke DP, Berton MT, Warren WD, Pierce SK (1995) CD40-CD40 ligand interactions stimulate B cell antigen processing. Eur J Immunol 25:3249-3255.

Fakruddin JM, Lempicki RA, Gorelick RJ, Yang J, Adelsberger JW, Garcia-Pineres AJ, Pinto LA, Lane HC, Imamichi T (2007) Noninfectious papilloma virus-like particles inhibit HIV-1 replication: implications for immune control of HIV-1 infection by IL-27. Blood 109:1841-1849.

Feng XM, Chen XL, Liu N, Chen Z, Zhou YL, Han ZB, Zhang L, Han ZC (2007) Interleukin-27 upregulates major histocompatibility complex class II expression in primary human endothelial cells through induction of major histocompatibility complex class II transactivator. Hum Immunol 68:965-972.

Feng XM, Liu N, Yang SG, Hu LY, Chen XL, Fang ZH, Ren Q, Lu SH, Liu B, Han ZC (2008) Regulation of the class II and class I MHC pathways in human THP-1 monocytic cells by interleukin-27. Biochem Biophys Res Commun 367:553-559.

Fitzgerald DC, Ciric B, Touil T, Harle H, Grammatikopolou J, Sarma JD, Gran B, Zhang GX, Rostami A (2007a) Suppressive Effect of IL-27 on Encephalitogenic

Th17 Cells and the Effector Phase of Experimental Autoimmune Encephalomyelitis. J Immunol 179:3268-3275.

Fitzgerald DC, Zhang GX, El-Behi M, Fonseca-Kelly Z, Li H, Yu S, Saris CJ, Gran B, Ciric B, Rostami A (2007b) Suppression of autoimmune inflammation of the central nervous system by interleukin 10 secreted by interleukin 27-stimulated T cells. Nat Immunol 8:1372-1379.

Gafa V, Lande R, Gagliardi MC, Severa M, Giacomini E, Remoli ME, Nisini R, Ramoni C, Di Francesco P, Aldebert D, Grillot R, Coccia EM (2006) Human dendritic cells following Aspergillus fumigatus infection express the CCR7 receptor and a differential pattern of interleukin-12 (IL-12), IL-23, and IL-27 cytokines, which lead to a Th1 response. Infect Immun 74:1480-1489.

Gaffen SL, Liu KD (2004) Overview of interleukin-2 function, production and clinical applications. Cytokine 28:109-123.

Gagro A, Servis D, Cepika AM, Toellner KM, Grafton G, Taylor DR, Branica S, Gordon J (2006) Type I cytokine profiles of human naive and memory B lymphocytes: a potential for memory cells to impact polarization. Immunology 118:66-77.

Geginat J, Sallusto F, Lanzavecchia A (2001) Cytokine-driven proliferation and differentiation of human naive, central memory, and effector memory CD4(+) T cells. J Exp Med 194:1711-1719.

Geginat J, Lanzavecchia A, Sallusto F (2003) Proliferation and differentiation potential of human CD8+ memory T-cell subsets in response to antigen or homeostatic cytokines. Blood 101:4260-4266.

Glimcher LH, Townsend MJ, Sullivan BM, Lord GM (2004) Recent developments in the transcriptional regulation of cytolytic effector cells. Nat Rev Immunol 4:900-911.

Godfrey DI, Rossjohn J, McCluskey J (2008) The fidelity, occasional promiscuity, and versatility of T cell receptor recognition. Immunity 28:304-314.

Goldberg R, Zohar Y, Wildbaum G, Geron Y, Maor G, Karin N (2004) Suppression of ongoing experimental autoimmune encephalomyelitis by neutralizing the function of the p28 subunit of IL-27. J Immunol 173:6465-6471.

Goldrath AW, Sivakumar PV, Glaccum M, Kennedy MK, Bevan MJ, Benoist C, Mathis D, Butz EA (2002) Cytokine requirements for acute and Basal homeostatic proliferation of naive and memory CD8+ T cells. J Exp Med 195:1515-1522.

Gomes AQ, Correia DV, Silva-Santos B (2007) Non-classical major histocompatibility complex proteins as determinants of tumour immunosurveillance. EMBO Rep 8:1024-1030.

Gonzalez-Rey E, Delgado M (2006) Therapeutic treatment of experimental colitis with regulatory dendritic cells generated with vasoactive intestinal peptide. Gastroenterology 131:1799-1811.

Gordon S (1986) Biology of the macrophage. J Cell Sci Suppl 4:267-286.

Gordon S, Taylor PR (2005) Monocyte and macrophage heterogeneity. Nat Rev Immunol 5:953-964.

Grant LR, Yao ZJ, Hedrich CM, Wang F, Moorthy A, Wilson K, Ranatunga D, Bream JH (2008) Stat4-dependent, T-bet-independent regulation of IL-10 in NK cells. Genes Immun.

Guilherme L, Ramasawmy R, Kalil J (2007) Rheumatic fever and rheumatic heart disease: genetics and pathogenesis. Scand J Immunol 66:199-207.

Guimond M, Fry TJ, Mackall CL (2005) Cytokine signals in T-cell homeostasis. J Immunother 28:289-294.

Haan C, Kreis S, Margue C, Behrmann I (2006) Jaks and cytokine receptors--an intimate relationship. Biochem Pharmacol 72:1538-1546.

Haczku A (2006) Role and regulation of lung collectins in allergic airway sensitization. Pharmacol Ther 110:14-34.

