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Innate and Adaptive Cellular Immunity in Chronic HCV and HIV-1 Infection

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As long as you are convinced of the meaning of your quest
you can conquer fear and exhaustion and take the next step.

Arlene Blum

ABSTRACT

Viral infections are initially countered by an innate immune response as a first line of defence followed by an adaptive immune response. However, certain viruses successfully evade cellular immune responses and establish chronic infection. Hepatitis C virus (HCV) and Human Immunodeficiency Virus 1 (HIV-1) are chronic viral infections on the rise globally. HCV/HIV-1 co-infection presents a formidable challenge to the human immune system. This thesis focuses on certain aspects of innate and adaptive immunity in chronic HCV/HIV-1 infection, and on the implications of aberrant immune responses for peg-IFNα and ribavirin treatment outcome.

NK cells and NKT cells most likely play important roles in protection from HCV and HIV-1 infection, and in the control of chronic infection. Here, HCV/HIV-1 co-infection was associated with a severely reduced NKT cell population that was not restored by HCV treatment. In contrast, conventional NK cells were largely unaffected with only a slight decrease in perforin content in CD56dim cells and an increased CD56bright immunoregulatory NK cell population. Interestingly, sharply elevated numbers of unconventional CD56-CD16+ NK cells, believed to be functionally impaired, accumulated in HCV/HIV-1 co-infected subjects, despite successful ART. A similar trend was seen in HCV mono-infected individuals suggesting a HCV-driven disturbance. CD56- NK cell numbers declined in parallel with HCV load in response to treatment with peg-IFNα and ribavirin. Furthermore, pre-treatment levels of CD56- NK cells correlated with treatment outcome. Patients with low levels of CD56- NK cells were more likely to clear HCV infection, and this was not directly linked to other viral and host factors known to influence treatment outcome.

Evaluation of adaptive immunity in HCV/HIV-1 co-infected subjects revealed a high level of activation, as measured by CD38 expression, in both CD4 and CD8 T cells. However, elevated T cell activation was not linked to altered differentiation and distribution of naïve, T_{CM}, T_{EM} and terminally differentiated cells. Reminiscent of CD56- NK cells, CD38+ T cells declined in response to peg-IFNα and ribavirin treatment. Furthermore, patients reaching a SVR had significantly lower CD8 T cell activation and higher HCV-specific T cell responses prior to treatment, as compared to patients who did not clear infection. Together, the data indicate that chronic HCV infection drives disturbances in both innate and adaptive cellular immunity in HCV/HIV-1 co-infection, which contributes to impaired control and clearance of HCV in this patient group.

Finally, we observed that chronic HCV mono-infection drives expansion of terminally differentiated CD8 T cells that express the Fc-receptor CD16. This population had NK cell-like properties and mediated ADCC towards target cells. This suggests that CD8 T cells in chronic HCV infection continue to differentiate beyond previously described stages of terminal effector cells to acquire NK-like functions. Taken together, the present thesis advances our knowledge of the immune system's relationship with HCV and HIV-1 infection, and identifies immunological biomarkers that correlate with HCV treatment outcome in HCV/HIV-1 co-infected patients.

Keywords: HCV, HIV-1, co-infection, adaptive immunity, innate immunity, NK cell, NKT cell, CD4 T cell, CD8 T cell, IFNα, ribavirin, immune activation.

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J Immunol Methods. 2008 vol. 330 (1-2) pp. 64-74

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Veronica D Gonzalez, Karolin Falconer, Jakob Michaëlsson, Markus Moll, Olle Reichard, Annette Alaeus, Johan K Sandberg.
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III. CD56 negative NK cells predicts pegylated interferon-a and ribavirin treatment outcome in chronic hepatitis C virus infection.
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Manuscript submitted.

V. Elevated numbers of FcγRIIIA+ (CD16+) effector CD8 T cells with NK cell-like function in chronic hepatitis C virus infection.
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LIST OF ABBREVIATIONS

αGalCer	α-galactoceramide
ADC	Analogue to digital signal conversion
ADCC	Antibody-dependent cellular cytotoxicity
AICD	Activation induced cell death
AIDS	Acquired immune deficiency syndrome
ALT	Alanine aminotransferase
APC	Allophycocyanin
APCs	Antigen presenting cells
ART	Antiretroviral treatment
CD	Cluster of differentiation molecules
CTL	Cytotoxic T lymphocyte
CCR	Chemokine (C-C motif) receptor
CMV	Cytomegalo virus
CXCR	Chemokine (X-C motif) receptor
DC	Dendritic cell
DNA	Deoxyribonucleic acid
dsRNA	Double stranded ribonucleic acid
EBV	Epstein-Barr virus
FACS	Fluorescence-activated cell sorting
FITC	Fluorescein isothiocyanate
FSC	Forward scatter
GFP	Green fluorescent protein
HCC	Hepatocellular carcinoma
HCV	Hepatitis C virus
HIV-1	Human immunodeficiency virus 1
HLA	Human leukocyte antigen
HSC	Hematopoietic stem cell
HTLV-III	Human T lymphotropic virus III
IFN	Interferon
IL	Interleukin
iNKR	Inhibitory natural killer cell receptor
IRF	Interferon regulatory factor

ISG	Interferon stimulated gene
IVDU	Intravenous drug use
KIR	Killer-cell immunoglobulin-like receptor
LAV	Lymphadenopathy-associated virus
LCMV	Lymphochoriomeningits viros
LN	Lymph node
LPS	Lipopolysaccharide
LTNP	Long-term non-progressor
MFI	Mean fluorescence intensity
MHC	Major histocompatibility complex
NANBH	Non-A, non-B hepatitis
NCR	Natural cytotoxicity receptor
NK Cell	Natural killer cell
NKG2D	NK group 2, member D
NKp	Natural killer cell protein
NKT Cell	Natural killer T cell
PAMP	Pathogen associated molecular patterns
PE	Phycoerythrin
PBMC	Peripheral blood mononuclear cell
pMHC	Peptide loaded MHC molecule
PMT	Photo multiplier tube
QD	Quantum dot
SSC	Side scatter
ssRNA	Single stranded ribonucleic acid
SVR	Sustained virological response
T_{CM}	Central memory T cell
TCR	T cell receptor
T_E	Effector T cell
T_{EM}	Effector memory T cell
TLR	Toll-like receptor
T_N	Naïve T cells
Treg	T regulatory cell
WHO	World health organization

1 INTRODUCTION

Essentially all living organisms are vulnerable to infectious agents and have through evolution developed defense mechanisms, of various degrees of sophistication. Even simple prokaryotes have the capacity to protect themselves against both infectious agents and environmental competition, with enzymes cleaving foreign DNA and secretion of antibiotics as well as antimicrobial peptides. With the evolution of multi-cellular organism came the gradual development of an innate immune system, later on, an adaptive immune system in higher organisms (1).

Antimicrobial peptides are probably the most ancient among immune defense mechanisms. It is present in plants, insects and mammals, although with varying physical and chemical properties. This suggests a common evolutionary process prior to the divergence of plants and animals. Next in evolution appeared the first immune defense designated receptors, the pattern recognition receptors such as Toll-like receptors (TLRs), and with that the development of phagocytic cells as an additional part of the innate immune system present in invertebrates as well as vertebrates (2, 3). The evolutionary background of the adaptive immune system is not entirely clear and systems allowing diversification of pathogen recognition receptors trough gene-rearrangement is believed to have arisen independently in vertebrates as well as some invertebrates. Subsequent evolution has refined the adaptive immune system and the key components found in all present vertebrates (4, 5).

The human immune system as we know it today reveals a tremendous complexity with the capacity to combat a vast universe of microorganisms, encountered throughout life. An immune response initiates an intricate cascade of events, including a network of cells, signaling molecules and effector mechanisms, crucial for our survival. For better understanding the work related in this thesis I will present basic concepts of innate and adaptive cellular immunity in relation to viral infections in humans.

1.1 INNATE AND ADAPTIVE CELLULAR IMMUNITY

The cellular immune system may be divided into two cooperative branches, known as the innate and adaptive arms of the immune system constituted by a variety of cells commonly termed as leukocytes. The innate cellular immune system constituted by granulocytes, macrophages, dendritic cells (DC), Natural Killer (NK) cells, survey the body and act as a first line of defense with immediate and early responses within hours of infection. The innate immune system acts in a non-specific manner recognizing general pathogen associated molecular patterns (PAMPs) and cellular stress signals indicating infection or cellular dysfunction, which enables invading pathogens and aberrant cells to be phagocytosed or killed through direct cytotoxicity.

The adaptive immune system with its B and T lymphocytes is able to act in a pathogen specific manner and establish an immunological memory. The adaptive immune system is activated late during an ongoing infection, complementing the innate immune system clearing pathogens and infected cells. Primary infection by an unknown agent often leads to establishment of an immunological memory, which allows for a rapid response upon a secondary infection by the same agent. Immunological memory can be sustained for several years and is the basis of vaccine development. Cells of the innate and adaptive immune system share features of development, activation as well as function as they synergize and interact in a complex network against immune challenges.

1.1.1 Leukocyte ontogeny

Leukocytes originate from pluripotent hematopoietic stem cells (HSC) in the bone marrow that give rise to common lymphoid and myeloid progenitor cells. The common myeloid progenitors develop into a variety of cells of the innate immune system as well as erythrocytes and platelets. Myeloid progenitor cells develop into granulocyte progenitor in the bone marrow that further develops into different granulocytes and monocytes residing in the blood. Monocytes in turn have the capacity to mature into macrophages and DCs upon entering the tissues (6, 7).

Common lymphoid progenitors can develop into DCs or prolymphoblasts with the capacity to become NK cells or small lymphocytes that further develop into NKT cells, T cells and B cells (Figure 1). T cells and B cells undergo selection and maturation in lymphoid organs before entering the blood and lymphatic system where they together with NK cells survey the body and upon pathogen encounter differentiate into effector cells. This thesis will further focus on NK and NKT cells of the innate immune system and CD4 and CD8 T cells of the adaptive cellular immune system in the setting of viral infection.

1.1.2 Natural Killer cells

NK cells develop in the bone marrow from HSC. Although their full maturation pathway remains unclear, it is known that NK cell are derived from the common lymphoid progenitor prior to gene rearrangement which commits cells to the T cell lineage (8, 9), with the requirement of Interleukin (IL) 15 for full differentiation (10, 11).

NK cells are a heterogenic population of granular cells constituting 5-20% of peripheral blood lymphocytes, generally defined as CD3- with heterogeneous expression of CD56 and CD16, a low affinity Fcγ receptor (FcγRIIIA) in humans. The definition of NK cells remains somewhat unclear due to the heterogeneous nature of this population and intra-individual variability in non-NK restricted surface marker expression. However, recent reports suggest

NKp46 as a marker defining NK cells across species (12). However, a small subset of NK cells does not express the receptor while a subset of T cells can be NKp46+ (13, 14).

There are two main subsets of NK cells with differential expression of NK receptors, adhesion molecules, cytokine and chemokine receptors and distinct function (15, 16). The majority of NK cells (≈90%) in the periphery is CD56dim and express high levels of CD16. They have the capacity for direct cytotoxicity and antibody-dependent cellular cytotoxicity (ADCC) (17, 18). The other major population constitute about 10% of NK cells in the periphery, but a majority in secondary lymphoid organs and are readily defined as CD56bright CD16- (Table 1). They have low cytotoxic capacity but abundant cytokine production and function as immunoregulatory cells (19, 20). Recently, an additional CD56- CD16+ NK cell population has been in focus in relation to chronic viral infections. They display a dysfunctional profile with poor cytokine production, low proliferative and cytotoxic capacity (21-25).

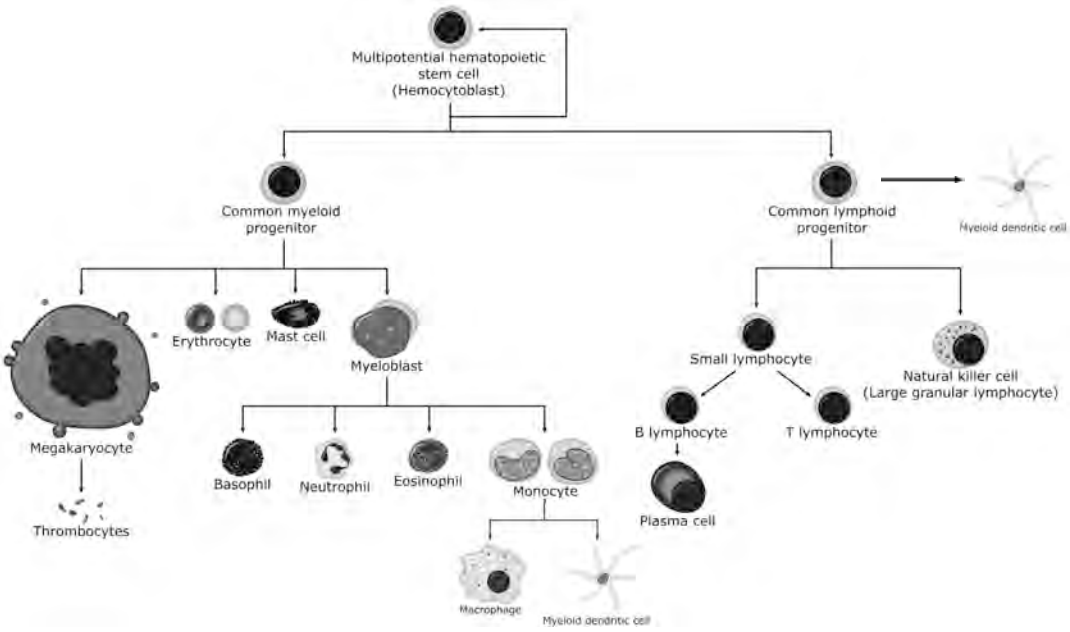

Figure 1. Schematic figure of human hematopoeisis. Development of lymphocytes, granulocutes, macrophages and dendritic cells from lymphoid and myeloid progenitors.

Characteristic	$CD56^{bright}CD16^{-}$	$CD56^{dim}CD16^{+}$	$CD56^{-}CD16^{+}$
Main location	LN	Blood	Blood
Cytotoxicity	+	+++	+
Perforin	+	+++	++
ADCC	-	+++	+
Cytokine production	+++	++	+
iKIR	+/-	+++	+++
NKG2D	+	+	+
NCR	+++	++	+/-
CD94	++	+	+
CCR7	+++	-	?
CXCR3	++	+	+
CD62L	++	+	+
IL7 receptor	High affinity	Low affinity	?
IL2 receptor	High affinity	Low affinity	?

Table 1. Phenotypic and functional properties of NK cell subsets.

1.1.3 NK cell surveillance and activation

NK cells where discovered more then 30 years ago as lymphocytes with an intrinsic capacity to kill target cells regardless of priming and expression of major histocompatibility complex (MHC) class I molecules (26-28). Since then the “missing-self hypothesis” was postulated by Ljunggren and Kärre (29) suggesting that lack of MHC class I surface expression would render cells susceptible to NK cell mediated killing. Today we now that a vast array of germ-line encoded activating and inhibitory cell-surface receptors regulate NK cell activity in a balanced recognition of self versus non-self in aberrant cells (30-32).

NK cells express two families of inhibitory NK cell receptors (iNKRs) highly specific for MHC class I molecules, the killer-cell immunoglobulin-like receptor (KIR) family and the C-type-lectin family of receptors. Interaction between iNKRs and MHC class I molecules regulates the inhibition of NK-cell activity (30, 33-35). Natural cytotoxicity receptors (NCRs) such as NKp30 (NK-cell protein 30), NKp44 and NKp46 are activating receptors that interact with ligands on target cells (36). Upon NK cell recognition of a target cell lacking MHC class I molecules NCR-ligand interactions will trigger activation of a variety of effector function and cytotoxicity (30, 33). NK group 2, member D (NKG2D) is another activating receptor, which binds endogenous ligands that are upregulated upon infection, and mediate target cell lysis (32, 37-39). Besides for activating and inhibitory receptors NK cells also have several co-receptors that regulate the strength and nature of the response by increasing either cytokine production or cytotoxicity-associated proteins. These include NKp80, CD161, CD96 and 2B4 (30, 33, 40).

Target cell recognition by NK cells occurs in three steps. The first step is missing-self recognition, which is a balance between inhibitory and activating signals. The dominant signal is given by iNKRs recognizing MHC class I molecules regularly expressed by all healthy cells. When cells are lacking self-proteins simultaneous engagement of NCRs overthrows the balance and initiates NK cell activation (39, 41). The second step occurs when NKG2D recognizes stress-induced self-proteins that are upregulated by infected cells or tumor cells, a process generally known as induced self-recognition (30, 33, 37, 39, 40, 42). The third step involves recognition of pathogen-encoded molecules and co-receptor engagement regulating the strength and directing effector function (31, 43-45).

1.1.4 Natural Killer T cells

NKT cells are a highly conserved immunoregulatory T cell subset with some innate characteristics. They carry an invariant T cell receptor (TCR) of α chain variable gene segment 24 (Vα24) and β chain variable gene segment 11 (Vβ11) in humans (46). They recognize self and non-self glycolipids presented by CD1d on DCs and monocytes and may play a role in a variety of immunological settings like autoimmunity, allergies, tumor immunity and antimicrobial immune responses (47).

NKT cells may be further subdivided in phenotypically and functionally distinct populations with the capacity to produce either Th1 or Th2 cytokines (48) with CD4- NKT cells displaying a more effector like phenotype residing in the periphery while CD4+ NKT in general cells seem to be immunoregulatory and circulate the secondary lymphoid organs (48, 49). NKT cells are able to both kill aberrant cells through perforin and granzyme mediated cytotoxicity as well as through Fas ligation, suggested to occur in response to certain bacterial infections (50, 51). Most importantly, NKT cells have the capacity to regulate and activate several other immune cells including DCs, NK cells, T and B cells (reviewed in (52)).

The importance of NKT cell in viral infections is poorly understood although evidence suggests that they play a role in the control of hepatitis B virus as well as cytomegalo virus (CMV) infection (53, 54). They are lost in infection with human immunodeficiency virus-1 (HIV-1) (55-57) and lymphocytic choriomeningitis virus (LCMV) (58). NKT cells have been of interest in immunotherapy both in regards to tumor immunology (59, 60), and in the setting of viral infections (61, 62). However, evidence suggests that they may also be involved in the immunopathogenesis of chronic hepatitis C virus (HCV) infection (63, 64).

1.1.5 Bridging innate and adaptive immunity

Cells of the innate and adaptive immune system interact in response to pathogens, and NKT cells are able to recruit and activate various immune cells. Their diverse function depend on their capacity to produce a broad array of cytokines and chemokines such as IL-2, IL-4, IL-10, IL-17, IL-21, IFNγ, TNFα, GM-CSF, TGFβ, MIP1α/β and RANTES (52). NK cells are capable of direct cytotoxicity but may also induce recruitment of adaptive immune cells as they produce abundant amounts of IFNγ. Recent advances in understanding the crosstalk between NK cells and DCs further underscores the role of NK cells in the initiation of proper adaptive immune responses. In a cooperative manner, immature DCs produce IL-12, IL-15 as well as IFNα/β inducing NK cell proliferation and activation that support immature DCs to mature in response to IFNγ and TNFα released from NK cells resulting in proficient antigen presenting cells (APCs) able to present peptides and provide co-stimulatory signals inducing activation and proliferation of T cells, initiating the adaptive immune cascade (65, 66).

1.1.6 T cell selection and maturation in the thymus

T cell development begins in the bone marrow where HSCs differentiate into lymphoid progenitors that migrate through the blood to the thymus where they mature and diverge into CD4 and CD8 T cells (reviewed in (67)). T cell precursors in the thymus undergo gene-rearrangement as they acquire their surface antigen TCR, composed of two linked heterodimeric polypeptide chains. In general the TCR comprises an α and β chain but a minority of the thymocytes have a TCR made up of a γ and δ chain and leave the thymus without further differentiation. Thymocytes of the αβ lineage have a TCR with a variable region that recognizes antigen. The TCR mediates intracellular signals through a transmembrane domain into a cytoplasmic tail linked to adaptor proteins. Gene-rearrangement of the TCR genes generates a broad range of TCRs with the capacity to recognize a variety of antigens presented by different MHC molecules. To prevent autoimmunity and tolerance induction thymocytes undergo positive and negative selection after becoming CD3+CD4+CD8+. Double positive thymocytes are subjected to positive selection where they encounter complexes of self-peptides presented by self-MHC molecules. If the MHC complex is not recognized, cells undergo apoptosis. In the case of recognition of either MHC class I or class II molecules, survival is mediated followed by CD4 or CD8 T cell commitment and MHC restriction. Too strong recognition also leads to apoptosis in a process called negative selection, and in the end only 1-3 % of all thymocytes survive the selection process to leave the thymus as naïve CD4 or CD8 T cells (68, 69). Naïve T cells circulate through the blood and lymphatic system to lymph nodes (LN) and secondary lymphoid tissues in search for their cognate antigen presented by APCs whereupon they differentiate into effector cells.

1.1.7 Activation and function of CD4 T cells

CD4 T cells are said to be MHC class II restricted because they recognize antigens only when presented by MHC class II molecules expressed by APCs. CD4 T cells are generally called helper T cells and produce a variety of cytokines in response to antigen recognition, directing and regulating immune responses. The nature of the antigen and the co-stimulatory signal encountered leads to activation of distinct CD4 T cell subsets with different cytokine profiles. This generates immune responses to be mediated against intracellular or extracellular pathogens. Early studies showed that CD4 T cells could be of either Th1 or Th2 profile, with Th1 cells producing IFNγ and IL-2 effective against intracellular pathogens as well as CD8 T cell activation and proliferation while Th2 CD4 T cells mainly produced IL-4, IL-5 and IL-10 supporting B cell activation fighting off extracellular pathogens (70-72). In the case of chronic HCV infection CD4 T cells has been reported to be biased towards a Th2 profile most likely facilitating immune evasion due to improper responses (73-75).

During the last decade regulatory CD4 T cells termed Tregs and recently a subset called Th17 have been described as additional CD4 T cell subsets (76-79). Tregs regulate CD4 and CD8 T cell activation, shutting down an immune response, preventing immunopathogenesis. Tregs are believed to be reduced in numbers or functionally impaired in certain disease settings where chronic immune activation is the key disease mediator (80). Tregs are CD4+ and believed to be a direct target of HIV-1 infection (81, 82). This could contribute to chronic activation, which may lead to CD8 T cell exhaustion and impaired functionality (83, 84).

Th17 cells has been a hot topic the last years in relation to autoimmunity and diseases with chronic inflammation (85) as they induce epithelial and stromal cells to produce chemokines recruiting neutrophils to the site of “infection”. This particular subset of CD4 T cells is also believed to play a role in late HIV-1 infection as preferential loss of Th17 cells in the gut leads to increased permeability and spread of commensal bacteria resulting in chronic immune activation and thereby exhausted T cells unable to control viral replication.

1.1.8 CD8 T cell activation and effector function

MHC class I molecules are expressed on the surface of virtually all nucleated cells and present 8-10 amino acid long peptides from a broad range of antigens, amongst others viral peptides, to be recognized by CD8 T cells. CD8 T cells leave the thymus as naïve lymphocytes and circulate in the periphery and LN where they upon activation undergo final maturation, differentiating into effector and memory cells.

Naïve CD8 T cells are primed upon encounter with their cognate antigen presented by APCs in LN. The initiation of an immune response requires two

signals; (i) activation through TCR engagement and antigen recognition and (ii) co-receptor-ligand interaction, mediating survival (86, 87). Engagement of the CD3/TCR complex to peptide loaded MHC (pMHC) molecules on APCs activates CD8 T cells but if they do not receive a secondary signal through co-receptor engagement the cells will become anergic and undergo apoptosis (88). Therefore a variety of co-stimulatory receptors may be found on the surface of CD8 T cells such as CD28, which binds to CD80 or CD86 present on APCs (89). An additional third signal is provided by cytokines from APCs and helper T cells mediating proliferation and "clonal expansion" with further maturation of the CD8 T cells. Upon expansion some CD8 T cells gain effector function becoming cytotoxic T lymphocytes (CTL) able to release granzyme, granulysin and perforin containing granules inducing apoptosis of target cells (90-92). Alternatively CD8 T cells can induce killing through death receptors such as TRAIL and FASL and produce an array of cytokines and chemokines like IFNγ, TNFα/β, IL2 and MIP1α/β, inducing an antiviral state in cells and allowing for recruitment of macrophages, NK cells and B cells (91). During the final stages of an immune response a majority of the CD8 T cells undergo apoptosis while a portion become long-lived memory cells that remain for years in the host providing a more rapid response to antigen upon re-encounter (86, 93). However, many viruses have evolved mechanisms for immunoevasion preventing immune recognition and impairing T cell differentiation thus allowing an infection to become chronic like in the case of CMV, HCV and HIV-1.

1.1.9 CD8 T cell maturation upon antigen recognition

CD8 T cell activation and differentiation is regulated by a vast array of homing molecules, co-stimulatory receptors as well as cytokine and chemokine receptors several models have been proposed to explain the maturation pathways of CD8 T cell responses and lineage relationship between effector and memory subsets, according the phenotype of the cells by different surface receptors. This field of T cell immunity is the core to understanding immune responses and the generation of protection in vaccine development. However, due to the wide heterogeneity of phenotypic markers in use and controversial classifications of different T cell subset has the understanding of T cell differentiation become somewhat confusing.

René van Lier pioneered in the description of phenotypic and functionally distinct T cell subpopulations based on the expression of CD27 and CD45RA describing naïve, memory and effector subsets that are functionally distinct (94). An early model suggested by Sallusto and Lanzavecchia proposed a linear differentiation pathway considering the homing capacity of distinct T cell subset with naïve CD8 T cells (T_N) developing into central memory (T_{CM}), effector memory (T_{EM}) and finally to effector cells (T_E). The linear differentiation pathway allowed the specific populations to be distinguished according to the surface expression of CD62L and CCR7, receptors essential for LN homing as well as CD45RA, a co-stimulatory receptor found in different isoforms on the cell surface depending on the level of activation. Further, the two memory

populations had distinct functions with not only different homing capacity but also different response rate, cytokine profile and ability to mediate killing of target cells (95). In the context of primary and chronic viral infections Appay et al described the differentiation pattern of virus specific CD8 T cells according to expression of CD27 and CD28 defining early, intermediate and late CD8 T cell subset. This study showed that each virus infection is characterized by enrichment of CD8 T cells with different phenotypes in a chronic setting but with similarities in the response during acute infection of both infections that are cleared and that become chronic (96).

Hitherto, several studies have followed, stressing the connection between impaired T cell differentiation and dysfunctional immune responses in relation to chronic viral infections (97-101). One of the first detailed characterizations of CD8 T cell used the combination of CD7, CD27, CD28 and CD45RO, all co-stimulatory receptors, and the homing receptors CCR7 and CD62L. This model further stressed the functional heterogeneity of different memory and effector subsets, addressing the cytokine profile of different CD8 T cell populations. This CD7 based model was also used to study CD8 T cell maturation in relation to untreated HIV-1 infection revealing a correlation between viral load and effector cell accumulation (97, 102).

With the development of polychromatic flow cytometry Mario Roederer (103, 104) initiated the use of high definition phenotyping and several markers relevant for T cell differentiation has since been included. This has resulted in a complex picture of T cell differentiation with a vast heterogeneity of different T cell subsets (Figure 2) (reviewed in (105)). During recent years progress has been done in combining phenotypic and functional properties in the understanding of T cell differentiation generating both cytolytic and cytokine producing subset relevant for vaccine development (106-110).

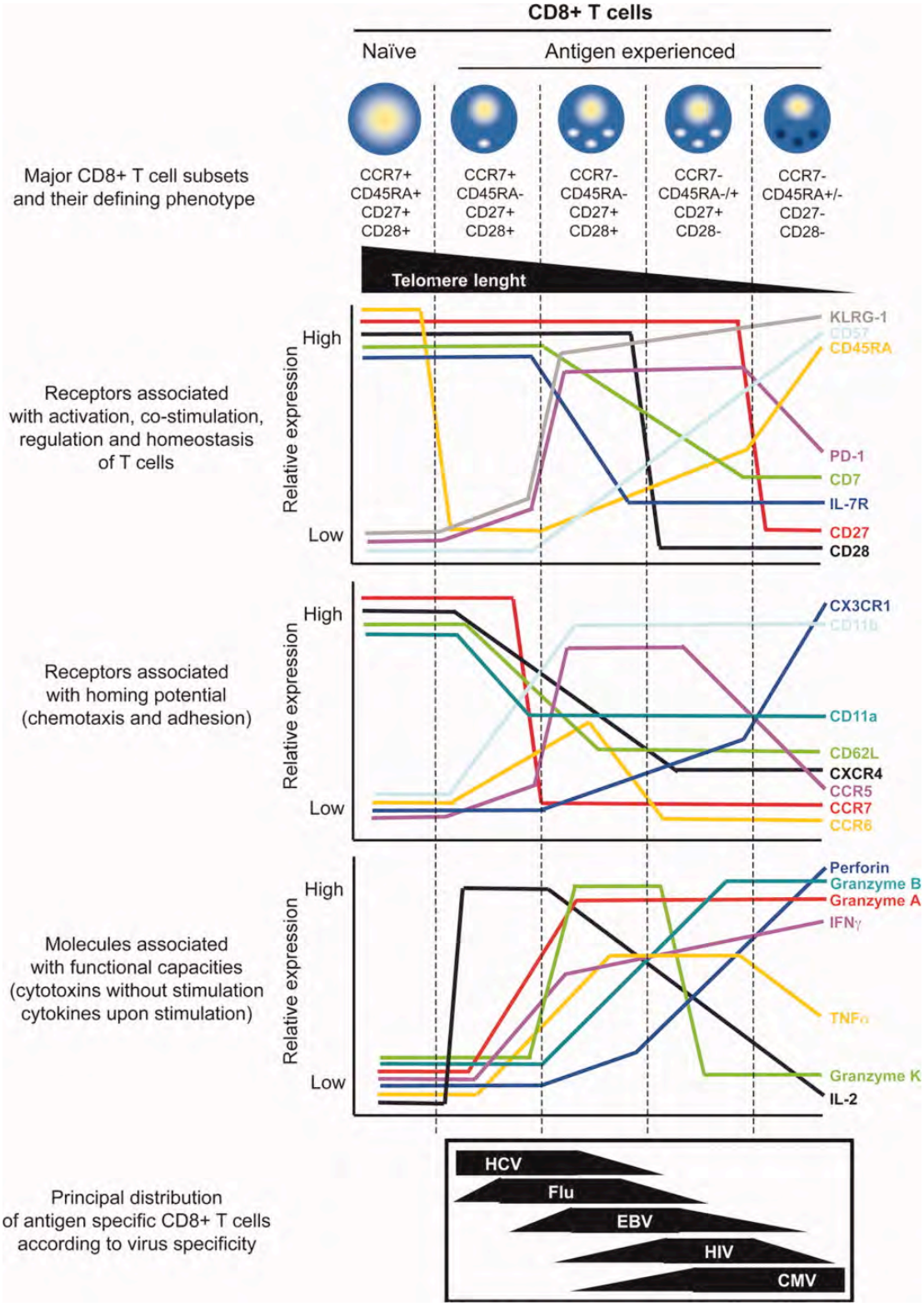

Figure 2. Overview of CD8 T cell maturation in response to antigen. Phenotypic and functional characteristics related to five distinct subsets defined according to the expression of CD27, CD28, CCR7 and CD45RA. Association of expression of a variety of receptors involved in homing, activation, regulation and homeostasis of CD8 T cells as well as maturation pattern during different viral infections. Adapted from (105).

2 VIRAL INFECTIONS - CLEARANCE AND PERSISTENCE

Invading microorganisms such as bacteria, fungi, parasites and viruses constantly challenge the human immune system. Many infections can be cleared by the innate and adaptive immune systems. Sometimes, however, both latent and active chronic infections can establish themselves in the host. Infections that are cleared usually lead to the generation of an immunological memory and a more rapid response upon a secondary encounter. In the case of latent infection there is constant immune activity ongoing, keeping the virus under control. From time to time the virus may re-emerge and a stronger immune response is then required to suppress the infection. Active chronic viral infections may cause disease directly, but also indirectly through chronic immune activation leading to immunopathogenesis. It is a complex interaction between host and viral factors that prevent viral clearance, and while certain viruses always establish latency or chronic infection some may also be cleared. In the following section I will focus on HCV and HIV-1, two viruses that to a large extent cause chronic infection, and their impact on innate and adaptive immune responses.

2.1 HEPATITIS C VIRUS

HCV was initially called non-A, non-B hepatitis (NANBH), as the unknown agent causing post-transfusion hepatitis, until it was renamed upon its discovery in 1989. HCV is a small single stranded positive sense RNA virus belonging to the Flaviviridae family and the hepacivirus genus. The virus genome with around 9600 nucleotides makes up the viral core surrounded by a nucleocapsid, further encapsulated in a cell derived lipid membrane with viral envelope proteins (Figure 3) (111, 112).

HCV has a tropism for hepatocytes in the liver where it undergoes a lytic replication cycle, although the possibility to infect monocytes and lymphocytes has been suggested (113-115). Replication takes place in the cytoplasm leading to translation of a single polyprotein further cleaved to produce three structural and seven non-structural proteins. HCV has a high rate of replication with approximately 10^{12} virion particles produced per day in the infected host. Due to lack of proofreading by the HCV RNA polymerase, HCV also has an exceptionally high mutation rate and therefore a high genetic variability (111, 112).

The HCV species is further classified into six genotypes (1-6), depending on genetic differences with several subtypes within each genotype (represented by letters) and a variation in global distribution of distinct HCV genotypes. Furthermore within every infected individual a variety of quasispecies of the specific subtype can be found and even super-infection with several genotypes is possible (116).

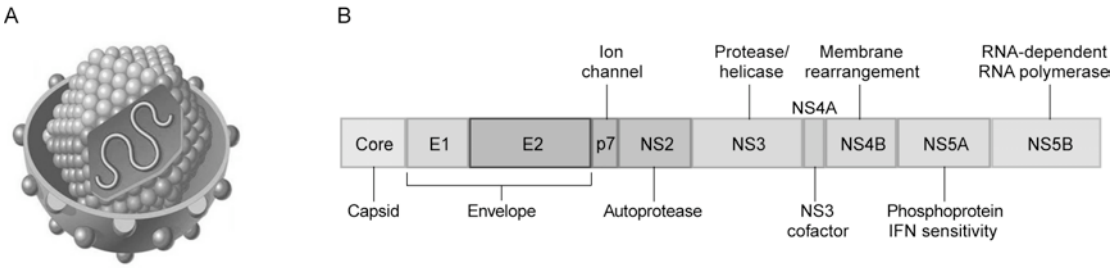

Figure 3. The hepatitis C virus. Schematic picture of a HCV particle containing a +ssRNA in a nucleocapsid surrounded by a cell-derived membrane envelope with glycoproteins (A). The HCV genome encodes a single polyprotein cleaved to yield three structural proteins (Core, E1 and E2) and seven non-structural proteins (p7, NS2, NS3, NS4A, NS4B, NS5A and NS5B) (B). Adapted from (112).

2.1.1 HCV epidemiology

The World Health Organisation (WHO) estimates that approximately 170 million people are infected with HCV worldwide and around 3-4 million are newly infected each year. The prevalence and distribution of HCV genotypes varies throughout the world with the highest incidence in Africa and Asia (Figure 4). Genotypes 4 and 5 are found almost exclusively in Africa with the highest prevalence of HCV infection ranging from 2-9%. Asia follows with a prevalence of 2-5% and besides for genotypes 1, 2 and 3, infection with genotype 6 dominates this geographic region. Australia, Western and Southern Europe as well as North and South America have a prevalence of 1-2% dominated by genotype 1 followed by genotypes 2 and 3 (116, 117). HCV genotype plays a significant role in response to treatment and maybe also in disease progression.