Hamano S, Himeno K, Miyazaki Y, Ishii K, Yamanaka A, Takeda A, Zhang M, Hisaeda H, Mak TW, Yoshimura A, Yoshida H (2003) WSX-1 is required for

resistance to Trypanosoma cruzi infection by regulation of proinflammatory cytokine production. Immunity 19:657-667.

Harrington LE, Hatton RD, Mangan PR, Turner H, Murphy TL, Murphy KM, Weaver CT (2005) Interleukin 17-producing CD4+ effector T cells develop via a lineage distinct from the T helper type 1 and 2 lineages. Nat Immunol 6:1123-1132.

Harty JT, Badovinac VP (2008) Shaping and reshaping CD8+ T-cell memory. Nat Rev Immunol 8:107-119.

Haynes AP, Daniels I, Abhulayha AM, Carter GI, Metheringham R, Gregory CD, Thomson BJ (2002) CD95 (Fas) expression is regulated by sequestration in the Golgi complex in B-cell lymphoma. Br J Haematol 118:488-494.

Hedegaard CJ, Krakauer M, Bendtzen K, Lund H, Sellebjerg F, Nielsen CH (2008) T helper cell type 1 (Th1), Th2 and Th17 responses to myelin basic protein and disease activity in multiple sclerosis. Immunology.

Heinrich PC, Behrmann I, Muller-Newen G, Schaper F, Graeve L (1998) Interleukin-6-type cytokine signalling through the gp130/Jak/STAT pathway. Biochem J 334 (Pt 2):297-314.

Hibbert L, Pflanz S, De Waal Malefyt R, Kastelein RA (2003) IL-27 and IFN-alpha signal via Stat1 and Stat3 and induce T-Bet and IL-12Rbeta2 in naive T cells. J Interferon Cytokine Res 23:513-522.

Hibi M, Murakami M, Saito M, Hirano T, Taga T, Kishimoto T (1990) Molecular cloning and expression of an IL-6 signal transducer, gp130. Cell 63:1149-1157.

Hirota K, Hashimoto M, Yoshitomi H, Tanaka S, Nomura T, Yamaguchi T, Iwakura Y, Sakaguchi N, Sakaguchi S (2007) T cell self-reactivity forms a cytokine milieu for spontaneous development of IL-17+ Th cells that cause autoimmune arthritis. J Exp Med 204:41-47.

Hisada M, Kamiya S, Fujita K, Belladonna ML, Aoki T, Koyanagi Y, Mizuguchi J, Yoshimoto T (2004) Potent antitumor activity of interleukin-27. Cancer Res 64:1152-1156.

Honda K, Nakamura K, Matsui N, Takahashi M, Kitamura Y, Mizutani T, Harada N, Nawata H, Hamano S, Yoshida H (2005) T helper 1-inducing property of IL-27/WSX-1 signaling is required for the induction of experimental colitis. Inflamm Bowel Dis 11:1044-1052.

Huber M, Steinwald V, Guralnik A, Brustle A, Kleemann P, Rosenplanter C, Decker T, Lohoff M (2008) IL-27 inhibits the development of regulatory T cells via STAT3. Int Immunol 20:223-234.

Huising MO, Kruiswijk CP, Flik G (2006) Phylogeny and evolution of class-I helical cytokines. J Endocrinol 189:1-25.

Inoue H, Kubo M (2004) SOCS proteins in T helper cell differentiation: implications for allergic disorders? Expert Rev Mol Med 6:1-11.

Judge AD, Zhang X, Fujii H, Surh CD, Sprent J (2002) Interleukin 15 controls both proliferation and survival of a subset of memory-phenotype CD8(+) T cells. J Exp Med 196:935-946.

Jung S (2004) Good, bad and beautiful--the role of dendritic cells in autoimmunity. Autoimmun Rev 3:54-60.

Kalliolias GD, Ivashkiv LB (2008) IL-27 Activates Human Monocytes via STAT1 and Suppresses IL-10 Production but the Inflammatory Functions of IL-27 Are Abrogated by TLRs and p38. J Immunol 180:6325-6333.

Kamimura D, Ishihara K, Hirano T (2003) IL-6 signal transduction and its physiological roles: the signal orchestration model. Rev Physiol Biochem Pharmacol 149:1-38.

Kamiya S, Owaki T, Morishima N, Fukai F, Mizuguchi J, Yoshimoto T (2004) An indispensable role for STAT1 in IL-27-induced T-bet expression but not proliferation of naive CD4+ T cells. J Immunol 173:3871-3877.

Kamiya S, Nakamura C, Fukawa T, Ono K, Ohwaki T, Yoshimoto T, Wada S (2007) Effects of IL-23 and IL-27 on osteoblasts and osteoclasts: inhibitory effects on osteoclast differentiation. J Bone Miner Metab 25:277-285.

Kastelein RA, Hunter CA, Cua DJ (2007) Discovery and Biology of IL-23 and IL-27: Related but Functionally Distinct Regulators of Inflammation. Annu Rev Immunol 25:221-242.

Kebir H, Kreymborg K, Ifergan I, Dodelet-Devillers A, Cayrol R, Bernard M, Giuliani F, Arbour N, Becher B, Prat A (2007) Human TH17 lymphocytes promote blood-brain barrier disruption and central nervous system inflammation. Nat Med 13:1173-1175.

Kennedy MK, Mohler KM, Shanebeck KD, Baum PR, Picha KS, Otten-Evans CA, Janeway CA, Jr., Grabstein KH (1994) Induction of B cell costimulatory function by recombinant murine CD40 ligand. Eur J Immunol 24:116-123.