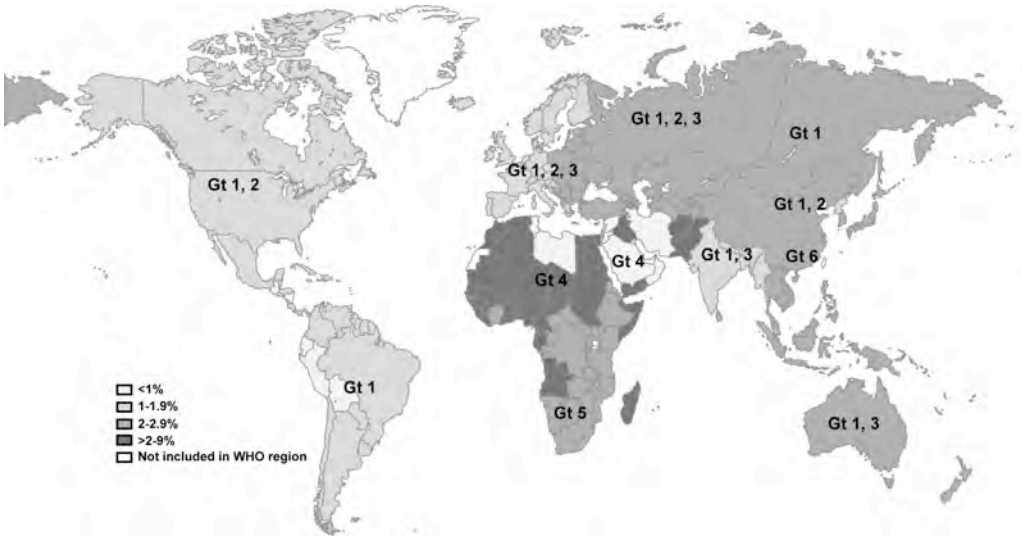

Figure 4. Estimated prevalence of HCV infection by WHO region (2005) and worldwide genotype distribution. Adapted from (116, 117).

2.1.2 HCV transmission and pathogenesis

HCV is a blood-borne virus that can be transmitted sexually, parentally and perinatally. Vertical and sexual transmission is very rare and the most common routes of infection are intravenous drug use (IVDU), through contaminated blood products, and during health care-related procedures. Since routine screening for HCV was implemented in the 1990's, transmission due to infected blood products and organ transplantation has been mostly eliminated in developing countries.

HCV infection can go undetected for decades because disease is often asymptomatic both during its acute and chronic phase despite high viral replication already one week after infection (118). Acute HCV infection is considered the first 6 months after infection and symptoms may be include decreased appetite, fatigue, jaundice and general flu-like symptoms that seldom lead to HCV detection of the causative agent. 15-30% of all individuals may spontaneously clear HCV during this phase which otherwise comes to establish a chronic infection in the liver (119-121).

The natural course of chronic HCV infection ranges from 15-30 years and longer, with chronic inflammation of the liver eventually leading to fibrosis, cirrhosis, and in one third of the cases to hepatocellular carcinoma (HCC). Symptoms specifically suggestive of liver disease are typically absent however HCV infection is a systemic disease and patients may experience a wide spectrum of clinical manifestations and long-term infection may give rise to extrahepatic diseases that can lead to detection of an underlying HCV infection (119-121).

2.1.3 Spontaneous clearance of acute HCV infection and treatment of chronic HCV infection

HCV can be spontaneously cleared by the host immune system. Nevertheless, in 60-80% of the cases a chronic infection is established and treatment is needed. Standard treatment against HCV is a combination of immunomodulatory pegylated IFNα and the antiviral guanosine analogue ribavirin (122). Despite available treatment though, a variety of viral and host factors influences treatment outcome and HCV eradication is only obtained in 35-50% of the cases (123).

Factors that have been reported to influence the rate of HCV disease progression, and in particular treatment outcome, include HCV genotype, high viral load and greater quasispecies diversity (123). In particular genotype 1 and 4 are difficult to treat and recommendations suggest 48 weeks of treatment, while 24 weeks is considered for other genotypes (116). Treatment during the acute phase of infection is also beneficial for a positive outcome, although previous practice was to not treat acute infections in case of spontaneous

clearance. Early treatment may be difficult to achieve since HCV is rarely detected early in non high-risk individuals (124).

Additional host factors influencing treatment are high age, male gender, African American ethnicity, poor uptake of ribavirin and co-morbidities such as alcohol or drug consumption, viral co-infections, obesity and renal disease (125-132). Although pretreatment levels of plasma chemokines can correlate with treatment outcome (133-135), direct cellular immunological correlates of treatment outcome have not been described.

Furthermore, specific human leukocyte antigen (HLA) class I and class II alleles, as well as a strong Th1 cytokine profile, can increase the likelihood to obtain a sustained virological response (SVR) to HCV treatment. Recent reports suggest that a high ribavirin concentration and rapid virological response can be predictive of SVR (132, 136). Sensitivity to IFN and the capacity to further induce interferon-stimulated genes (ISG) has been shown to be of importance for treatment enhanced immune responses and SVR (130, 137, 138).

2.1.4 Innate immune responses in acute and chronic HCV infection

HCV targets hepatocytes in the liver where NKT cells and NK cells are abundant. They are able to mount a first line of defense together with DCs and liver resident macrophages, the so-called Kupffer cells. The first viral components available for immune detection are the HCV core and dsRNA that upon recognition induces a rapid type I IFN response in the cells. Whether the hepatocytes them selves or DCs and Kupffer cells are the first IFN producer upon HCV infection remains unknown (139). However, endogenous IFNα/β induce a cascade of IFN regulatory factors (IRF), leading to up-regulation of ISGs that generates an antiviral states in cells to prevent spread of infection and activating innate immune cells able to kill infected cells (140, 141).

NK cells and NKT cells may contribute to spontaneous eradication of acute HCV infection through IFNγ production and non-cytolytic killing, even in the absence of virus-specific T cells. NKT cells recognize lipid antigens presented by CD1d, which is upregulated on in biliary cells in the liver upon HCV infection, supporting NKT cell activation (142). However, the precise role of NKT cells in HCV infection remains elusive with conflicting data regarding the frequency and function of NKT cell both in liver and blood, as well as their possible involvement in pathogenesis (143-145).

NKT cells are involved in Th1/Th2 skewing of immune responses with different subsets producing Th1 cytokines such as IFNγ, or Th2 cytokines such as IL-4 and IL-13. Reports indicate that already early in HCV infection, NKT cells are biased towards Th2 cytokine production, which in the long run is pro-fibrogenic, contributing to the liver disease seen in advanced HCV infection (146). Although it is unknown why NKT cells are predominantly of Th2 profile in HCV

infection, this may have implications for the induction of appropriate adaptive cellular immune responses and the establishment of chronic HCV. In addition, treatment with peg-IFNa and ribavirin may increase the frequency of non-classical NKT cells (62), and shift NKT responses from Th2 to Th1. This may be relevant for obtaining SVR in response to treatment (147).

NK cells constitute a majority of the lymphocytes in the liver and may contribute to prevent and control HCV infection. *In vitro* studies in a HCV subgenomic replicon system suggested that NK cells inhibit HCV replication in an IFNγ dependent manner, inducing IFNα production, without cytolytic killing of infected cells (147-150). However, several HCV proteins have immunosuppressive activity directed towards NK cells and type I interferon signaling.

HCV protein E2 is suggested to inhibit NK cell signaling through cross-linking of the tetraspannin CD81 (151, 152). Furthermore, indirect inhibition of NK cell activity has been reported to occur through upregulation of MHC class I by hepatocytes and stabilization of HLA-E expression preventing NK cell mediated killing (153-155). In addition HCV downregulates stress related MICA and MICB expression by DCs, preventing self-induced recognition through NKG2D (156, 157). Moreover, HCV protein NS5 has been reported to inhibit IFN induced protein kinase (PKR) (158, 159), blocking type I interferon signaling and IFNα mediated NK cell activation, which is important for cooperative DC stimulation (160). NK-DC interactions are required for effective induction of adaptive immune response. Thus, NK cell dysfunction may lead to improper DC maturation and insufficient T cell priming, which in the long run may facilitate establishment of chronic HCV infection and lack of control of viral replication.

2.1.5 Adaptive cellular immune responses in acute and chronic HCV infection

Adaptive cellular immune responses by CD4 and CD8 T cells are important for immune control of chronic viral infections. HCV is cleared by the host immune system in 15-30% of the cases and spontaneous resolution has been correlated to strong, broad and persistent HCV specific T cell responses during acute infection (161, 162).

T cell responses in HCV infection are often delayed by several weeks after infection even in the case of viral clearance, for reasons yet unknown (163). Virus specific CD4 T cells has been detected in resolved acute HCV and recurrence of viremia was associated with CD4 T cell loss (161, 162, 164). However, detectable CD4 T cell responses do not guarantee HCV resolution, as impaired function with low IFNγ production may prevent clearance (73, 163). Few studies have addressed CD4 T cell responses in the liver during acute infection, although evidence from post liver transplantation further underline their importance in suppressing HCV replication (165-168).

The role of CD4 helper T cells in chronic HCV infection seems to be established already during the acute phase of infection and poor responses do not spontaneously improve. On the contrary broad responses are often lost and virus-specific cells can be found in low frequencies in the blood targeting a few epitomes (164). Moreover, evidence suggests that CD4 T cells isolated from liver in chronic infection predominately produce Th2 cytokines upon HCV antigen stimulation. This may in turn promote viral persistence and liver disease progression (73-75). Further, treatment with peg-IFNα and ribavirin may improve CD4 T cell help through an unknown mechanism, shifting responses towards a Th1 profile after reduced viral replication (128, 169-171).

CD8 T cell responses in acute HCV infection can be associated with at least partial control of viral replication and transient increase of serum alanine transaminase (ALT) levels in liver, probably due to the cytolytic killing of infected cells. CTLs are expanded during acute infection and although presence of virus-specific cells does not necessarily correlate with viral clearance, absence of such cells appears to lead to the establishment chronic infection (172). However, viral clearance may occur without increased ALT levels, suggesting non-cytolytic control of infection (166, 173-175). IFNγ has been suggested as a major contributor to viral clearance after *in vitro* studies revealed IFNγ dependent inhibition of subgenomic HCV replication (148, 176-178). Moreover, a majority of the responding CD8 T cells have a “stunned” phenotype in chronic HCV infection with low IFNγ production (175, 179, 180).

Evidence suggests that there is a prolonged lag period after T cell activation before full effector function is achieved (162), but to what extent this may contribute to establishment of chronic infection is not fully understood. Studies in both humans and chimpanzees stress the importance of broad CD8 T cell responses to achieve viral clearance with failure correlating with fewer targeted epitopes (166, 174, 181, 182). However, additional factors are probably involved because broad immune responses may be seen in chronic infection as well (183-187).

The mechanisms behind impaired CD8 T cell function in chronic HCV are not fully understood. Viral proteins inhibit proper T cell activation and antigen presentation, by blocking of ISGs. Furthermore, immune escape mutations (188-192), incomplete differentiation of CD4 and CD8 T cells to memory and effector cells as well as immune exhaustion influences adaptive immune responses (reviewed in (193)) and disease progression (96, 101, 162, 163, 194-196).

The evidence that both CD4 and CD8 T cell responses are common in spontaneous HCV clearance suggests a cooperative interaction to control viral replication (175, 181, 197). Despite the fact that HCV-specific T cells can be found in persistent infection, their role in controlling viral replication and contribution to the immunopathology is unclear. Although established memory against HCV does not protect against re-infection, pre-existing immunity diminishes the risk for a re-infection to become chronic emphasizing the importance of adaptive immune responses (173, 198-202).

2.2 HUMAN IMMUNODEFICIENCY VIRUS-1

HIV-1 is a lentivirus belonging to the Retroviridae family and the causative agent of acquired immunodeficiency syndrome (AIDS). HIV-1, was first identified by Dr. Luc Montagnier and his group in France 1983 (203) and the finding was further confirmed by Dr. Robert Gallo in USA (204).

HIV-1 is composed of two copies of positive single stranded (ss) RNA enclosed in a capsid, surrounded by a cell-derived phospholipid envelope with embedded viral glycoproteins. The HIV-1 genome consists of 9 genes encoding for 15 viral proteins in total. Gag and env are the two major structural genes, pol encodes for enzymatic proteins and the remaining six tat, rev, nef, vif, vpr and vpu are regulatory genes for proteins involved in infection, replication and immune evasion (Figure 5) (205).

HIV was initially called lymphadenopathy-associated virus (LAV) and later human T-lymphotropic virus-III (HTLV-III) because of its tropism for CD4 T cells (206, 207). However it has the ability to infect a variety of immune cells expressing the receptors used for viral entry, CD4 and the chemokine co-receptors CCR5 or CXCR4 (208, 209).

Viral replication occurs after the viral capsid has been released into the cytoplasm. The viral ssRNA is transcribed, through reverse transcription into double stranded (ds) DNA. It is then transported into the nucleus where it integrates into the host genome where it may lie dormant or be replicated into viral particles in a non-lytic replication cycle. Productive HIV-1 infection can produce up to 10^{10} virions per day, and because the process of reverse transcription is highly error prone, HIV has a high mutation rate and a very high genetic variability (205). Three groups of HIV-1 have been identified on the basis of differences in env; the M, N, and O groups. Group M is the most prevalent and is subdivided into eight subtypes, termed clades, based on the whole genome The different clades vary in their infectiousness, pathogenesis and geographical distribution. Subtype B dominates in North America and Europe. A and D are common in Africa, and D is believed to be more pathogenic. Subtype C is found mainly in Africa and Asia (210).

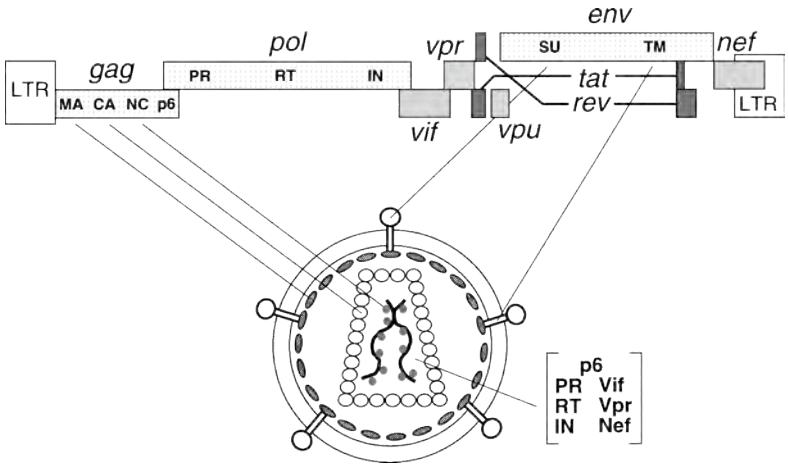

Figure 5. Schematic overview of the HIV-1 virion and genome. The gag gene encodes for the main structural proteins matrix (MS), capsid (CA) and nucleocapsid (NC). Pol encodes for three enzymatic proteins; protease (PR), reverse transcriptase (RT) and integrase (IN). The env gene encodes for the glycoprotein subunits gp 120 (SU) and gp41 (TM). Following six genes encodes for accessory and regulatory proteins; viral infectivity factor (vif), negative effector (nef, p24), viral protein U (vpu), viral protein r (vpr), tat and rev. Adapted from (205).

2.2.1 The HIV-1 pandemic

The HIV-1 we find in humans today originated in non-human primates in sub-Saharan Africa and transferred to humans early in the 20th century. The spread of HIV in the western world is believed to have begun in New York and California, USA, in 1981 where cases of opportunistic infections normally related with severely immuno-compromised patients was observed in a number of young homosexual men (211, 212). Health authorities soon realized that the same opportunistic infections were also reported among hemophiliacs, heterosexual intravenous drug users, and Haitian immigrants (213, 214).

Today HIV-1 is a global epidemic with around 40 million infected worldwide and around 3-6 million newly infected each year, and WHO estimates that around 25 million people have died of HIV and AIDS since its recognition in 1981. Sub-Saharan Africa remains by far the worst affected region, with over 60% of all HIV infected people living in this area. The highest prevalence of HIV-1 is found in South Africa and Nigeria with over 15% of the population being infected. South & South East Asia are the second-worst affected with 5-12 million infected individuals and is together with Eastern Europe the region with the most rapidly increasing incidence of HIV-1. The prevalence of HIV in Europe, North and South America ranges from 0.5-2% of the population and is increasing despite access to information, means of prevention and treatment (215).

2.2.2 HIV-1 transmission and prevention

HIV-1 is transmitted mainly by three routes; sexual, mother-to-child and blood-to-blood contact. The majority of infections are acquired through unprotected heterosexual intercourse when the virus is transmitted over genital, oral, or rectal mucous membranes. Appropriate use of condoms may radically reduce the risk of sexual transmission and during recent years several trials with microbicide gels applied to the female genital tract have been undertaken as an alternative mean of protection. Studies with SIV exposures in microbicide treated monkeys showed some promise but still not sufficiently effective for real life usage (216, 217).

The majority of HIV-1 infected individuals reside in Africa WHO and UNAIDS has investigated the importance of circumcision in the prevention of HIV spread. Recent trials conducted in South Africa, Kenya and Uganda showed promising results with an up to 60% reduced risk for heterosexual transmission (218-220), and WHO has recognized and recommends circumcision as a preventive measure.

Vertical transmission of the virus from mother to child can occur *in utero* during pregnancy, at childbirth and through breast-feeding. However with access to antiretroviral drug treatment (ART) and Caesarean section the risk can be reduced from 25% to less than 1% as long as breast- feeding is not practiced (221). This remains a major problem in poor countries due to problems with social stigma and poor economic means to buy milk powder.

2.2.3 Pathogenesis and disease progression of HIV-1 infection

HIV-1 infection can be divided into three stages of disease; (i) acute infection, (ii) chronic asymptomatic stage and (iii) AIDS, with individual variation in length, symptoms and severity. Upon initial infection with HIV there is a short incubation period before the establishment of primary infection and rapid viral replication during the first 2-4 weeks. The massive viral load is followed by a dramatic loss of CD4 T cell numbers in the gut and later also in blood. The acute viremia may go undetected, but is often associated with general flu-like symptoms such as myalgia, fever, nausea and rash. Even in severe cases with the need for hospitalization HIV may go undetected due to the nonspecific nature of the symptoms (222, 223).

Early CD8+ T cell responses are generally credited for controlling the initial peak of viral replication leading to establishment of a viral set point and partial recovery of CD4 T cells in the periphery (224, 225). During the chronic infection there is a balance act between the immune system establishing strong and broad cellular immune responses and HIV-1 trying to escape through mutations and evasion mechanism, a phase that can last for many years. When CD4+ T cell numbers decline below a critical level, cell-mediated immunity is lost, and infections with a variety of opportunistic microbes appear

with the patient entering the final stage of disease, AIDS (Figure 6). The first symptoms often include moderate and weight loss, recurring respiratory tract infections, common opportunistic infections and tumors eventually leading to death (222, 223).

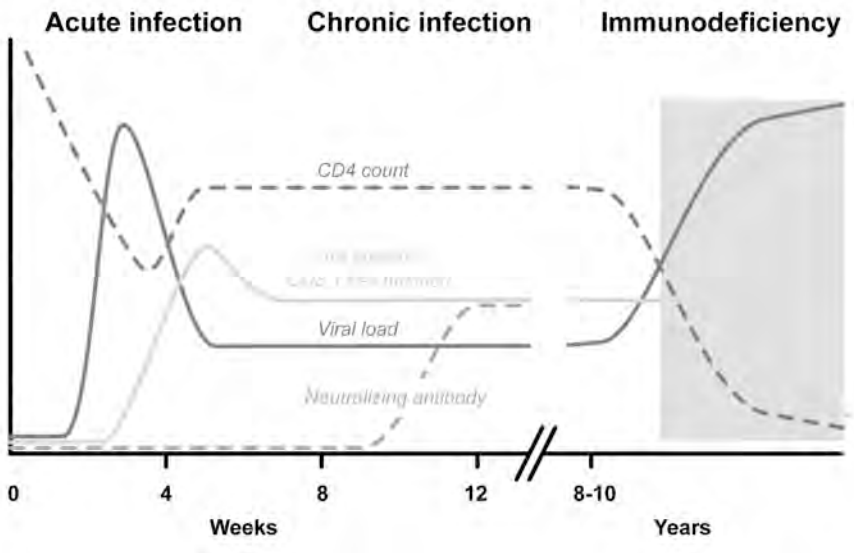

Figure 6. Natural course of HIV-1 infection. The diagram illustrates relationship between HIV-1 viral load, peripheral bloodCD4 count as well as cellular and humoral immune responses during primary, chronic and late stage disease. Figure provide by Dr Aandahl.

2.2.4 HIV-1 infection in the era of antiretroviral treatment

Currently there is no vaccine or cure for HIV, but the development of ART as effective therapy has substantially reduced disease progression and mortality in countries where these drugs are available. ART was introduced 1996 as a combination therapy when non-nucleoside reverse transcriptase inhibitor (NNRTI) and protease inhibitor (PI) were approved as HIV-1 drugs complementing already available nucleoside analogue reverse transcriptase inhibitors (226) (NRTI) (227). Due to sever side-effects and the risk for viral escape mutations leading to drug resistance, ART is usually not initiated until the CD4 count falls below a certain threshold in the range of 200-350 cells/µl, depending on country-specific recommendations (228). Promising new treatments include HIV fusion inhibitors (229) and recombinases able to exert the integrated viral DNA from the human genome (230, 231). However, a lot remains to be done in this field and access of drugs to third world countries must be a priority.

2.2.5 Innate immune responses in primary and chronic HIV-1 infection

NK cells may not only be the first line of defense by targeting and killing infected cells but are most likely also involved in preventing HIV-1 infection

altogether (232-234). NK cells produce large amounts of MIP1α, MIP1β and RANTES, ligands for CCR5. Through competitive inhibition of receptor binding they block viral entry to target cells by R5-tropic viruses *in vitro* as well as *in vivo* (208, 209, 234-238). Further, chemokine mediated blocking of HIV-1 replication has been observed to inversely correlate with HIV-1 viremia (236).

The importance of NK cells in controlling HIV-1 infection is further supported by the finding that host genotype may play a role in control of viral replication and disease progression. Expression of certain HLA-B alleles and specific KIR haplotypes may through epistatic association influence control of HIV viremia and either slows down or promotes disease progression (239-243).

Early *in vitro* studies of HIV-1 infected tumor cell lines showed that NK cells may lyse target cells both directly and through ADCC (244-246). HIV-1 infection down-regulates HLA-A and HLA-B MHC class I molecules on the cell surface of infected cells rendering them susceptible to NK cell mediated killing (247). In spite of this, studies of NK cells targeting HIV-1 infected autologous T cell blast *in vitro* revealed protection from lysis (248, 249). Through selective retention of HLA-C and HLA-E expression in infected cells HIV-1 is able to prevent cell lysis and in the long run even skew the NK cell repertoire towards cells with HLA-C specific iKIRs (247, 250-252).

Although the mechanisms involved in the interaction between NK cells and HIV-1 are somewhat unclear, evidence suggest multiple phenotypic and functional defects in chronic infection (253, 254). Viremic HIV-1 infection may lead to up-regulation of various inhibitory receptors, such as CD94 and LIR1, thereby inhibiting cytotoxicity. Despite that several NCRs and activating receptors (NKG2D, NKp46, 2B4 and NKp80) are maintained in patients with ongoing viral replication some may be downregulated (NKp30 and NKp44) that, in addition to altered iKIR expression further impairs NK cell function (251, 255-261). Furthermore, active HIV-1 replication leads to altered distribution of NK cell subsets with fewer CD56dim cytotoxic NK cells, and accumulation of an irregular CD56-CD16+ NK cell population (21-25). The expansion of this unconventional NK cell population is reverted upon effective ART-mediated suppression of viremia, and so are also many of the phenotypic alterations observed, and this indirectly may lead to restored NK cell function (262-264). Though evidence suggests that NK cells may aid control of HIV-1 replication and disease progression a lot remains to be understood.

2.2.6 Adaptive cellular immune responses in HIV-1 infection

Massive depletion of CD4 T cells occurs very early in the acute phase of HIV-1 infection. The early loss of CD4 T cells is most significant in the gut, and later in infection loss becomes significant also in the periphery and LNs (265, 266). Although CD4 T cell count recovers slightly after the initial loss and the establishment of a viral set point, progressive depletion of CD4 T cells occurs throughout disease (267). The mechanisms behind CD4 T cell depletion are

not fully understood and since primary infection is difficult to study in humans a lot has been learned from monkey models and SIV infection.

HIV-1 infects mainly activated and memory CD4 T cells expressing the viral co-receptor CCR5. Since a majority of the cells in the gut are of memory phenotype this may explain the massive loss at this site (265, 268). High antigen load may lead to activation of a large fraction of cells rendering them susceptible not only to HIV-1 infection, but also to activation induced cell death (AICD). However, T helper cell depletion is not explained solely by death through HIV cytopathicity, or by CTL-mediated killing (269, 270). Enhanced turnover due to chronic immune activation together with reduced thymic output seems to be the main mechanism behind the gradual CD4 T cell depletion (271-275). When more then half of the initial CD4 T cell population is lost the risk of opportunistic infection and tumors is increased. In the late stage of disease, near total loss of CD4 T cells eventually leads to a decline in CTL activity, increased viral replication and progression to AIDS (Figure 6).

Besides for being depleted throughout infection, CD4 T cells also show signs of dysfunction. Chronic antigen exposure leads to skewed CD4 T cell maturation with an accumulation of T_{CM} and T_{EM} populations with reduced proliferative capacity and low IL-2 production (ref Jordan Virology 2006) (276-280). Maintenance of a CD4 T cell population with healthy characteristics has been shown to correlate with lower viral load and slower disease progression together with stronger CTL responses in long-term non-progressors (LTNPs) (281-283). Thus, an early prevention of CD4 T cell loss may be of importance to induce proper CTL responses and prevent viral replication and disease progression.

The appearance of CD8 T cell responses during primary infection is associated with a rapid decline in viremia and the establishment of a viral set point (224, 284). The importance of CTLs in controlling viral replication has been evident in studies of non-human primates were antibody-mediated depletion of CD8 T cells *in vivo,* prior to SIV infection, leads to uncontrolled viremia and rapid disease progression (285, 286). Furthermore, it has been shown that HIV-specific CD8 T cells isolated from infected individuals may inhibit viral replication in autologous CD4 T cells *in vitro* (287).

The central role of CD8 T cells in controlling HIV-1 replication is emphasized by the influence of different HLA types on disease progression. HIV-1 epitopes presented by certain HLA types, such as HLA-B27 and HLA-B57, are associated with slower disease progression and this is likely due to more efficient immune responses. Other HLA types may, on the other hand, increase susceptibility to infection and increase disease progression with narrow CTL responses (288-291). The total HIV-1 specific responses may take up a significant part of the CD8 T cell population and can be maintained throughout infection (292, 293). However, HIV-1 has evolved a variety of mechanisms to evade CTL responses. To prevent CTL recognition, HIV-1 has the ability to down-regulate MHC class I molecules as well as co-receptors necessary to

prevent AICD and anergy upon antigen recognition (247, 294). In addition, narrow and strong CD8 T cell responses often promote to viral escape via mutations parts of the genome coding for viral epitopes (295-297).

HIV-1 does not only evade CTL recognition, but may also functionally impair CD8 T cells through mechanisms still not fully understood (298, 299). Phenotypic and functional characterization of HIV-1 specific CD8 T cells has revealed low perforin and reduced killing as well as skewed maturation (94, 96, 300-302). Recently, late-stage disease has been associated with exhausted CD8 T cells due to systemic immune activation and upregulation of inhibitory receptors like PD-1(303-305). Chronic immune activation of the CD8 T cell population in general in believed to be caused at least in part by microbial translocation from the gut (266, 306, 307).

2.3 HCV/HIV-1 CO-INFECTION

HIV-1 and HCV are two major viral epidemics globally today and HCV/HIV-1 co-infection is becoming increasingly common with 15-30% of all HIV-1 infected individuals estimated to be co-infected with HCV (308, 309). The pathogenesis of this co-infection is poorly understood but differs clearly from the setting of mono-infection. A more rapid disease progression has been documented with increased liver related morbidity and higher HCV RNA levels (310-313). A pre-existing HIV-1 infection also reduces the likelihood of spontaneous HCV clearance and establishment of chronic HCV infection occurs more often than in mono-infection.

HCV responses in blood are generally low and even scarcer in co-infection, probably because of loss of CD4 T cells, which in turn influences the presence of HCV-specific CD8 T cells (314, 315). Several reports indicate that co-infection further impairs T cell-mediated immunity with not only fewer HCV-specific T cells in comparison to HCV mono-infection, but also with skewed cytokine profile, reduced proliferation and capacity of simultaneous cytokine production (316-319).

HCV/HIV-1 co-infection complicates treatment of both infections with the need to avoid drug interactions and severe side effects damaging the liver. Still, although HIV-1 infection is not cured by ART, viral replication can be successfully suppressed and disease progression to AIDS delayed in the setting of co-infection. However, CD4 T cell reconstitution upon ART can be reduced in HCV/HIV-1 co-infected subjects compared to HIV-1 mono-infection (320) and treatment may even lead to increased HCV RNA (321, 322).

3 FLOW CYTOMETRY

Flow cytometry is a widely used technique for counting, examining, and sorting cells and microorganisms while suspended in a stream of fluid. It allows simultaneous multiparametric analysis of both physical and chemical characteristics of single cells and particles.

Flow cytometry was developed from microscopy and Gucker built the very first instrument in 1947 for detection of bacteria in a laminar sheath stream of air (323). Since then, engineers and scientist such as L. Kamentsky, M. Fulwyler and W. Coulter have contributed towards developing the technology as we now it today. The first basic fluorescence activated cell sorter (FACS) was developed in the late 1960s by Bonner, Sweet, Hulett and Herzenberg, amongst others at Stanford University, USA. The first instruments had the possibility to do flow cytometry and cell sorting with one laser and two light detectors, one for forward scatter and one for fluorescence detection. The first commercially available instruments came out 1974 produced by Becton Dickinson Immunocytometry Systems in close collaboration with the Herzenberg group (324, 325). Around the same time Wolfgang Göhde from the University of Münster, Germany developed a fluorescence-based flow cytometry device, later commercially available through the German developer and manufacturer Partec. During the 1970s, FACS and pulse cytophotometry became alternative techniques to fluorescence microscopy, and in 1976 at the Conference of the American Engineering Foundation in Pensacola, Florida, the technique was commonly termed flow cytometry (326).

Early flow cytometers were generally experimental devices used for the early basic studies of lymphocyte subsets (324, 327-331). In time, the deeper understanding of the complexity of the immune system required more advanced machinery with the ability to detect up to three fluorescence parameters to be developed (332, 333). During the late 1990s technique development opened the doors to a veritable explosion in available instrumentation, rapidly increasing the capacity to simultaneously detect 6-12 colors (103, 104, 334-337). Flow cytometry-based assays were developed and with that came the need for new reagents used in analysis (292, 338-342), such as fluorescently labeled antibodies and analysis software (335). Today, modern flow cytometers have multiple lasers with up to 18 fluorescence detectors, and are able to analyze several thousand particles per second in "real time". Sorters can actively separate and isolate particles having specified properties (343, 344).

3.1 PRINCIPLES OF FLOW CYTOMETRY

A flow cytometer is composed of mainly five components (i) a flow cell, (ii) an optical system including lasers and detectors, (iii) a detector and analogue to digital conversion (ADC) system, (iv) an amplification system and (v) a

computer for analysis (Figure 7). The principle behind flow cytometry is that a single cell suspension is hydro-dynamically focused in a stream of fluid which aligns cells to pass in single file trough a flow cell (i) where they are exposed to light beams from lasers of a certain wavelength. Thereby, fluorophore-conjugated antibodies and fluorescent chemicals found in the particle or attached to the particle may be excited into emitting light at a higher wavelength than the light source. A number of detectors, photo multiplier tubes (PMTs), are then aimed at the point where the stream passes through the light beam; one in line with the light beam detecting particle size (Forward Scatter/ FSC) and several perpendicular to it, one measuring granularity and inner complexity (Side Scatter/SSC) and others detecting fluorescence (ii). A combination of filters and mirrors directs light signals reflected by the cells in form of fluorescence signals at different wavelengths to their specific detectors. The detectors take up the light as voltage pulses which in turn are converted by the ADC system (iii) into electrical signals that are amplified (iv) and processed by a computer (v) for analysis. By analyzing fluctuations in brightness at each detector for a specific fluorescence emission peak it is then possible to derive various types of information about each individual particle passing through the flow cell (345).

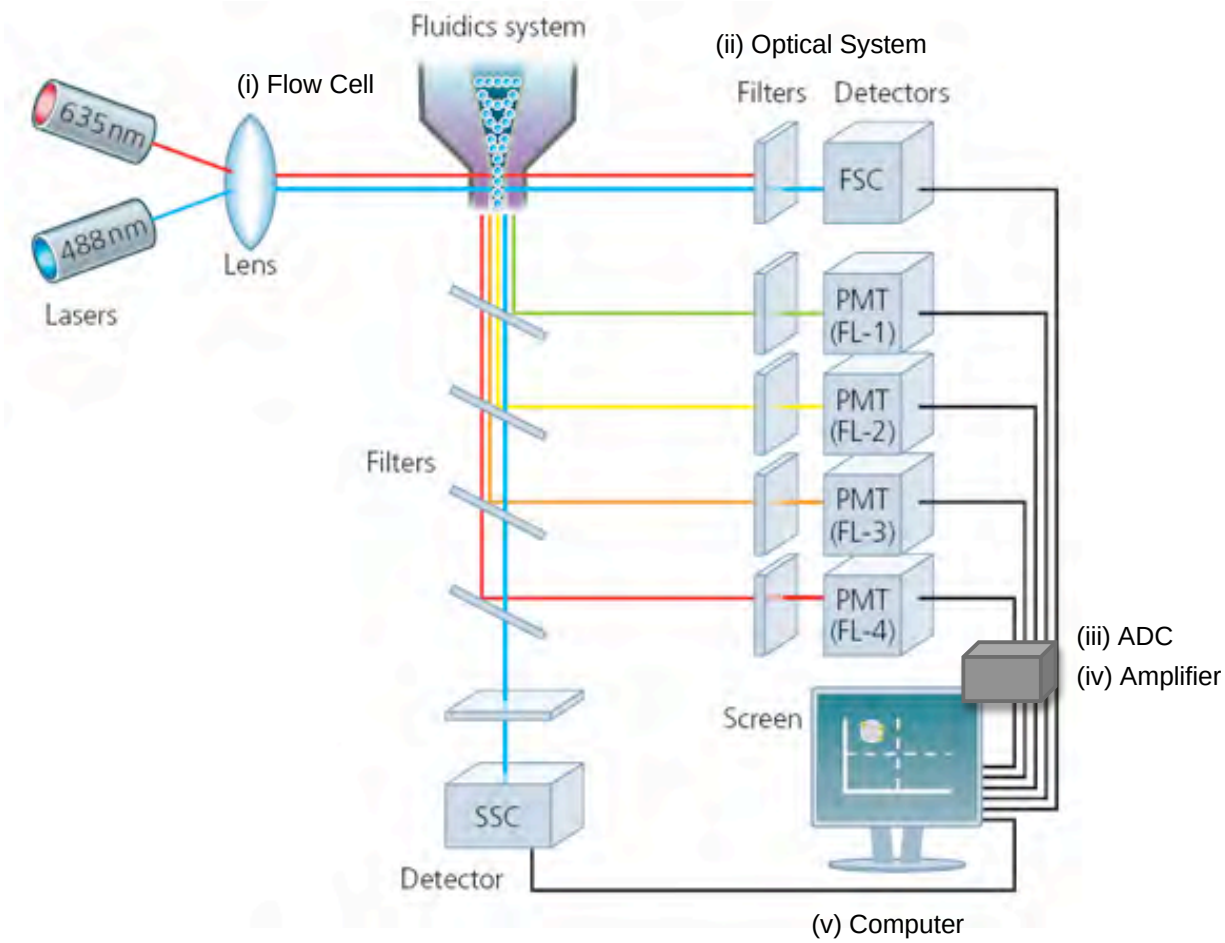

Figure 7. Schematic overview of a flow cytometer. Adapted from (345).