Kieper WC, Tan JT, Bondi-Boyd B, Gapin L, Sprent J, Ceredig R, Surh CD (2002) Overexpression of interleukin (IL)-7 leads to IL-15-independent generation of memory phenotype CD8+ T cells. J Exp Med 195:1533-1539.

Kondrack RM, Harbertson J, Tan JT, McBreen ME, Surh CD, Bradley LM (2003) Interleukin 7 regulates the survival and generation of memory CD4 cells. J Exp Med 198:1797-1806.

Lane P (2000) Role of OX40 signals in coordinating CD4 T cell selection, migration, and cytokine differentiation in T helper (Th)1 and Th2 cells. J Exp Med 191:201-206.

Lantz O, Grandjean I, Matzinger P, Di Santo JP (2000) Gamma chain required for naive CD4+ T cell survival but not for antigen proliferation. Nat Immunol 1:54-58.

Lanzavecchia A (1998) Immunology. Licence to kill. Nature 393:413-414.

Larousserie F, Pflanz S, Coulomb-L'Hermine A, Brousse N, Kastelein R, Devergne O (2004) Expression of IL-27 in human Th1-associated granulomatous diseases. J Pathol 202:164-171.

Larousserie F, Charlot P, Bardel E, Froger J, Kastelein RA, Devergne O (2006) Differential effects of IL-27 on human B cell subsets. J Immunol 176:5890-5897.

Larousserie F, Bardel E, Pflanz S, Arnulf B, Lome-Maldonado C, Hermine O, Bregeaud L, Perennec M, Brousse N, Kastelein R, Devergne O (2005) Analysis

of interleukin-27 (EBI3/p28) expression in Epstein-Barr virus- and human T-cell leukemia virus type 1-associated lymphomas: heterogeneous expression of EBI3 subunit by tumoral cells. Am J Pathol 166:1217-1228.

Larregina A, Morelli A, Kolkowski E, Fainboim L (1996) Flow cytometric analysis of cytokine receptors on human Langerhans' cells. Changes observed after short-term culture. Immunology 87:317-325.

Larsen L, Ropke C (2002) Suppressors of cytokine signalling: SOCS. APMIS 110:833-844.

Lee CK, Smith E, Gimeno R, Gertner R, Levy DE (2000) STAT1 affects lymphocyte survival and proliferation partially independent of its role downstream of IFN-gamma. J Immunol 164:1286-1292.

Levy DE, Darnell JE, Jr. (2002) Stats: transcriptional control and biological impact. Nat Rev Mol Cell Biol 3:651-662.

Li J, Huston G, Swain SL (2003) IL-7 promotes the transition of CD4 effectors to persistent memory cells. J Exp Med 198:1807-1815.

Li J, Gran B, Zhang GX, Rostami A, Kamoun M (2005) IL-27 subunits and its receptor (WSX-1) mRNAs are markedly up-regulated in inflammatory cells in the CNS during experimental autoimmune encephalomyelitis. J Neurol Sci 232:3-9.

Lin J, Weiss A (2001) T cell receptor signalling. J Cell Sci 114:243-244.

Liu J, Guan X, Ma X (2007) Regulation of IL-27 p28 gene expression in macrophages through MyD88- and interferon-gamma-mediated pathways. J Exp Med 204:141-152.

Lucas S, Ghilardi N, Li J, de Sauvage FJ (2003) IL-27 regulates IL-12 responsiveness of naive CD4+ T cells through Stat1-dependent and -independent mechanisms. Proc Natl Acad Sci U S A 100:15047-15052.

Mackey MF, Barth RJ, Jr., Noelle RJ (1998) The role of CD40/CD154 interactions in the priming, differentiation, and effector function of helper and cytotoxic T cells. J Leukoc Biol 63:418-428.

Mangan PR, Harrington LE, O'Quinn DB, Helms WS, Bullard DC, Elson CO, Hatton RD, Wahl SM, Schoeb TR, Weaver CT (2006) Transforming growth factor-beta induces development of the T(H)17 lineage. Nature 441:231-234.

Masutani K, Akahoshi M, Tsuruya K, Tokumoto M, Ninomiya T, Kohsaka T, Fukuda K, Kanai H, Nakashima H, Otsuka T, Hirakata H (2001) Predominance of Th1 immune response in diffuse proliferative lupus nephritis. Arthritis Rheum 44:2097-2106.

Matsui M, Moriya O, Belladonna ML, Kamiya S, Lemonnier FA, Yoshimoto T, Akatsuka T (2004) Adjuvant activities of novel cytokines, interleukin-23 (IL-23) and IL-27, for induction of hepatitis C virus-specific cytotoxic T lymphocytes in HLA-A*0201 transgenic mice. Journal of virology 78:9093-9104.

Matsukawa A (2007) STAT proteins in innate immunity during sepsis: lessons from gene knockout mice. Acta Med Okayama 61:239-245.

Mayer KD, Mohrs K, Reiley W, Wittmer S, Kohlmeier JE, Pearl JE, Cooper AM, Johnson LL, Woodland DL, Mohrs M (2008) Cutting edge: T-bet and IL-27R are critical for in vivo IFN-gamma production by CD8 T cells during infection. J Immunol 180:693-697.

McGeachy MJ, Cua DJ (2008) Th17 cell differentiation: the long and winding road. Immunity 28:445-453.

Melief CJ (2003) Mini-review: Regulation of cytotoxic T lymphocyte responses by dendritic cells: peaceful coexistence of cross-priming and direct priming? Eur J Immunol 33:2645-2654.