3.1.1 Polychromatic flow cytometry

Flow cytometry requires not only a good instrument but also fluorophore-conjugated antibodies and chemicals to detect cell-associated markers, mostly proteins. The first fluorescent dyes to become available were fluorescein isothiocyanate (FITC) and rhodamine, but with the development of 4-color instruments the need for additional fluorophores became clear. New fluorophores were developed from naturally available fluorescent proteins from seaweed and bacteria such as the phycobilinprotein family with commonly used phycoerythrin (PE) and allophycocyanin (APC), as well as jellyfish derived green fluorescent protein (GFP) (325). However, as the development of flow cytometers rapidly advanced during the 1990s, with improved software, hardware and laser technology the capacity to detect up to 17 colors required new flow cytometry reagents.

Besides for the expansion of new organic dyes, recent years have seen the development of tandem-dyes and more recently in-organic dyes called quantum dots (QDs). Tandem-dyes were developed by coupling cyanins (Cy5, Cy5.5 and Cy7) to common fluorophores such FITC, PE and APC which trough transfer of resonance energy allowed the proteins to be excited by their regular laser but emitting at a longer wavelength (335). However, tandem conjugates are extremely light sensitive and prone to decay, which limits their half-life, and increases the risk of positive detection in the wrong wavelength. QDs on the other hand are semiconductors nanocrystals with narrow emission and broad excitation spectra, which permits for a single laser source to excite a broad range of QDs (346, 347). Therefore, in contrast to organic dyes that have an asymmetric emission, QDs narrow symmetric Gaussian emission profile makes them particularly useful for polychromatic flow cytometry because emission overlap is minimized.

The repertoire of fluorochromes has broadened (Figure 8) giving scientists more options to optimize detection and to implement new applications like phosphospecific flow cytometry were intracellular signaling through phosphorylation is instantly recognized by flurophore-conjugated antibodies (348, 349). Other reagents include epitope-specific HLA tetramers, cell proliferation reagents, amine reactive dyes that allows for exclusion of dead cells even in fixed samples, and reagents for intracellular staining that can be found in a broad range of wavelengths (292, 338-342). The era of polychromatic flow cytometry has allowed the implementation of new applications especially in the field of T cell immunology and vaccine development, were the detection of more than 100 functionally distinct populations has increased our understanding of the complexity of the immune system (104, 343). However, with the advancement of technology, proper execution and analysis has become more complicated requiring expertise knowledge behind fluorchrome selection and combination.

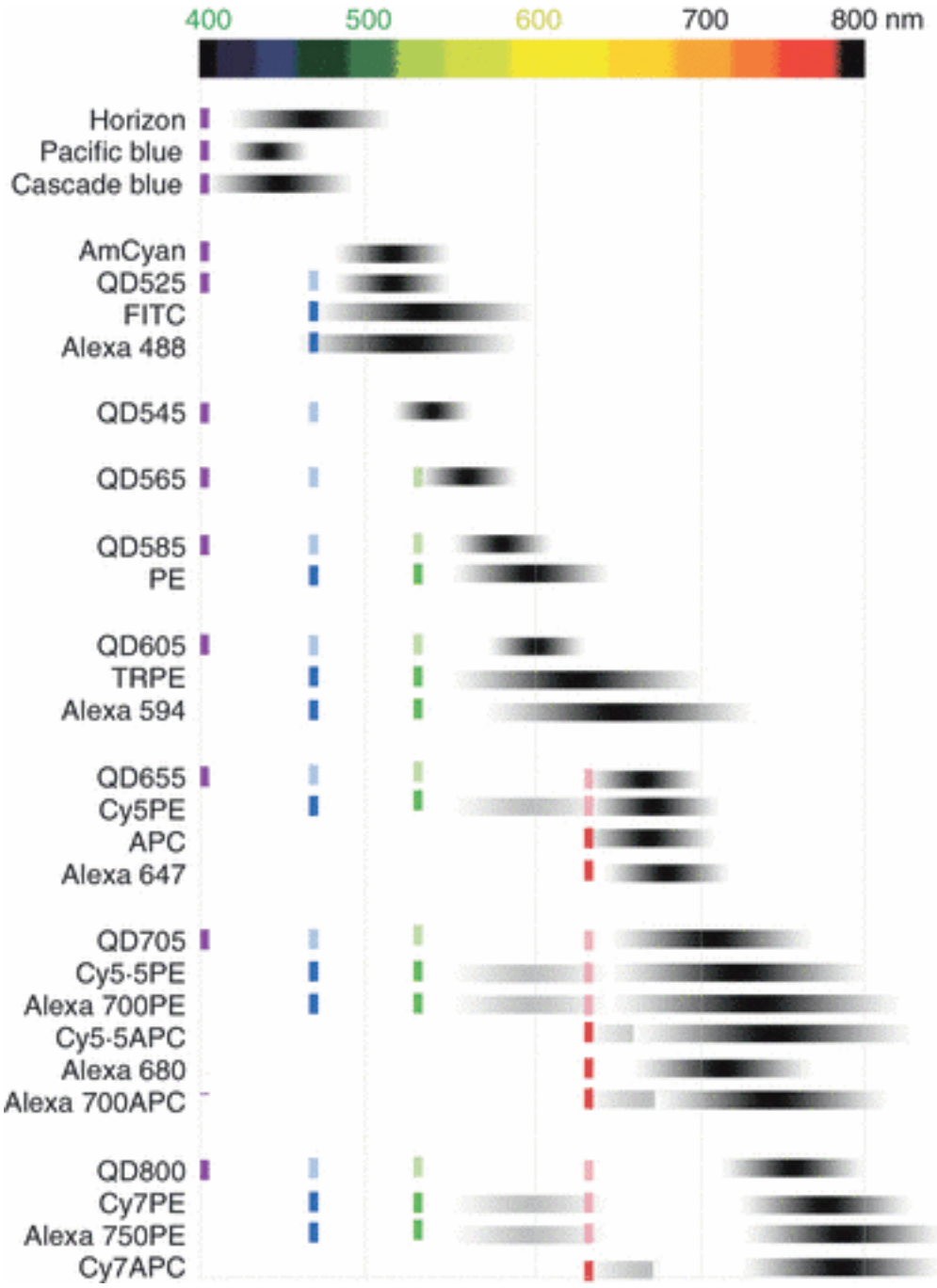

Figure 8. A selection of commonly used fluorchromes in flow cytometry. The optimal laser source for excitation of each fluorochrome is indicated by colored dark lines (Violet = 405nm, blue = 488 nm, green = 532 nm and red = 633 nm) Dim-colored lines indicates suboptimal excitation source of quantum dots. The greyblack bars represent each flurochromes emission range with the darkest area corresponding to peak emission. Adapted from (335).

3.1.2 Compensation and analysis of flow cytometry data

The biggest difficulty with polychromatic flow cytometry has become the spectral overlaps and data analysis. Each fluorochrome will have spectral overlap, contributing to a signal over several detectors. Therefore the contribution in a specific detector that derives from fluorochromes not assigned to that channel must be subtracted in a process called compensation. Compensation is unavoidable despite the use of appropriate filters collecting the maximum amount of light from the primary fluorochrome and as little as possible from nearby emitting fluorophores (335, 350-352).

Regardless of accessible tools, compensation is still probably the least understood process behind proper flow cytometry data analysis and with increasing flow cytometry applications, data analysis is becoming an increasing source of miss-interpretation despite standardization of instruments and assays (353, 354). Compensation was initially done by hardware in between adjacent detectors, prior to signal conversion from analogue to digital, but this proved limiting partly because strong fluorescence signals may spread over more than two detectors indirectly leading over-compensation in subsequent detectors. Modern instruments on the other hand, allows for compensation to occur after data collection. With the help of software, spectral overlap inter- and intra-laser over all detectors is removed mathematically. However, software compensation is limited by the fact that conversion of the electronic signal from linear to logarithmic signal is approximate and thereby sometimes inaccurate, especially for very low signals, and long wavelength signals were signal uptake is low. This may result in visual artifacts upon data display with increased risk of over- and under compensation the more flurochromes are included in the analysis (355).

The data generated by flow cytometers can be plotted in one dimension, to produce a histogram, or in two dimensional dot plots on a 4-5 decade logarithmic scale. Events of low fluorescence may not be properly displayed further complicating analysis of polychromatic flow cytometry data. Recent advances in data display and software development allows for logicle scaling (bio-exponential display) which combines a logarithmic scaling for high fluorescence and a linear scaling for fluorescence signals approaching zero (350, 356, 357). Data display thereby visually becomes more clear and interpretation of data more accurate in the definition of negative and positive populations as every acquired event is visualized and accounted for.

The topic of compensation, data analysis and presentation may take up an entire thesis but due to time constraints and limited space I will leave this topic with the recommendation to read at least two reviews on the subject by Herzenberg (357) and Roederer (351).

4 AIMS OF THE THESIS

The general aim of this thesis was to investigate the impact of chronic infection with HCV and HIV-1 on NK cells and NKT cells of the innate immune system, as well as adaptive cellular immune responses by CD4 and CD8 T cells. Furthermore, the aim was to assess differences in immunity and pathogenesis between mono- and co-infected subjects. The specific objectives were:

To develop and establish polychromatic flow cytometry assays for the study of NK cells, NKT cells, and T cells in human infectious disease research (**paper I**).

To study the impact on NK cells and NKT cells by long-term chronic HCV infection in subjects with and without HIV-1 co-infection, to understand their role in HCV infection as well as in viral clearance upon treatment with peg-IFNα and ribavirin (**paper II and III**).

To investigate CD4 and CD8 T cell responses to HCV in HCV/HIV-1 co-infection in comparison to HCV mono-infection and how they are influenced by peg-IFNα and ribavirin treatment. Furthermore, to assess the relationship between immune responses and level of T cell activation as well as differentiation (**paper IV**).

To determine host innate and adaptive immune correlates predictive of HCV treatment outcome (**paper III and IV**).

To investigate the unique aspects of CD8 T cell effector maturation and function in chronic HCV mono-infection (**paper V**).

5 RESULTS AND DISCUSSION

5.1 POSSIBILITIES AND LIMITATIONS OF FLOW CYTOMETRY IN IMMUNOLOGICAL STUDIES

Polychromatic flow cytometry has increased our understanding of the complexity of the immune system and our knowledge about the vast array of receptors and cytokines involved in regulating cellular immunity and their role in T cell activation and differentiation (104, 343). Recent advances in instrument, software and reagents have allowed the implementation of new applications especially in the field of T cell immunology and vaccine development.

In **paper I** we have exemplified the usefulness of polychromatic flow cytometry in the study of innate and adaptive cellular immune responses. The ability to detect up to nine markers simultaneously is highly advantageous when dealing with limited research material like human biopsies. Figure 1, **paper I**, demonstrates the complex data achieved from high definition phenotypic analysis of virus-specific CD8 T cells. However, creating a complex panel requires thorough thought of the available flurochromes and the markers of interest. Fluorochromes with low emission intensity are not optimal for detection of markers with low expression because you risk a negative result where there should at least some positive detection. Strong emission intensity fluorophores on the other hand are better not used for high expression markers since it increases the risk of off-scale signaling with higher background stain and increased spectral overlap. Nevertheless, a strong signal may be useful to better detect a varying degree of expression since the presence of a marker is not always a yes or no question (358).

Spectral overlap should also be considered when creating a panel and markers that you may wish to analyze against each other should not be stained for with a combination of fluorochromes that requires a high degree of compensation in between them. The reason for this is that an incorrect compensation matrix may result in false double-positive events that really ought to be single positive for either marker. Therefore, fluorochromes with wide emission spectra are best put to use in exclusion channels, to prevent compensation from giving false positive results. Although it may be difficult to exclude the use of fluorophores with wide emission spectra there may be alternatives detected by the same PMT but with less need for compensation like QD 605 (em 580-630) instead of PE-Texas Red (em 580-700) (355). Using proper controls prevents inaccurate compensation, as well as misinterpretation of data. Compensation controls needs to be specific for a certain panel because different antibodies conjugated to the same fluorophore may not always have the same fluorochrome to antibody ratio. Further, the use of single-stained cells is not optimal since compensation then becomes not only based on the intensity of a fluorochrome but also on the expression level of the marker stained for. Therefore, Ig-coated beads should be used not only to save sample but also to create an accurate compensation matrix. Thus, creating a polychromatic flow

cytometry panel requires not only knowledge about your sample and the markers you wish to address but you also need to know your instrument and understand how the qualities of different fluorochromes may influence your analysis. Of course unexpected interactions in between specific antibodies can occur and you may need to try out a variety of combinations before achieving the optimal result.

Polychromatic flow cytometry does also allow for assessment of functional properties in combination with high definition phenotyping of a specific cell population. One application of this approach is the detection of intracellular cytokine production upon stimulation, as shown in **paper I** where NK cell recognition of HLA-E expressing target cells leads to de-granulation and cytokine production (Figure 3, **paper I**). Although this is a commonly used method it has limitations in detection sensitivity and certain cytokines and chemokines are not readily detectable by flow. In addition, the quantity produced can only be measured indirectly by the mean fluorescence intensity (MFI). Besides the practical limitations of flow cytometry you may also face analytical limitations. When looking at rare-cell events like virus-specific T cells (Figure 1, **paper I**) or NKT cell (Figure 2, **paper I**), it may be difficult break down the population of interest into subpopulations when doing high-definition phenotyping. This may be overcome by acquiring many events on your instrument, but only to a certain extent as too many events may crowd your plots and make gating difficult. Although polychromatic flow cytometry is a very powerful in immunological studies one should be aware of the limitations of this methodology and prevent as to the best of your abilities mistakes that might result in faulty data.

5.2 INNATE CELLULAR IMMUNITY IN CHRONIC HCV AND HIV-1 CO-INFECTION

NK cells and NKT cells are important in cell-mediated innate immunity against both HCV and HIV-1. HCV/HIV-1 co-infection is common, but little is known about the impact of this co-infection on NK cells and NKT cells in comparison to mono-infection. In **paper II** we have investigated these two cellular compartments in individuals with chronic HCV, on a background of ART treated HIV-1 infection. Furthermore, we assessed the impact of HCV treatment with peg-IFNα and ribavirin on the innate cellular immune responses.

NKT cells were significantly reduced in co-infected subjects, in comparison to healthy individuals, to a similar extent as in mono-infected control-groups. NKT cell are known to be decreased or lost in chronic HIV-1 infection (55-57). A probable contributing factor to this is that many NKT cells express CD4 and CCR5. Some patients with chronic HIV-1 infection retain almost healthy numbers of NKT cells. We have recently found that these cells display impaired function as assessed by cytokine production and proliferation, and that they express elevated levels of the inhibitory receptor PD-1 (359). It is thus clear that the impact of HIV-1 infection on the CD1d-restricted NKT cells is

multifaceted, with a severe NKT cell loss in many subjects and significant NKT cell dysfunction in subjects who retain relatively normal numbers of these cells.

In HCV infection, conflicting data speak of unchanged, decreased as well as increased numbers of NKT cells (143, 144). Some of these discrepancies however, can possibly be explained by differences in study populations, duration of infection, and the detailed definition of NKT cells. The reduction of NKT cells in co-infection occurred to a similar extent as in mono-infected control groups (Figure 5B and 5C, **paper II**). However, the real difference between mono-infection and co-infection was in the overall presence of NKT cells. A detectable population of NKT cells was more commonly observed in mono-infected control groups than in HCV/HIV-1 co-infection (Figure 5D, **paper II**). Thus, HCV/HIV-1 co-infection might impact NKT cells by various different, and still unknown, mechanisms leading to a strong reduction of this lymphocyte population. Our observations support a HCV-related depletion of both CD4+ and CD4- NKT cells in the periphery. However, because HCV infection is a hepatic disease and a large fraction of NKT cells reside in the liver, detection of NKT cells in the periphery may not tell the entire truth and reduction might at least partially be due to redistribution to organs other than the blood.

Recently, it was shown that NKT cells in primary HIV-1 infection could be restored by combination treatment with IL-2 and ART, but not with ART alone (61). Also, therapy with α-galactoceramide (α-GalCer), a glycosphingolipid derived from a marine sponge, can act as an adjuvant in vaccination trials in mice and increase NKT cell-mediated activation of NK cells and DCs (53, 60, 360, 361). HCV treatment with peg-IFNα and ribavirin does however not lead to restoration of NKT cells in co-infected subjects (Figure 5E and 5F, **paper II**). A similar finding was done by others in HCV mono-infected individuals (146). Permanent loss of NKT cells may hamper NK cell activation, and may in the setting HCV/HIV-1 co-infection contribute to increased susceptibility to opportunistic infections, higher HCV viral load and a more rapid disease progression.

In contrast to NKT cells, conventional CD3-CD56+ NK cell numbers were not significantly altered in HCV/HIV-1 co-infected subjects in comparison to healthy and mono-infected subjects (Figure 1, **paper II**). There was a trend towards altered distribution as well as function of NK cell subsets, with more numerous CD56brightCD16- NK cells and reduced perforin in CD56+CD16+ NK cells. Treatment against HCV did not improve perforin expression, although it did lead to increased numbers of CD56brightCD16- NK cells. CD56bright NK cells have been suggested to be important in early immune responses both in shaping and regulating adaptive immune responses. However, several clinical conditions are associated with increased numbers of CD56bright NK cells (reviewed in (362)). An increased CD56bright population has also seen in a cohort of chronically HCV infected women, but the significance of this finding remains unknown (363). Interestingly, CD56bright NK cells are capable producers of IL-10 in response to monokine stimulation. IL-10 is believed to be involved in the immunoregulatory function of this population (19, 364, 365).

Therefore, more numerous CD56bright NK cells may be a natural way to suppress innate and adaptive cellular immune responses. However, instead of preventing HCV-mediated immunopathogenesis, due to an over active immune system, increased numbers of regulatory NK cells may in the long run impair HCV-specific immune responses (365). This is supported by previously published data suggesting that low IL-10 levels are related to virological response and SVR upon HCV treatment (169, 366, 367). Thus, more remains to be done before the role of CD56bright NK cells in this disease is understood.

5.3 HCV INFECTION DRIVES EXPANSION OF CD56- NK CELLS – CORRELATE WITH IFNα+RIBAVIRIN TREATMENT OUTCOME

In addition to conventional CD3-CD56+ NK cells we have studied a population of CD3-CD56-CD16+ NK cells in **paper II and III**. Accumulation of CD56-CD16+ NK cells with an altered functional profile has been observed in viremic HIV-1 infection (21, 23-25). The expansion of this unconventional NK cell population is reverted upon effective antiviral treatment, suggesting that high levels of viral replication are responsible for driving and maintaining increased numbers of these cells (262-264). However, these cells had not previously been studied in relation to HCV infection.

CD56-CD16+ NK cells were sharply elevated in HCV/HIV-1 co-infected patients in comparison to healthy donors, despite successful ART (Figure 3A and 3B, **paper II**). Data suggested an HCV-driven accumulation of this irregular NK cell population, since a similar pattern was seen in HCV mono-infected subjects although not to the same extent (Figure 3D and 3E, **paper II**; Figure 1B, **paper III**). It thus seems plausible that the CD56- NK cells generated in the presence of high loads of RNA virus may be actively engaged in the antiviral response, and may have down-regulated CD56 in response to recent activation. The rather poor cytolytic function of these cells could in this scenario result from a temporary activation-induced hyporeactivity. Alternatively, the CD56- NK cells expanded during these circumstances may be rendered dysfunctional by a hitherto unknown viral immune evasion mechanism. The differences between mono- and co-infected subjects could at least in part be due to the significantly higher HCV load in subjects with HIV-1 co-infection (Table 1, **paper II**), although no correlation was found between HCV RNA and CD56- NK cell numbers (data not shown).

Treatment with peg-IFNα and ribavirin reduced HCV viremia followed by a decline in CD56-CD16+ NK cell numbers (Figure 3D and 3E, **paper II**; figure 3A, **paper IV**). This was reminiscent of the effect of ART in HIV-1 infection. It seems that it is the HCV viremia in it self that associated with the CD56- NK cells. IFNα did not seem to affect the presence of CD56-CD16+ NK cells per se, as *in vitro* cultures of CD56-CD16+ NK cells showed no upregulation of CD56 surface expression nor increased the susceptibility to apoptosis in CD56- NK cells (Figure 4, **paper II**).

In contrast to HIV-1 infection, chronic HCV infection can be cleared by combination treatment with peg-IFNα and ribavirin. However, HCV eradication is only obtained in 30-80% of the cases. Interestingly, when studying CD56-CD16+ NK cells we could detect a high intra-group variability amongst HCV infected subjects (Figure 1B, **paper III**). We therefore investigated whether this intra-group variability before initiation of HCV therapy could be associated with the variable treatment outcome. When grouping subjects who obtained a SVR and subjects that were either non-responders or response-relapser, it was clear that patients not reaching SVRs had significantly higher numbers of CD56-CD16+ NK cells prior to treatment, in comparison to those reaching SVR (Figure 1C, **paper III**). This suggests that the high intra-group variability could be related to treatment outcome. Next, we divided our two patient groups according to levels of CD56-CD16+ NK cells in the periphery, comparing treatment response rates between high and low levels of these cells. Data showed that patients with low numbers of CD56-CD16+ NK cells had a significantly higher response rate to treatment, in comparison to patients with a more pronounced accumulation of CD56- NK cells (Figure 1D, **paper IV**). Thus, it appears that chronic HCV infection drives a disturbance in the NK cell compartment that can affect disease and influence treatment outcome.

5.4 HIGHLY ELEVATED CHRONIC IMMUNE ACTIVATION IN CD8 T CELLS IN HCV/HIV-1 CO-INFECTED SUBJECTS ON EFFECTIVE ART

Chronic HCV infection is often associated with poor T cell responses with high rate of immune escape mutations, incomplete effector and memory T cell maturation as well as immune exhaustion (96, 101, 162, 163, 194, 196). In **paper IV** we have investigated the cellular adaptive immune responses to HCV, and the state of immune activation, in chronically co-infected patients with a background of ART-treated HIV-1 infection. Furthermore, we assessed how these factors relate to the outcome of HCV treatment with peg-IFNα and ribavirin.

The CD8 T cell compartment was significantly expanded in both relative and absolute terms in both HIV-1 mono-infected subjects and co-infected subjects (Figure 1A and 1B, **paper IV**). However, the massive expansion of CD8 T cells did not affect the overall state of T cell maturation, when this was assessed by defining subsets of naïve, T_{CM}, T_{EM} and terminally differentiated effector cells (Figure 1C, **paper IV**). In contrast to CD8 T cells, the CD4 T cell compartment in HCV/HIV-1 co-infected subjects displayed reduced numbers of central memory cells in comparison to healthy controls and HCV mono-infected subjects (Figure 1F, **paper IV**).

Next we evaluated T cell activation, as measured by CD38 expression in mono- and co-infected subjects. This is of interest because high level of chronic immune activation is a hallmark of chronic HIV-1 infection. Excessive immune activation can result in functional anergy, AICD, and excessive T cell turnover in response to high antigen load (96, 101, 162, 163, 194, 368, 369). In

HIV-1 infection, immune activation has been found to correlate better with disease progression than CD4 T cell count or viral load (370-373). Successful ART with reduced HIV-1 viremia is associated with lower CD38 expression in CD8 T cells (374-376). Reminiscent of chronic HIV-1 infection, chronic HCV infection has been shown to induce high levels of T cell activation and CD38 expression that correlates with HCV viremia (196, 377). Furthermore, HCV/HIV-1 co-infection without ART has been shown to result in an even higher degree of T cell activation than HCV mono-infection (196). We have observed that CD38 expression is strongly elevated in CD8 T cells in HCV/HIV-1 co-infected subjects despite successful ART, compared to both healthy and mono-infected control groups (Figure 2A, **paper IV**). Similar to CD8 T cells, CD4 T cells were highly activated not only in co-infected subjects but also in HCV mono-infected subjects, compared to healthy controls (Figure 2B, **paper IV**). The higher degree of CD38 expression in co-infected subjects compared to HCV mono-infected subjects could possibly be due to a significantly higher HCV load in the co-infected subjects (Figure 3A, **paper IV**). Investigating whether HCV treatment with peg-IFNα and ribavirin, in analogue to ART in HIV-infection, could reduce T cell activation we observed that CD38 expression was reduced in both CD4 and CD8 T cells in parallel with HCV RNA decline (Figure 3, **paper IV**). Thus consistent with previously published data, our data indicate that a high level of T cell activation in co-infected subjects is driven by high antigen exposure.

CD38 expression has been suggested as a marker of treatment efficiency in HIV-1 infection, and sustained viral replication has been associated with consistent T cell activation (reviewed in (378)). Since chronic HCV infection can be cleared by peg-IFNα and ribavirin treatment we set out to investigate whether CD38 expression prior to treatment might correlate with treatment outcome in HCV/HIV-1 co-infected subjects. Although CD38 expression was reduced in both CD4 and CD8 T cells in parallel with HCV RNA decline (Figure 3, **paper IV**), HCV RNA did not correlate with T cell activation. However, patients who did not obtain a SVR had significantly higher pre-treatment levels CD8+CD38+ T cells compared to patients who reached SVR. T cell activation in the latter group was consistently high throughout treatment (Figure 4A, **paper IV**). A similar trend was seen in the CD4 T cell compartment but it did not reach statistical significance (Figure 4B, **paper IV**) despite a direct correlation between CD38 expression in CD4 and CD8 T cells (Figure 2C, **paper IV**). Thus, chronic immune activation seems to be an important component in the pathogenesis of HCV infection, particularly in the context of HIV-1 co-infection. Furthermore, data suggest that reduced antigen exposure may not be sufficient to reverse activation induced T cell exhaustion.

5.5 CAN CD56-NK CELLS AND CD38+ CD8 T CELLS BE CLINICALLY USEFUL BIOMARKERS OF TREATMENT FAILURE?

Cellular markers are used to diagnose disease and follow disease progression in a variety of conditions including HIV-1. Direct cellular immunological markers

have not previously been observed in HCV infection although pretreatment levels of plasma chemokines can correlate with treatment outcome (133-135). Our data on the accumulation of CD56- NK cells and CD38+ CD8 T cells show interesting promise as cellular markers for the prediction of HCV treatment outcome. However, several host and viral factors influence the effectiveness of HCV treatment with peg-IFNα and ribavirin and thereby also treatment outcome.

To address whether high levels of CD56- NK cells were associated with previously described factors influencing treatment outcome, we analyzed possible relationships between some known risk factors and the levels of CD56-CD16+ NK cells in peripheral blood. We observed higher HCV loads in the HCV/HIV-1 co-infected subjects compared to mono-infected subjects, but this difference between the groups with high or low percentages of CD56- NK cells was not statistically significant (Figure 9A). Neither did baseline levels of CD56- NK cells directly correlate with viral load or ALT levels (data not shown). Although the patient cohort did not have an equal distribution of HCV genotypes, the data indicated that CD56- NK cell levels above the threshold were not directly related to a specific viral genotype (Figure 9B). Elderly and male individuals tended to have higher levels of CD56- NK cells, but this difference did not reach statistical significance (Figure 9C and 9D). Thus none of the known risk factors addressed here were directly linked to higher levels of CD56-CD16+ NK cells, suggesting that CD56- NK cells might be an independent biomarker for predicting the outcome of antiviral HCV treatment. However, a degree of association among such predictive factors would be expected in a larger prospective trial, and such a trial would be necessary to test the hypothesis that CD56- NK cell levels can be an independent biomarker for HCV treatment outcome.

The notion that CD38 expression is a relevant marker for treatment outcome is supported by data indicating that a high level of activation of IFNα-inducible genes before the start of IFNα and ribavirin treatment is associated with an inability to respond to IFNα, resulting in poor treatment outcome (137). This suggests a possible link between a high background level immune activation with ongoing IFNα responses in some patients that are unable to control the virus, where adding IFNα+ribavirin treatment has a limited effect. The present study did not allow the assessment of other markers suggestive of activation induced T cell exhaustion like PD-1 and CTLA-4 (303, 379, 380). Furthermore, gender and genotype, factors known to influence HCV treatment could not be properly evaluated in relation to CD38 expression due to biased gender and genotype distribution within the patient group (Table 1, **paper IV**).

Together, the data indicate that chronic HCV infection drives a disturbance in both innate and adaptive cellular immune responses in patients with HCV/HIV-1 co-infection, which contributes to impaired control and clearance of HCV in this patient group. Whether there is a direct link between defective NK cell responses and insufficient T cell responses remains to be understood although we did not find a correlation between accumulation of CD56- NK cells and

CD38 expression in CD8 T cells in the subgroup of patients where both parameter could be evaluated (data not shown). A combined assessment of viral genotype, and T cell activation or CD56- NK cell content may allow identification of patients in need of higher doses and longer treatment and those perhaps most likely to benefit from novel combination regimens including HCV protease inhibitors.

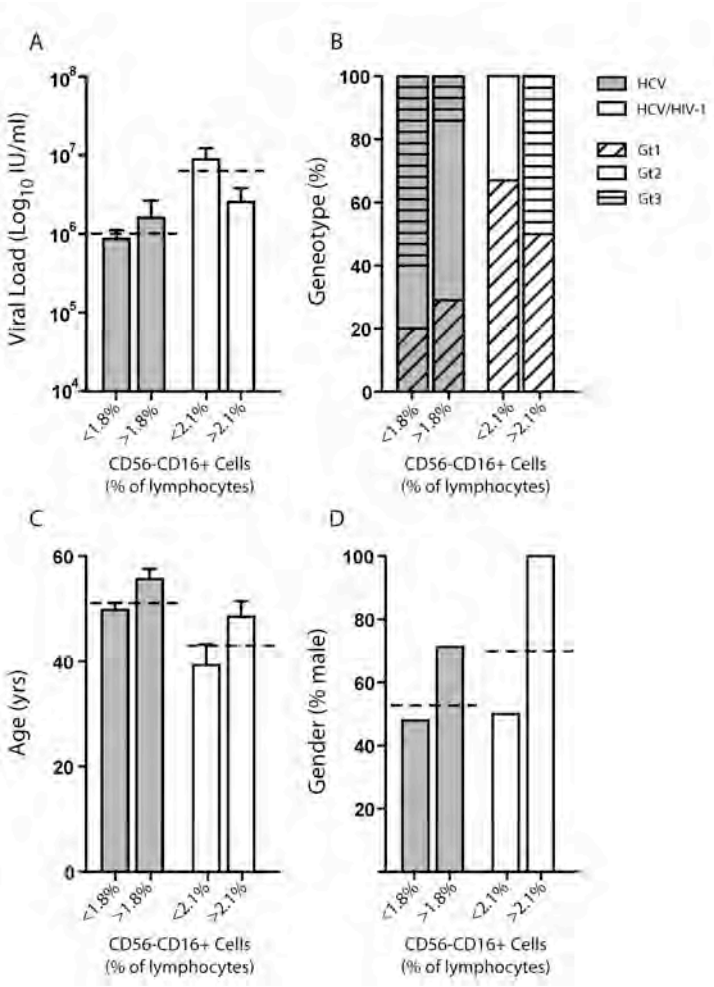

Figure 9. Distribution of known risk factors for failed treatment in subjects with high and low CD56- NK cell percentages. The bar charts represent cumulative data from HCV mono-infected subjects (n = 32) and HCV/HIV-1 co-infected subjects (n = 10) with low or high CD56- NK cell numbers. High numbers of CD56- NK cells indicated for HCV mono-infected subjects in grey, and for HCV/HIV-1 co-infected individuals in white. No significant differences emerged in (A) viral load, (B) genotype, (C) age or, (D) gender distribution between HCV mono-infected or HCV/HIV-1 co-infected subjects with low and high numbers of CD56- NK cells. Dotted line represents mean of viral load, age and gender respectively in the entire group. Statistical analyses performed were Mann-Whitney test (A, C) and Fisher's exact test (D).

5.6 HCV DRIVES THE EMERGENCE OF CD8 T CELLS WITH INNATE-LIKE FUNCTION

Chronic HCV infection is associated with aberrant differentiation of CD8 T cells to memory and effector cells, as well as immune exhaustion influencing

adaptive immune responses in viral clearance (reviewed in (193)) and disease progression (96, 101, 162, 163, 194, 195). Our assessment of CD4 and CD8 T cell differentiation in HCV mono- and HCV/HIV-1 co-infection (Figure 1C and 1D, **paper IV**) did not reveal any major disturbances in the overall distribution of naïve, T_{CM}, T_{EM} and terminally differentiated effector cells. However, in **paper V** we report a HCV-driven accumulation of a CD16+ CD8 T cell population in mono-infected patients.

CD16 expression in T cells has been previously observed, but earlier studies have mainly been restricted to $\gamma\delta$ T cells (381-385). Here, a significantly increased proportion of CD8 $\alpha\beta$ T cells were observed to express CD16 in HCV infected subjects in comparison to healthy controls (Figure 1B, 1C, 1D and 2A, **paper V**). Characterization of this T cell population revealed a terminally differentiated phenotype and a significantly higher content of perforin and granzyme B compared to CD16- T cells (Figure 2B-2E, **paper V**). CD16 is a low affinity Fcγ receptor (FcγRIIIA) best characterized for its role in ADCC mediated by NK cells. Assessment of the functional capacity of CD16 on CD8 T cells displayed a response level similar to NK cells even in the absence of TCR triggering (Figure 5, **paper V**). Genetic transfer of a CD16 receptor gene has *in vitro* shown to increase T cells cytotoxic capacity in tumor directed ADCC (386). Furthermore, a functional CD16+ effector memory CTL population has been observed in relation to Epstein-Barr virus (EBV) infection. Our efforts to assess virus-specificity of the expanded CD16+ CD8 T cells in HCV infection were unsuccessful (data not shown). However, it is likely that these cells are HCV-specific. Alternatively, there may be unique aspects of HCV infection that promotes acquisition of innate-like characteristics in CD8 T cells, perhaps in the local inflammatory hepatic environment.

Expansion of a CD16+ CD8 T cell population may be driven by high antigen exposure. Isolation of CD16- naïve, T_{CM} and T_{EM} cultured in the presence of rIL-2 in vitro showed upon CD16 upregulation in T_{EM} already after 3 days of culture (Figure 2F, **paper V**). Additional data support activation-induced proliferation with a majority of the cells expressing the senescence marker CD57 (Figure 6C, **paper V**). Furthermore, a high level of activation was indicated by CD56 and CD38 expression, with a positive correlation between frequency of CD38+ CD8 T cells and frequency of CD16+ CD8 T cells (Figure 2G and 2H, **paper V**). Interestingly, our unpublished data revealed a similar pattern of expansion of CD16+ CD8 T cells in both untreated and treated HIV-1 infection (Figure 10). Thus, all the present data together suggests that terminal antigen-driven differentiation of effector memory CD8 T cells leads to acquisition of NK cell-like properties. However, further studies are warranted to elucidate the disease relevance of these findings, and the involvement of these CD16+ CD8 T cells in immunopathogenesis and control of viral replication.