Mescher MF, Curtsinger JM, Agarwal P, Casey KA, Gerner M, Hammerbeck CD, Popescu F, Xiao Z (2006) Signals required for programming effector and memory development by CD8+ T cells. Immunol Rev 211:81-92.

Miyazaki Y, Inoue H, Matsumura M, Matsumoto K, Nakano T, Tsuda M, Hamano S, Yoshimura A, Yoshida H (2005) Exacerbation of experimental allergic asthma by augmented Th2 responses in WSX-1-deficient mice. J Immunol 175:2401-2407.

Molle C, Nguyen M, Flamand V, Renneson J, Trottein F, De Wit D, Willems F, Goldman M, Goriely S (2007) IL-27 Synthesis Induced by TLR Ligation Critically Depends on IFN Regulatory Factor 3. J Immunol 178:7607-7615.

Morishima N, Owaki T, Asakawa M, Kamiya S, Mizuguchi J, Yoshimoto T (2005) Augmentation of effector CD8+ T cell generation with enhanced granzyme B expression by IL-27. J Immunol 175:1686-1693.

Mueller YM, Makar V, Bojczuk PM, Witek J, Katsikis PD (2003) IL-15 enhances the function and inhibits CD95/Fas-induced apoptosis of human CD4+ and CD8+ effector-memory T cells. Int Immunol 15:49-58.

Murphy CA, Langrish CL, Chen Y, Blumenschein W, McClanahan T, Kastelein RA, Sedgwick JD, Cua DJ (2003) Divergent pro- and antiinflammatory roles for IL-23 and IL-12 in joint autoimmune inflammation. J Exp Med 198:1951-1957.

Nguyen KB, Cousens LP, Doughty LA, Pien GC, Durbin JE, Biron CA (2000) Interferon alpha/beta-mediated inhibition and promotion of interferon gamma: STAT1 resolves a paradox. Nat Immunol 1:70-76.

Niedbala W, Wei XQ, Cai B, Hueber AJ, Leung BP, McInnes IB, Liew FY (2007) IL-35 is a novel cytokine with therapeutic effects against collagen-induced arthritis through the expansion of regulatory T cells and suppression of Th17 cells. Eur J Immunol 37:3021-3029.

Niedbala W, Cai B, Wei X, Patakas A, Leung BP, McInnes IB, Liew FY (2008) Interleukin-27 attenuates collagen-induced arthritis. Ann Rheum Dis.

Nieuwenhuis EE, Neurath MF, Corazza N, Iijima H, Trgovcich J, Wirtz S, Glickman J, Bailey D, Yoshida M, Galle PR, Kronenberg M, Birkenbach M, Blumberg RS (2002) Disruption of T helper 2-immune responses in Epstein-Barr virus-induced gene 3-deficient mice. Proc Natl Acad Sci U S A 99:16951-16956.

O'Dwyer MJ, Mankan AK, White M, Lawless MW, Stordeur P, O'Connell B, Kelleher DP, McManus R, Ryan T (2008) The human response to infection is associated with distinct patterns of interleukin 23 and interleukin 27 expression. Intensive Care Med 34:683-691.

O'Shea JJ, Gadina M, Schreiber RD (2002) Cytokine signaling in 2002: new surprises in the Jak/Stat pathway. Cell 109 Suppl:S121-131.

Oberg HH, Wesch D, Grussel S, Rose-John S, Kabelitz D (2006) Differential expression of CD126 and CD130 mediates different STAT-3 phosphorylation in CD4+CD25- and CD25high regulatory T cells. Int Immunol 18:555-563.

Owaki T, Asakawa M, Fukai F, Mizuguchi J, Yoshimoto T (2006a) IL-27 induces Th1 differentiation via p38 MAPK/T-bet- and intercellular adhesion molecule-1/LFA-1/ERK1/2-dependent pathways. J Immunol 177:7579-7587.

Owaki T, Asakawa M, Kamiya S, Takeda K, Fukai F, Mizuguchi J, Yoshimoto T (2006b) IL-27 suppresses CD28-mediated [correction of medicated] IL-2 production through suppressor of cytokine signaling 3. J Immunol 176:2773-2780.

Owaki T, Asakawa M, Morishima N, Hata K, Fukai F, Matsui M, Mizuguchi J, Yoshimoto T (2005) A role for IL-27 in early regulation of Th1 differentiation. J Immunol 175:2191-2200.

Pearce EL, Mullen AC, Martins GA, Krawczyk CM, Hutchins AS, Zediak VP, Banica M, DiCioccio CB, Gross DA, Mao CA, Shen H, Cereb N, Yang SY, Lindsten T, Rossant J, Hunter CA, Reiner SL (2003) Control of effector CD8+ T cell function by the transcription factor Eomesodermin. Science 302:1041-1043.

Pflanz S, Hibbert L, Mattson J, Rosales R, Vaisberg E, Bazan JF, Phillips JH, McClanahan TK, de Waal Malefyt R, Kastelein RA (2004) WSX-1 and glycoprotein 130 constitute a signal-transducing receptor for IL-27. J Immunol 172:2225-2231.

Pflanz S et al. (2002) IL-27, a heterodimeric cytokine composed of EBI3 and p28 protein, induces proliferation of naive CD4(+) T cells. Immunity 16:779-790.

Pradhan A, Lambert QT, Reuther GW (2007) Transformation of hematopoietic cells and activation of JAK2-V617F by IL-27R, a component of a heterodimeric type I cytokine receptor. Proc Natl Acad Sci U S A 104:18502-18507.