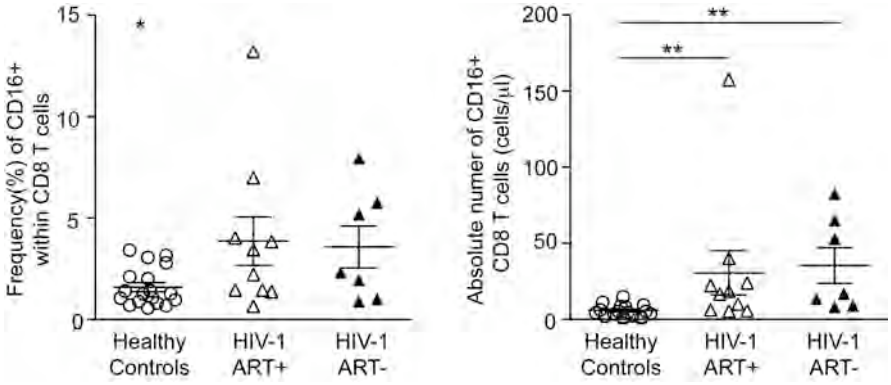

Figure 10. CD16+CD8 T cell expansion in treated and untreated HIV-1 infection. The scatter plots represent cumulative data from healthy controls (n = 18), ART treated HIV-1 infected subjects (n = 10) and untreated HIV-1 infected subjects (n = 7). Statistical analyses performed were one way ANOVA on ranks followed by Dunn's multiple comparison test, where * $p<0.05$ and ** $p<0.01$.

5.7 WHAT IS SO SPECIAL WITH HCV INFECTION ON A BACKGROUND OF ART-TREATED HIV-1 INFECTION?

HCV/HIV-1 co-infection presents a formidable challenge to the human immune system. Whereas the pathogenesis of this co-infection is poorly understood, it differs clearly from the corresponding mono-infections. HCV/HIV-1 co-infection has been documented to promote increased liver related morbidity and higher HCV RNA levels (310-313). A pre-existing HIV-1 infection also reduces the likelihood of spontaneous HCV clearance, and establishment of chronic HCV infection thus occurs more often than in mono-infection.

One possible scenario is that the damage to the immune system caused by HIV leads to impaired immune control of HCV. This then leads to higher HCV loads, that in turn feed TLR-mediated IFNα responses and immunopathology, and the high levels of immune activation observed in co-infected compared to HCV mono-infected subjects (in this study and (196)). A relationship between HIV-1 infection and poor control of HCV infection is supported by data showing a CD4 count dependent presence and magnitude of HCV-specific CD8 T cells (314, 315). Chronic immune activation is a major driver of HIV-1 disease progression (378, 387). However, it is not entirely clear how this works, and what is the driver of activation. One hypothesis is that the destruction of the immune system in the gut already in acute HIV infection leads to leakage of microbial products into the circulation, promoting activation of immunity via innate pattern recognition pathways (306). Plasma lipopolysaccharide (LPS) levels, indicative of microbial translocation, has been observed to be higher in AIDS patients co-infected with HCV compared to HCV negative subjects in a study investigating microbial translocation in relation to monocyte activation and dementia in AIDS patients (388).

The liver plays an important role in LPS clearance (reviewed in (389)). However, chronic HCV infection has been shown to induce lack of TLR

tolerance towards LPS resulting in chronic activation of liver residing monocytes and macrophages. Hyperresponsive monocytes and macrophages in turn lead to higher plasma LPS levels and increased TNFα production promoting persistent liver inflammation (390). Increased immune responses to LPS due to HCV mediated loss of tolerance, together with increased levels of LPS due to microbial translocation indirectly promoted by HIV-1 infection might account for the higher level of activation in HCV/HIV-1 co-infected subjects. This theory is supported by a recent study by Balagopal et al. (391). Thus, HCV related immune defects together with HIV-1 related immune defects might result in synergistic effects leading to a more rapid disease progression and the observed increase in liver-related morbidity in co-infected subjects. This is one of several possible scenarios, keeping in mind that HCV/HIV-1 co-infection causes many alterations in both innate and adaptive immunity as observed in this thesis. Co-infection promotes a combination of synergistic or antagonistic interactions (Figure 11) resulting in a complex disease setting differing from respective mono-infection. Therefore, HCV/HIV-1 infection warrants further studies to improve the basic understanding of these complex diseases, as well as the clinical care of this patient group.

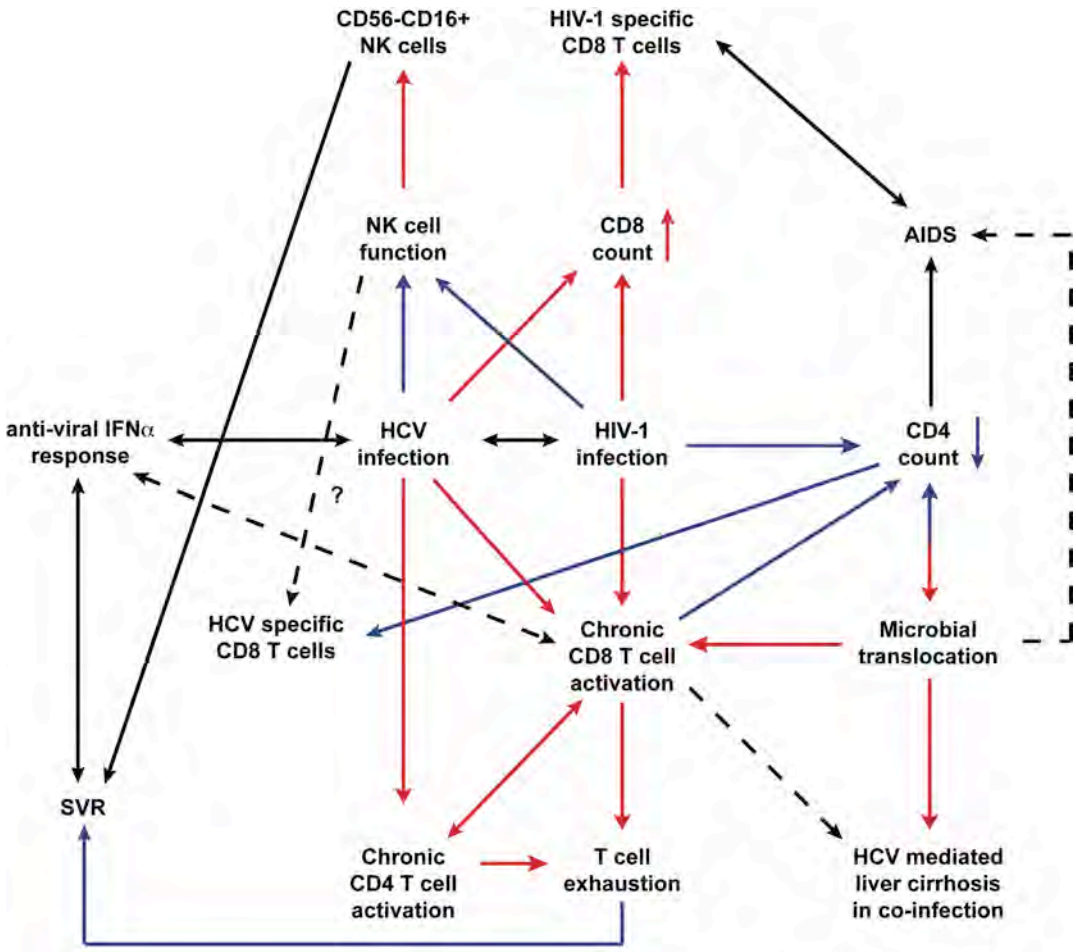

Figure 11. Schematic overview of possible synergistic and antagonistic effects on the immune system by HCV and HIV-1 infection. Red arrows indicate increased action and blue arrows decreased action directly or indirectly caused by HCV or HIV-1 infection. Black arrows indicate an observed connection while dashed arrows indicate possible interactions.

6 CONCLUDING REMARKS

The scientific progress presented in this thesis contributes to increase our understanding of HCV and HIV-1 pathogenesis. It is clear that HCV/HIV-1 co-infection is a very complex condition with a multifaceted pathogenesis, severely altering innate and adaptive cellular immune responses. A common denominator of HIV-1 and HCV is high antigen exposure, which seems to directly and/or indirectly contribute to exhaustion of the immune system and a more rapid disease progression. Still the mechanisms involved in a synergistic disease progression are poorly understood.

Together, the data presented in this thesis indicate that chronic HCV infection drives disturbances in both innate and adaptive cellular immune responses in patients with HCV/HIV-1 co-infection. The elevated levels of chronic immune activation and accumulation of defective NK cells are likely to contribute to impaired control of HCV in this patient group. The expansion of CD16+ CD8 T cells with NK-like functions appears to be linked to the elevated immune activation. The protective or pathogenic roles of this subset remain obscure at this point in time. Several other important questions remain to be answered. What are the key elements in establishment of chronic HCV? Is there a link between defective NK cell responses and insufficient T cell responses? Why are immune activation levels so high in HCV/HIV-1 co-infected patients despite that they are on effective ART?

Future research in this field is strongly needed and could be of high clinical relevance to improve treatment strategies in co-infected subjects. An increased understanding of the synergistic and antagonistic mechanisms involved in HCV/HIV-1 co-infection may aid in preventing and reversing immune defects as well as the immunopathogenesis associated with this co-infection.

7 POPULÄRVETENSKAPLIG SAMMANFATTNING

Världshälsoorganisation WHO uppskattar att 120-170 miljoner individer är HCV infekterade och ca 40 miljoner bär på en kronisk HIV-1 infektion runt om i världen. HIV-1 kännetecknas av det infekterar immunförsvarets CD4 T celler och orsakar en obalans i immunsystemet som med tiden leder till AIDS. Trots över 30 års forskning finns det fortfarande inget botemedel eller vaccin mot HIV-1. Däremot finns det idag bromsmediciner, antiretroviral behandling, som förhindrar omfattande virusreplikation och fördröjer sjukdomsprocessen. HCV ger upphov till en kronisk infektion i upp till 80% av alla infekterade individer. HCV infektion är ofta asymptomatisk men leder i längden till leverrelaterade sjukdomar som kan komma att kräva levertransplantation. HCV infektion kan behandlas med en kombination av IFNα och ribavirin men behandling är endast framgångsrik i ca 50% av fallen. 15-30% av alla HIV-1 infekterade individer uppskattas vara co-infekterade med HCV. HCV/HIV-1 co-infektion ger upphov till komplikationer utöver dem observerade i respektive sjukdomsfall. Co-infektion ger ofta en snabbare sjukdomsprocess och sämre behandlingsresultat. I denna avhandling har vi försökt förstå hur HCV och HIV-1 co-infektion påverkar det medfödda och adaptiva immunförsvaret. Samt hur eventuella förändringar influerar IFNα och ribavirin behandling av HCV infektion.

NK celler utgör en del av det medfödda immunsystemet och är troligen viktiga för att förhindra HCV och HIV-1 infektion samt begränsa virusreplikation i redan infekterade individer. NK celler kan delas upp i flera subpopulationer med olika funktioner. En del NK celler har till uppgift att döda infekterade celler antigen utsöndring av signalämnen eller genom cell-cell kontakt som signalerar till den infekterade cellen att genomgå apoptos, så kallad programmerad celldöd. Andra NK celler är viktiga för att aktivera och stänga av olika delar av immunsystemet genom att släppa ut olika signalämnen. Både HCV och HIV-1 har visats ha negativa effekter på NK celler som leder till bristfällig kontroll av infektionen. Undersökning av NK celler hos HCV/HIV-1 co-infekterade patienter visade att CD56+ NK celler till stor del förblev opåverkade. Däremot observerade vi en abnorm expansion av CD56- NK celler, vilka tidigare har observerats vara expanderade och dysfunktionella i samband med obehandlad HIV-1 infektion med höga virusmängder. Ansamling av CD56- NK celler i samband med co-infektion trots antiretroviral behandling mot HIV-1 tydde på en HCV driven expansion. Detta stöddes av en liknande trend hos HCV mono-infekterade patienter samt observationen att antalet CD56- NK celler reducerades till följd av IFNα och ribavirin behandling parallellt med att HCV mängden minskade. Dessutom fann vi att höga mängder med CD56- NK celler innan behandling korrelerade med sämre chans till utläkning av HCV efter behandling. Detta mönster var till synes oberoende av andra kända faktorer som kan påverka behandling.

I samband med HCV/HIV-1 co-infektion undersökte vi även adaptiva immunresponser från T celler. Dessa celler är en typ av vita blodkroppar som

känner igen och eliminerar virusinfekterade celler. T celler delas in i framförallt två grupper, cytotoxiska CD8 T celler och hjälpar CD4 T celler, som samverkar i försvar mot virusinfektioner. Tidigare forskning visar tydligt att starka T cells svar är nödvändiga för att få bukt med HCV infektion. I och med att HIV-1 infektion leder till förlust av CD4 T celler, får man sämre CD8 T cells svar och därmed svårare att bekämpa infektioner. Detta är framförallt ett problem vid HCV/HIV-1 co-infektion vilket troligen bidrar till extremt hög HCV replikation. Vidare kan hög virus exponering leda till mycket hög kronisk T cells aktivering vilket i sin tur kan resultera i ett utmattat immunsystem, som inte kan kontrollera den pågående infektionen. Vi fann att HCV/HIV-infektion leder till högre kronisk immunaktivering av T celler än mono-infektion. Vidare kunde den höga immunaktiveringen reduceras till följd av IFNα och ribavirin medierad virus suppression. Dessutom visade det sig att hög immunaktivering före behandling korrelerade med sämre svar på behandling samt lägre funktionalitet hos T cellerna. I relation till detta visade studier av HCV mono-infekterade patienter expansion av en population CD8 T celler med NK cells liknande egenskaper. Detta är troligen ytterligare en effekt av hög virusexponering som resulterar i expansion av abnorma populationer. Den fulla betydelsen av dessa förändringar och deras specifika roll i viruskontroll och patogenes återstår dock att fastställa.

Co-infektion är en multifasetterad och komplex utmaning för det humana immunsystemet. Våra resultat visar att kronisk HCV infektion inducerar störningar både i det medfödda och adaptiva immunförsvaret hos patienter med HCV/HIV-1 co-infektion med allvarlig påverkan på både sjukdomsförlopp och behandling. Ytterligare forskning behövs för att förstå de synergistiska och antagonistiska mekanismerna involverade i HCV/HIV-1 co-infektion. Ökad kunskap kring denna co-infektion är också nödvändig och relevant ur ett kliniskt perspektiv för att förbättra behandlingen av denna patientgrupp.

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9 REFERENCES

1. P. Murphy, K., P. Travers, M. Walport, C. Janeway, M. Ehrenstein, and C. Mauri. 2007. Janeway's Immunobiology: 887.
2. Beutler, B. 2004. Innate immunity: an overview. *Mol Immunol* 40:845-859.
3. Kimbrell, D.A., and B. Beutler. 2001. The evolution and genetics of innate immunity. *Nat Rev Genet* 2:256-267.
4. Cooper, M.D., and M.N. Alder. 2006. The evolution of adaptive immune systems. *Cell* 124:815-822.
5. Alder, M.N., I.B. Rogozin, L.M. Iyer, G.V. Glazko, M.D. Cooper, and Z. Pancer. 2005. Diversity and function of adaptive immune receptors in a jawless vertebrate. *Science* 310:1970-1973.
6. Wagers, A.J., J.L. Christensen, and I.L. Weissman. 2002. Cell fate determination from stem cells. *Gene Ther* 9:606-612.
7. Blom, B., and H. Spits. 2006. Development of human lymphoid cells. *Annu Rev Immunol* 24:287-320.
8. Shibuya, A., K. Nagayoshi, K. Nakamura, and H. Nakauchi. 1995. Lymphokine requirement for the generation of natural killer cells from CD34+ hematopoietic progenitor cells. *Blood* 85:3538-3546.
9. Miller, J.S., C. Verfaillie, and P. McGlave. 1992. The generation of human natural killer cells from CD34+/DR- primitive progenitors in long-term bone marrow culture. *Blood* 80:2182-2187.
10. Suzuki, H., G.S. Duncan, H. Takimoto, and T.W. Mak. 1997. Abnormal development of intestinal intraepithelial lymphocytes and peripheral natural killer cells in mice lacking the IL-2 receptor beta chain. *J Exp Med* 185:499-505.
11. Kennedy, M.K., M. Glaccum, S.N. Brown, E.A. Butz, J.L. Viney, M. Embers, N. Matsuki, K. Charrier, L. Sedger, C.R. Willis, K. Brasel, P.J. Morrissey, K. Stocking, J.C. Schuh, S. Joyce, and J.J. Peschon. 2000. Reversible defects in natural killer and memory CD8 T cell lineages in interleukin 15-deficient mice. *J Exp Med* 191:771-780.
12. Walzer, T., S. Jaeger, J. Chaix, and E. Vivier. 2007. Natural killer cells: from CD3(-)NKp46(+) to post-genomics meta-analyses. *Curr Opin Immunol* 19:365-372.
13. Meresse, B., S.A. Curran, C. Ciszewski, G. Orbelyan, M. Setty, G. Bhagat, L. Lee, M. Tretiakova, C. Semrad, E. Kistner, R.J. Winchester, V. Braud, L.L. Lanier, D.E. Geraghty, P.H. Green, S. Guandalini, and B. Jabri. 2006. Reprogramming of CTLs into natural killer-like cells in celiac disease. *J Exp Med* 203:1343-1355.
14. Walzer, T., M. Bléry, J. Chaix, N. Fuseri, L. Chasson, S.H. Robbins, S. Jaeger, P. André, L. Gauthier, L. Daniel, K. Chemin, Y. Morel, M. Dalod, J. Imbert, M. Pierres, A. Moretta, F. Romagné, and E. Vivier. 2007. Identification, activation, and selective in vivo ablation of mouse NK cells via NKp46. *Proc Natl Acad Sci USA* 104:3384-3389.
15. Caligiuri, M.A. 2008. Human natural killer cells. *Blood* 112:461-469.
16. Cooper, M.A., and M.A. Caligiuri. 2004. Isolation and characterization of human natural killer cell subsets. *Current protocols in immunology / edited by John E Coligan [et al]* Chapter 7:Unit 7.34.
17. Lanier, L.L., A.M. Le, C.I. Civin, M.R. Loken, and J.H. Phillips. 1986. The relationship of CD16 (Leu-11) and Leu-19 (NKH-1) antigen expression on human peripheral blood NK cells and cytotoxic T lymphocytes. *J Immunol* 136:4480-4486.
18. Lanier, L.L., A.M. Le, S. Cwirla, N. Federspiel, and J.H. Phillips. 1986. Antigenic, functional, and molecular genetic studies of human natural killer cells and cytotoxic T lymphocytes not restricted by the major histocompatibility complex. *Fed Proc* 45:2823-2828.
19. Cooper, M.A., T.A. Fehniger, S.C. Turner, K.S. Chen, B.A. Ghaheri, T. Ghayur, W.E. Carson, and M.A. Caligiuri. 2001. Human natural killer cells: a unique innate immunoregulatory role for the CD56(bright) subset. *Blood* 97:3146-3151.
20. Cooper, M.A., T.A. Fehniger, and M.A. Caligiuri. 2001. The biology of human natural killer-cell subsets. *Trends Immunol* 22:633-640.
21. Alter, G., N. Teigen, B.T. Davis, M.M. Addo, T.J. Suscovich, M.T. Waring, H. Streeck, M.N. Johnston, K.D. Staller, M.T. Zaman, X.G. Yu, M. Lichterfeld, N. Basgoz, E.S.