Prlic M, Williams MA, Bevan MJ (2007) Requirements for CD8 T-cell priming, memory generation and maintenance. Curr Opin Immunol 19:315-319.

Ranheim EA, Kipps TJ (1993) Activated T cells induce expression of B7/BB1 on normal or leukemic B cells through a CD40-dependent signal. J Exp Med 177:925-935.

Risueno RM, Schamel WW, Alarcon B (2008) T Cell Receptor Engagement Triggers Its CD3epsilon and CD3zeta Subunits to Adopt a Compact, Locked Conformation. PLoS ONE 3:e1747.

Rival C, Theas MS, Guazzone VA, Lustig L (2006) Interleukin-6 and IL-6 receptor cell expression in testis of rats with autoimmune orchitis. J Reprod Immunol 70:43-58.

Roark CL, French JD, Taylor MA, Bendele AM, Born WK, O'Brien RL (2007) Exacerbation of collagen-induced arthritis by oligoclonal, IL-17-producing gamma delta T cells. J Immunol 179:5576-5583.

Robinson DS (2000) Th-2 cytokines in allergic disease. Br Med Bull 56:956-968.

Rodriguez-Pinto D (2005) B cells as antigen presenting cells. Cell Immunol 238:67-75.

Rodriguez C, Grosgeorge J, Nguyen VC, Gaudray P, Theillet C (1995) Human gp130 transducer chain gene (IL6ST) is localized to chromosome band 5q11 and possesses a pseudogene on chromosome band 17p11. Cytogenet Cell Genet 70:64-67.

Ross JA, Nagy ZS, Cheng H, Stepkowski SM, Kirken RA (2007) Regulation of T cell homeostasis by JAKs and STATs. Arch Immunol Ther Exp (Warsz) 55:231-245.

Roy K, Chaudhuri A (2008) Influence of socioeconomic status, wealth and financial empowerment on gender differences in health and healthcare utilization in later life: evidence from India. Soc Sci Med.

Ruckerl D, Hessmann M, Yoshimoto T, Ehlers S, Holscher C (2006) Alternatively activated macrophages express the IL-27 receptor alpha chain WSX-1. Immunobiology 211:427-436.

Russell JH, Ley TJ (2002) Lymphocyte-mediated cytotoxicity. Annu Rev Immunol 20:323-370.

Salcedo R, Stauffer JK, Lincoln E, Back TC, Hixon JA, Hahn C, Shafer-Weaver K, Malyguine A, Kastelein R, Wigginton JM (2004) IL-27 mediates complete regression of orthotopic primary and metastatic murine neuroblastoma tumors: role for CD8+ T cells. J Immunol 173:7170-7182.

Sallusto F, Geginat J, Lanzavecchia A (2004) Central memory and effector memory T cell subsets: function, generation, and maintenance. Annu Rev Immunol 22:745-763.

Scheller J, Schuster B, Holscher C, Yoshimoto T, Rose-John S (2005) No inhibition of IL-27 signaling by soluble gp130. Biochem Biophys Res Commun 326:724-728.

Schluns KS, Kieper WC, Jameson SC, Lefrancois L (2000) Interleukin-7 mediates the homeostasis of naive and memory CD8 T cells in vivo. Nat Immunol 1:426-432.

Schmidt C, Giese T, Ludwig B, Mueller-Molaian I, Marth T, Zeuzem S, Meuer SC, Stallmach A (2005) Expression of interleukin-12-related cytokine transcripts in inflammatory bowel disease: elevated interleukin-23p19 and interleukin-27p28 in Crohn's disease but not in ulcerative colitis. Inflamm Bowel Dis 11:16-23.

Schnurr M, Toy T, Shin A, Wagner M, Cebon J, Maraskovsky E (2005) Extracellular nucleotide signaling by P2 receptors inhibits IL-12 and enhances IL-23 expression in human dendritic cells: a novel role for the cAMP pathway. Blood 105:1582-1589.

Seddon B, Tomlinson P, Zamoyska R (2003) Interleukin 7 and T cell receptor signals regulate homeostasis of CD4 memory cells. Nat Immunol 4:680-686.

Seder RA, Ahmed R (2003) Similarities and differences in CD4+ and CD8+ effector and memory T cell generation. Nat Immunol 4:835-842.

Seder RA, Darrah PA, Roederer M (2008) T-cell quality in memory and protection: implications for vaccine design. Nat Rev Immunol 8:247-258.

Shainheit MG, Saraceno R, Bazzone LE, Rutitzky LI, Stadecker MJ (2007) Disruption of interleukin-27 signaling results in impaired gamma interferon

production but does not significantly affect immunopathology in murine schistosome infection. Infect Immun 75:3169-3177.

Shevach EM, Tran DQ, Davidson TS, Andersson J (2008) The critical contribution of TGF-beta to the induction of Foxp3 expression and regulatory T cell function. Eur J Immunol 38:915-917.

Shimizu M, Shimamura M, Owaki T, Asakawa M, Fujita K, Kudo M, Iwakura Y, Takeda Y, Luster AD, Mizuguchi J, Yoshimoto T (2006) Antiangiogenic and antitumor activities of IL-27. J Immunol 176:7317-7324.

Shuai K, Liu B (2003) Regulation of JAK-STAT signalling in the immune system. Nat Rev Immunol 3:900-911.

Skurkovich B, Skurkovich S (2007) Autoimmune diseases are connected with disturbances in cytokine synthesis, and therapy with IFN-gamma blockers is their main pathogenetic treatment. Ann N Y Acad Sci 1109:167-177.

Sonobe Y, Yawata I, Kawanokuchi J, Takeuchi H, Mizuno T, Suzumura A (2005) Production of IL-27 and other IL-12 family cytokines by microglia and their subpopulations. Brain Res 1040:202-207.

Sonoda KH, Yoshimura T, Takeda A, Ishibashi T, Hamano S, Yoshida H (2007) WSX-1 plays a significant role for the initiation of experimental autoimmune uveitis. Int Immunol 19:93-98.

Sprecher CA, Grant FJ, Baumgartner JW, Presnell SR, Schrader SK, Yamagiwa T, Whitmore TE, O'Hara PJ, Foster DF (1998) Cloning and characterization of a novel class I cytokine receptor. Biochem Biophys Res Commun 246:82-90.

Strauss-Ayali D, Conrad SM, Mosser DM (2007) Monocyte subpopulations and their differentiation patterns during infection. J Leukoc Biol 82:244-252.

Stumhofer JS, Silver JS, Laurence A, Porrett PM, Harris TH, Turka LA, Ernst M, Saris CJ, O'Shea JJ, Hunter CA (2007) Interleukins 27 and 6 induce STAT3-mediated T cell production of interleukin 10. Nat Immunol 8:1363-1371.

Stumhofer JS, Laurence A, Wilson EH, Huang E, Tato CM, Johnson LM, Villarino AV, Huang Q, Yoshimura A, Sehy D, Saris CJ, O'Shea JJ, Hennighausen L, Ernst M, Hunter CA (2006) Interleukin 27 negatively regulates the

development of interleukin 17-producing T helper cells during chronic inflammation of the central nervous system. Nat Immunol 7:937-945.

Suto A, Nakajima H, Tokumasa N, Takatori H, Kagami S, Suzuki K, Iwamoto I (2005) Murine plasmacytoid dendritic cells produce IFN-gamma upon IL-4 stimulation. J Immunol 175:5681-5689.

Szabo SJ, Sullivan BM, Peng SL, Glimcher LH (2003) Molecular mechanisms regulating Th1 immune responses. Annu Rev Immunol 21:713-758.

Takeda A, Hamano S, Yamanaka A, Hanada T, Ishibashi T, Mak TW, Yoshimura A, Yoshida H (2003) Cutting edge: role of IL-27/WSX-1 signaling for induction of T-bet through activation of STAT1 during initial Th1 commitment. J Immunol 170:4886-4890.

Tan JT, Ernst B, Kieper WC, LeRoy E, Sprent J, Surh CD (2002) Interleukin (IL)-15 and IL-7 jointly regulate homeostatic proliferation of memory phenotype CD8+ cells but are not required for memory phenotype CD4+ cells. J Exp Med 195:1523-1532.

Tan JT, Dudl E, LeRoy E, Murray R, Sprent J, Weinberg KI, Surh CD (2001) IL-7 is critical for homeostatic proliferation and survival of naive T cells. Proc Natl Acad Sci U S A 98:8732-8737.

Tebbutt NC, Giraud AS, Inglese M, Jenkins B, Waring P, Clay FJ, Malki S, Alderman BM, Grail D, Hollande F, Heath JK, Ernst M (2002) Reciprocal regulation of gastrointestinal homeostasis by SHP2 and STAT-mediated trefoil gene activation in gp130 mutant mice. Nat Med 8:1089-1097.

Tofaris GK, Patterson PH, Jessen KR, Mirsky R (2002) Denervated Schwann cells attract macrophages by secretion of leukemia inhibitory factor (LIF) and monocyte chemoattractant protein-1 in a process regulated by interleukin-6 and LIF. J Neurosci 22:6696-6703.

van Lier RA, ten Berge IJ, Gamadia LE (2003) Human CD8(+) T-cell differentiation in response to viruses. Nat Rev Immunol 3:931-939.

Veckman V, Miettinen M, Pirhonen J, Siren J, Matikainen S, Julkunen I (2004) Streptococcus pyogenes and Lactobacillus rhamnosus differentially induce

maturation and production of Th1-type cytokines and chemokines in human monocyte-derived dendritic cells. J Leukoc Biol 75:764-771.

Veldhoen M, Hocking RJ, Atkins CJ, Locksley RM, Stockinger B (2006) TGFbeta in the context of an inflammatory cytokine milieu supports de novo differentiation of IL-17-producing T cells. Immunity 24:179-189.

Villarino A, Hibbert L, Lieberman L, Wilson E, Mak T, Yoshida H, Kastelein RA, Saris C, Hunter CA (2003) The IL-27R (WSX-1) is required to suppress T cell hyperactivity during infection. Immunity 19:645-655.

Villarino AV, Huang E, Hunter CA (2004) Understanding the pro- and anti-inflammatory properties of IL-27. J Immunol 173:715-720.

Villarino AV, Stumhofer JS, Saris CJ, Kastelein RA, de Sauvage FJ, Hunter CA (2006) IL-27 limits IL-2 production during Th1 differentiation. J Immunol 176:237-247.

Villarino AV, Larkin J, 3rd, Saris CJ, Caton AJ, Lucas S, Wong T, de Sauvage FJ, Hunter CA (2005) Positive and negative regulation of the IL-27 receptor during lymphoid cell activation. J Immunol 174:7684-7691.

Vivien L, Benoist C, Mathis D (2001) T lymphocytes need IL-7 but not IL-4 or IL-6 to survive in vivo. Int Immunol 13:763-768.

Ward SG (1996) CD28: a signalling perspective. Biochem J 318 (Pt 2):361-377.

Werlen G, Palmer E (2002) The T-cell receptor signalosome: a dynamic structure with expanding complexity. Curr Opin Immunol 14:299-305.

Wilson NJ, Boniface K, Chan JR, McKenzie BS, Blumenschein WM, Mattson JD, Basham B, Smith K, Chen T, Morel F, Lecron JC, Kastelein RA, Cua DJ, McClanahan TK, Bowman EP, de Waal Malefyt R (2007) Development, cytokine profile and function of human interleukin 17-producing helper T cells. Nat Immunol 8:950-957.

Wirtz S, Tubbe I, Galle PR, Schild HJ, Birkenbach M, Blumberg RS, Neurath MF (2006) Protection from lethal septic peritonitis by neutralizing the biological function of interleukin 27. J Exp Med 203:1875-1881.

Wirtz S, Becker C, Fantini MC, Nieuwenhuis EE, Tubbe I, Galle PR, Schild HJ, Birkenbach M, Blumberg RS, Neurath MF (2005) EBV-induced gene 3 transcription is induced by TLR signaling in primary dendritic cells via NF-kappa B activation. J Immunol 174:2814-2824.

Wong CK, Lit LC, Tam LS, Li EK, Wong PT, Lam CW (2008) Hyperproduction of IL-23 and IL-17 in patients with systemic lupus erythematosus: Implications for Th17-mediated inflammation in auto-immunity. Clin Immunol.

Wu Y, Xu J, Shinde S, Grewal I, Henderson T, Flavell RA, Liu Y (1995) Rapid induction of a novel costimulatory activity on B cells by CD40 ligand. Curr Biol 5:1303-1311.

Yamada H, Nakashima Y, Okazaki K, Mawatari T, Fukushi JI, Kaibara N, Hori A, Iwamoto Y, Yoshikai Y (2007) Th1 but not Th17 cells predominate in the joints of patients with rheumatoid arthritis. Ann Rheum Dis.

Yamanaka A, Hamano S, Miyazaki Y, Ishii K, Takeda A, Mak TW, Himeno K, Yoshimura A, Yoshida H (2004) Hyperproduction of proinflammatory cytokines by WSX-1-deficient NKT cells in concanavalin A-induced hepatitis. J Immunol 172:3590-3596.

Yang L, Anderson DE, Baecher-Allan C, Hastings WD, Bettelli E, Oukka M, Kuchroo VK, Hafler DA (2008) IL-21 and TGF-beta are required for differentiation of human T(H)17 cells. Nature 454:350-352.

Yoshida H, Hamano S, Senaldi G, Covey T, Faggioni R, Mu S, Xia M, Wakeham AC, Nishina H, Potter J, Saris CJ, Mak TW (2001) WSX-1 is required for the initiation of Th1 responses and resistance to L. major infection. Immunity 15:569-578.

Yoshimoto T, Yoshimoto T, Yasuda K, Mizuguchi J, Nakanishi K (2007) IL-27 suppresses Th2 cell development and Th2 cytokines production from polarized Th2 cells: a novel therapeutic way for Th2-mediated allergic inflammation. J Immunol 179:4415-4423.

Yoshimoto T, Okada K, Morishima N, Kamiya S, Owaki T, Asakawa M, Iwakura Y, Fukai F, Mizuguchi J (2004) Induction of IgG2a class switching in B cells by IL-27. J Immunol 173:2479-2485.

Yoshimura T, Takeda A, Hamano S, Miyazaki Y, Kinjyo I, Ishibashi T, Yoshimura A, Yoshida H (2006) Two-sided roles of IL-27: induction of Th1 differentiation on naive CD4+ T cells versus suppression of proinflammatory cytokine production including IL-23-induced IL-17 on activated CD4+ T cells partially through STAT3-dependent mechanism. J Immunol 177:5377-5385.

Zajonc DM, Kronenberg M (2007) CD1 mediated T cell recognition of glycolipids. Curr Opin Struct Biol 17:521-529.

Zhang X, Sun S, Hwang I, Tough DF, Sprent J (1998) Potent and selective stimulation of memory-phenotype CD8+ T cells in vivo by IL-15. Immunity 8:591-599.

Zitzmann K, Brand S, De Toni EN, Baehs S, Goke B, Meinecke J, Spottl G, Meyer HH, Auernhammer CJ (2007) SOCS1 silencing enhances antitumor activity of type I IFNs by regulating apoptosis in neuroendocrine tumor cells. Cancer Res 67:5025-5032.

APPENDIX I

Supplementary data: TCCR expression by different cell subsets via Western blot

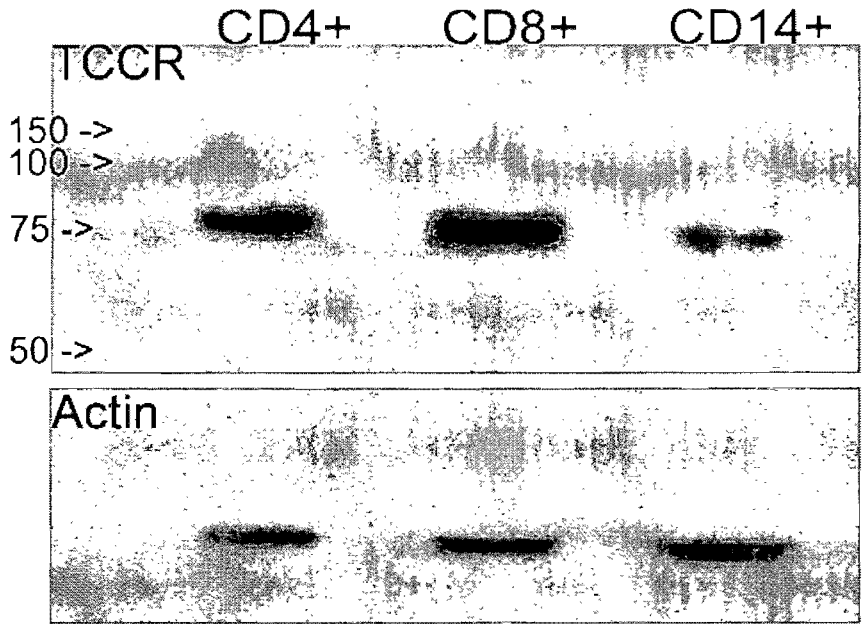


Figure 15. TCCR expression by different cell subsets via Western blot

Purified CD4+ and CD8+ T cells and monocytes (CD14+) were isolated and disrupted for protein extraction, which was consecutively subjected to gel electrophoresis and incubated with polyclonal biotinyalted anti-TCCR goat polyclonal antibody, followed by streptavidin-HRP and then revealed with ECL Plus. Experiments were performed by Diane Beauseigle. Purity of each subset > 98%. Data representative of 3 healthy donors. Numbers indicate position of ladder markers.

Teodora YANEVA

[redacted]
Montreal, [redacted] Quebec
Phone.: [redacted]
e-mail [redacted]

Date of birth: [redacted]

Citizenship: [redacted]

Languages written and spoken: English, French, Bulgarian

Computer Literacy: Word, Power Point, Excel, Internet, Graph Prism, Endnote, FlowJo

Education:

Sept. 2006 – Sept. 2008 ***M.Sc. in Microbiology and Immunology***
University of Montreal
Montreal, Quebec, ***Canada***
Supervisor: D[r]. Nathalie Arbour

Maintained sterile cell cultures, performed MACS cell sorting of human peripheral blood mononuclear cells, RNA and protein extraction, ELISA, Flow Cytometry, real time-PCR, Western blot.

Diploma Thesis Work:

«Effects of Interleukin-27 (IL-27) on human CD8 T cells »

1995 – 2000 ***B.Sc. and M.Sc. in Molecular Biology***
Specialisation: Virology
University of Sofia
Sofia, ***Bulgaria***

2000 ***M.Sc. Research Practical Work***
Referent Laboratory of Immunology
National Centre of Infectious and Parasitic Diseases
Sofia, ***Bulgaria***

Diploma Thesis Work:

«Early changes of CD8+ T cell subsets after antiretroviral therapy of HIV/AIDS patients»

Awards and Fellowships:

2006 – 2007 Neuroinflammation Training Program Fellowship, ***Canada*** (CIHR)
1995-1997 Merit Fellowship, University of Sofia, ***Bulgaria***

Teodora YANEVA
Phone.: [redacted]
e-mail: [redacted]

Posters:

1. Schneider, R., P. Saikali, C. Pittet, T. Yaneva, P. Duquette, and N. Arbour. Roles of Interleukin-15 in MS: enhanced peripheral expression and greater impact on CD8 T cell cytotoxicity. World Congress on Treatment and Research in multiple sclerosis (ACTRIMS + ECTRIMS + LACTRIMS). September 17-20, 2008. Montreal, Canada.
2. Yaneva T., D.Beauseigle, CS Jack, and N. Arbour. Interleukin-27 (IL-27), potentially provided by microglia, enhances human CD8 T cell responses. 21st annual meeting of the Canadian Society of Immunology. Mont-Tremblant, QC, Canada. April 11-14, 2008.
3. Yaneva T., D. Beauseigle, C.S. Jack, and N. Arbour. Interleukin-27 (IL-27), potentially provided by microglia, enhances human CD8 T cell responses. The Canadian endMS Research Conference, Banff, Alberta Canada, December 10-13, 2007.
4. Yaneva T., D. Beauseigle, C.S. Jack, and N. Arbour. Interleukin-27 (IL-27), potentially provided by microglia, enhances human CD8 T cell responses. Retreat Centre d'excellence en Neuromics. January 25 – 26, 2008. Hôtel Le Chantecler - Sainte-Adèle
5. Yaneva T., D. Beauseigle, C.S. Jack, and N. Arbour. Interleukin-27 (IL-27), potentially provided by microglia, enhances human CD8 T cell responses. CRCHUM Research Day. December 18, 2007.

Relevant Experience:

2006 – 2008 ***M.Sc. student***
Laboratory of Neuroimmunology
CHUM, Notre-Dame Hospital
Montreal, Quebec, ***Canada***

2004 – 2006 ***Molecular Biologist***
Department «Biological Products – biological and microbiological control of drugs»
Bulgarian Drug Agency
Sofia, ***Bulgaria***

Performed Limulus Amebocyte Lysate endotoxin test, high performance liquid chromatography of albumins and immunoglobulins, immunodiffusion of vaccines; wrote SOPs, prepared assessment reports upon drugs' documentation.

July 2002 – Sept. 2002 ***Administrative Assistant***
Antisel Selidis Bros
Sofia, ***Bulgaria***

Maintained inventory of chemicals and media; prepared firm's documentation for calls for tenders; correspondence with clients and other distributing firms.

Volunteer Work:

1998 – 1999 ***Volunteer Laboratory Assistant***
Laboratory of Virology
University Paediatric Hospital
Sofia, Bulgaria

Maintained documentation; trained in ELISA; assistance in performance of Coxsakie virus assay.