Rosenberg, and M. Altfeld. 2005. Sequential deregulation of NK cell subset distribution and function starting in acute HIV-1 infection. *Blood* 106:3366-3369.

22. Tarazona, R., J.G. Casado, O. Delarosa, J. Torre-Cisneros, J.L. Villanueva, B. Sanchez, M.D. Galiani, R. Gonzalez, R. Solana, and J. Peña. 2002. Selective depletion of CD56(dim) NK cell subsets and maintenance of CD56(bright) NK cells in treatment-naive HIV-1-seropositive individuals. *J Clin Immunol* 22:176-183.
23. Søndergaard, S.R., H. Ullum, and B.K. Pedersen. 2000. Proliferative and cytotoxic capabilities of CD16+CD56- and CD16+/-CD56+ natural killer cells. *APMIS* 108:831-837.
24. Hu, P.F., L.E. Hultin, P. Hultin, M.A. Hausner, K. Hirji, A. Jewett, B. Bonavida, R. Detels, and J.V. Giorgi. 1995. Natural killer cell immunodeficiency in HIV disease is manifest by profoundly decreased numbers of CD16+CD56+ cells and expansion of a population of CD16dimCD56- cells with low lytic activity. *J Acquir Immune Defic Syndr Hum Retrovirol* 10:331-340.
25. Mavilio, D., G. Lombardo, J. Benjamin, D. Kim, D. Follman, E. Marcenaro, M.A. O'Shea, A. Kinter, C. Kovacs, A. Moretta, and A.S. Fauci. 2005. Characterization of CD56-/CD16+ natural killer (NK) cells: a highly dysfunctional NK subset expanded in HIV-infected viremic individuals. *Proc Natl Acad Sci USA* 102:2886-2891.
26. Herberman, R.B., M.E. Nunn, H.T. Holden, and D.H. Lavrin. 1975. Natural cytotoxic reactivity of mouse lymphoid cells against syngeneic and allogeneic tumors. II. Characterization of effector cells. *Int J Cancer* 16:230-239.
27. Kiessling, R., E. Klein, and H. Wigzell. 1975. "Natural" killer cells in the mouse. I. Cytotoxic cells with specificity for mouse Moloney leukemia cells. Specificity and distribution according to genotype. *Eur J Immunol* 5:112-117.
28. Kiessling, R., E. Klein, H. Pross, and H. Wigzell. 1975. "Natural" killer cells in the mouse. II. Cytotoxic cells with specificity for mouse Moloney leukemia cells. Characteristics of the killer cell. *Eur J Immunol* 5:117-121.
29. Ljunggren, H.G., and K. Kärre. 1990. In search of the 'missing self': MHC molecules and NK cell recognition. *Immunol Today* 11:237-244.
30. Moretta, A., C. Bottino, M. Vitale, D. Pende, C. Cantoni, M.C. Mingari, R. Biassoni, and L. Moretta. 2001. Activating receptors and coreceptors involved in human natural killer cell-mediated cytolysis. *Annu Rev Immunol* 19:197-223.
31. Bryceson, Y.T., M.E. March, H.G. Ljunggren, and E.O. Long. 2006. Activation, coactivation, and costimulation of resting human natural killer cells. *Immunol Rev* 214:73-91.
32. Cerwenka, A., and L.L. Lanier. 2001. Natural killer cells, viruses and cancer. *Nat Rev Immunol* 1:41-49.
33. Moretta, L., C. Bottino, C. Cantoni, M.C. Mingari, and A. Moretta. 2001. Human natural killer cell function and receptors. *Current opinion in pharmacology* 1:387-391.
34. Colonna, M., F. Navarro, T. Bellón, M. Llano, P. García, J. Samaridis, L. Angman, M. Cella, and M. López-Botet. 1997. A common inhibitory receptor for major histocompatibility complex class I molecules on human lymphoid and myelomonocytic cells. *J Exp Med* 186:1809-1818.
35. Braud, V.M., D.S. Allan, C.A. O'Callaghan, K. Söderström, A. D'Andrea, G.S. Ogg, S. Lazetic, N.T. Young, J.I. Bell, J.H. Phillips, L.L. Lanier, and A.J. McMichael. 1998. HLA-E binds to natural killer cell receptors CD94/NKG2A, B and C. *Nature* 391:795-799.
36. Moretta, A., R. Biassoni, C. Bottino, M.C. Mingari, and L. Moretta. 2000. Natural cytotoxicity receptors that trigger human NK-cell-mediated cytolysis. *Immunol Today* 21:228-234.
37. Ogasawara, K., and L.L. Lanier. 2005. NKG2D in NK and T cell-mediated immunity. *J Clin Immunol* 25:534-540.
38. Lanier, L.L. 2005. NKG2D in innate and adaptive immunity. *Adv Exp Med Biol* 560:51-56.
39. Raulet, D.H. 2004. Interplay of natural killer cells and their receptors with the adaptive immune response. *Nat Immunol* 5:996-1002.
40. Lanier, L.L. 2005. NK cell recognition. *Annu Rev Immunol* 23:225-274.
41. Carena, I., A. Shamshiev, A. Donda, M. Colonna, and G.D. Libero. 1997. Major histocompatibility complex class I molecules modulate activation threshold and early signaling of T cell antigen receptor-gamma/delta stimulated by nonpeptidic ligands. *J Exp Med* 186:1769-1774.

42. Bottino, C., R. Castriconi, L. Moretta, and A. Moretta. 2005. Cellular ligands of activating NK receptors. *Trends Immunol* 26:221-226.
43. Smith, H.R., J.W. Heusel, I.K. Mehta, S. Kim, B.G. Dorner, O.V. Naidenko, K. Iizuka, H. Furukawa, D.L. Beckman, J.T. Pingel, A.A. Scalzo, D.H. Fremont, and W.M. Yokoyama. 2002. Recognition of a virus-encoded ligand by a natural killer cell activation receptor. *Proc Natl Acad Sci USA* 99:8826-8831.
44. Bryceson, Y.T., M.E. March, D.F. Barber, H.G. Ljunggren, and E.O. Long. 2005. Cytolytic granule polarization and degranulation controlled by different receptors in resting NK cells. *J Exp Med* 202:1001-1012.
45. Mandelboim, O., N. Lieberman, M. Lev, L. Paul, T.I. Arnon, Y. Bushkin, D.M. Davis, J.L. Strominger, J.W. Yewdell, and A. Porgador. 2001. Recognition of haemagglutinins on virus-infected cells by NKp46 activates lysis by human NK cells. *Nature* 409:1055-1060.
46. Godfrey, D.I., K.J. Hammond, L.D. Poulton, M.J. Smyth, and A.G. Baxter. 2000. NKT cells: facts, functions and fallacies. *Immunol Today* 21:573-583.
47. van der Vliet, H.J., J.W. Molling, B.M. von Blomberg, N. Nishi, W. Kölgen, A.J. van den Eertwegh, H.M. Pinedo, G. Giaccone, and R.J. Scheper. 2004. The immunoregulatory role of CD1d-restricted natural killer T cells in disease. *Clin Immunol* 112:8-23.
48. Hammond, K.J., S.B. Pelikan, N.Y. Crowe, E. Randle-Barrett, T. Nakayama, M. Taniguchi, M.J. Smyth, I.R. van Driel, R. Scollay, A.G. Baxter, and D.I. Godfrey. 1999. NKT cells are phenotypically and functionally diverse. *Eur J Immunol* 29:3768-3781.
49. Sandberg, N. Bhardwaj, and D.F. Nixon. 2003. Dominant effector memory characteristics, capacity for dynamic adaptive expansion, and sex bias in the innate Valpha24 NKT cell compartment. *Eur J Immunol* 33:588-596.
50. Kinjo, Y., and M. Kronenberg. 2005. Valpha14i NKT cells are innate lymphocytes that participate in the immune response to diverse microbes. *J Clin Immunol* 25:522-533.
51. Mattner, J., K.L. Debord, N. Ismail, R.D. Goff, C. Cantu, D. Zhou, P. Saint-Mezard, V. Wang, Y. Gao, N. Yin, K. Hoebe, O. Schneewind, D. Walker, B. Beutler, L. Teyton, P.B. Savage, and A. Bendelac. 2005. Exogenous and endogenous glycolipid antigens activate NKT cells during microbial infections. *Nature* 434:525-529.
52. Kronenberg, M., and L. Gapin. 2002. The unconventional lifestyle of NKT cells. *Nat Rev Immunol* 2:557-568.
53. van Dommelen, S.L., H.A. Tabarias, M.J. Smyth, and M.A. Degli-Esposti. 2003. Activation of natural killer (NK) T cells during murine cytomegalovirus infection enhances the antiviral response mediated by NK cells. *J Virol* 77:1877-1884.
54. Kakimi, K., L.G. Guidotti, Y. Koezuka, and F.V. Chisari. 2000. Natural killer T cell activation inhibits hepatitis B virus replication in vivo. *J Exp Med* 192:921-930.
55. Sandberg, N.M. Fast, E.H. Palacios, G. Fennelly, J. Dobroszycki, P. Palumbo, A. Wiznia, R.M. Grant, N. Bhardwaj, M.G. Rosenberg, and D.F. Nixon. 2002. Selective loss of innate CD4(+) V alpha 24 natural killer T cells in human immunodeficiency virus infection. *J Virol* 76:7528-7534.
56. Motsinger, A., D.W. Haas, A.K. Stanic, L. Van Kaer, S. Joyce, and D. Unutmaz. 2002. CD1d-restricted human natural killer T cells are highly susceptible to human immunodeficiency virus 1 infection. *J Exp Med* 195:869-879.
57. van der Vliet, H.J., B.M. von Blomberg, M.D. Hazenberg, N. Nishi, S.A. Otto, B.H. van Benthem, M. Prins, F.A. Claessen, A.J. van den Eertwegh, G. Giaccone, F. Miedema, R.J. Scheper, and H.M. Pinedo. 2002. Selective decrease in circulating V alpha 24+V beta 11+ NKT cells during HIV type 1 infection. *J Immunol* 168:1490-1495.
58. Hobbs, J.A., S. Cho, T.J. Roberts, V. Sriram, J. Zhang, M. Xu, and R.R. Brutkiewicz. 2001. Selective loss of natural killer T cells by apoptosis following infection with lymphocytic choriomeningitis virus. *J Virol* 75:10746-10754.
59. Smyth, M.J., K.Y. Thia, S.E. Street, E. Cretney, J.A. Trapani, M. Taniguchi, T. Kawano, S.B. Pelikan, N.Y. Crowe, and D.I. Godfrey. 2000. Differential tumor surveillance by natural killer (NK) and NKT cells. *J Exp Med* 191:661-668.
60. Nakagawa, R., K. Motoki, H. Ueno, R. Iijima, H. Nakamura, E. Kobayashi, A. Shimosaka, and Y. Koezuka. 1998. Treatment of hepatic metastasis of the colon26 adenocarcinoma with an alpha-galactosylceramide, KRN7000. *Cancer Res* 58:1202-1207.

61. Moll, J. Snyder-Cappione, G. Spotts, F.M. Hecht, Sandberg, and D.F. Nixon. 2006. Expansion of CD1d-restricted NKT cells in patients with primary HIV-1 infection treated with interleukin-2. *Blood* 107:3081-3083.
62. Yamagiwa, S., Y. Matsuda, T. Ichida, Y. Honda, M. Takamura, S. Sugahara, T. Ishikawa, S. Ohkoshi, Y. Sato, and Y. Aoyagi. 2008. Sustained response to interferon-alpha plus ribavirin therapy for chronic hepatitis C is closely associated with increased dynamism of intrahepatic natural killer and natural killer T cells. *Hepatol Res* 38:664-672.
63. Kita, H. 2005. A role of NKT cells in HCV infection and development of hepatocellular carcinoma: Are they protective or destructive? *Hepatol Res* 32:197-199.
64. Ahmad, A., and F. Alvarez. 2004. Role of NK and NKT cells in the immunopathogenesis of HCV-induced hepatitis. *J Leukoc Biol* 76:743-759.
65. Cooper, M.A., T.A. Fehniger, A. Fuchs, M. Colonna, and M.A. Caligiuri. 2004. NK cell and DC interactions. *Trends Immunol* 25:47-52.
66. Hoebe, K., E. Janssen, and B. Beutler. 2004. The interface between innate and adaptive immunity. *Nat Immunol* 5:971-974.
67. Bosselut, R. 2004. CD4/CD8-lineage differentiation in the thymus: from nuclear effectors to membrane signals. *Nat Rev Immunol* 4:529-540.
68. Starr, T.K., S.C. Jameson, and K.A. Hogquist. 2003. Positive and negative selection of T cells. *Annu Rev Immunol* 21:139-176.
69. Takahama, Y. 2006. Journey through the thymus: stromal guides for T-cell development and selection. *Nat Rev Immunol* 6:127-135.
70. Romagnani, S. 2000. T-cell subsets (Th1 versus Th2). *Ann Allergy Asthma Immunol* 85:9-18; quiz 18, 21.
71. Romagnani, S. 1999. Th1/Th2 cells. *Inflamm Bowel Dis* 5:285-294.
72. Romagnani, S. 1996. Understanding the role of Th1/Th2 cells in infection. *Trends Microbiol* 4:470-473.
73. Ulsenheimer, A., J.T. Gerlach, N.H. Gruener, M.C. Jung, C.A. Schirren, W. Schraut, R. Zachoval, G.R. Pape, and H.M. Diepolder. 2003. Detection of functionally altered hepatitis C virus-specific CD4 T cells in acute and chronic hepatitis C. *Hepatology* 37:1189-1198.
74. Tsai, S.L., Y.F. Liaw, M.H. Chen, C.Y. Huang, and G.C. Kuo. 1997. Detection of type 2-like T-helper cells in hepatitis C virus infection: implications for hepatitis C virus chronicity. *Hepatology* 25:449-458.
75. MacDonald, A.J., M. Duffy, M.T. Brady, S. McKiernan, W. Hall, J. Hegarty, M. Curry, and K.H. Mills. 2002. CD4 T helper type 1 and regulatory T cells induced against the same epitopes on the core protein in hepatitis C virus-infected persons. *J Infect Dis* 185:720-727.
76. Steinman, L. 2007. A brief history of T(H)17, the first major revision in the T(H)1/T(H)2 hypothesis of T cell-mediated tissue damage. *Nat Med* 13:139-145.
77. Dong, C. 2008. TH17 cells in development: an updated view of their molecular identity and genetic programming. *Nat Rev Immunol* 8:337-348.
78. Mottet, C., and D. Golshayan. 2007. CD4+CD25+Foxp3+ regulatory T cells: from basic research to potential therapeutic use. *Swiss medical weekly : official journal of the Swiss Society of Infectious Diseases, the Swiss Society of Internal Medicine, the Swiss Society of Pneumology* 137:625-634.
79. Wilczynski, J.R., M. Radwan, and J. Kalinka. 2008. The characterization and role of regulatory T cells in immune reactions. *Front Biosci* 13:2266-2274.
80. Rouse, B.T., P.P. Sarangi, and S. Suvas. 2006. Regulatory T cells in virus infections. *Immunol Rev* 212:272-286.
81. Baker, C.A., R. Clark, F. Ventura, N.G. Jones, D. Guzman, D.R. Bangsberg, and H. Cao. 2007. Peripheral CD4 loss of regulatory T cells is associated with persistent viraemia in chronic HIV infection. *Clin Exp Immunol* 147:533-539.
82. Oswald-Richter, K., S.M. Grill, N. Shariat, M. Leelawong, M.S. Sundrud, D.W. Haas, and D. Unutmaz. 2004. HIV infection of naturally occurring and genetically reprogrammed human regulatory T-cells. *PLoS Biol* 2:E198.
83. Ndhlovu, L.C., C.P. Loo, G. Spotts, D.F. Nixon, and F.M. Hecht. 2008. FOXP3 expressing CD127lo CD4+ T cells inversely correlate with CD38+ CD8+ T cell activation levels in primary HIV-1 infection. *J Leukoc Biol* 83:254-262.

84. Holmes, D., Q. Jiang, L. Zhang, and L. Su. 2008. Foxp3 and Treg cells in HIV-1 infection and immuno-pathogenesis. *Immunol Res* 41:248-266.
85. Ouyang, W., J.K. Kolls, and Y. Zheng. 2008. The biological functions of T helper 17 cell effector cytokines in inflammation. *Immunity* 28:454-467.
86. Williams, M.A., and M.J. Bevan. 2007. Effector and memory CTL differentiation. *Annu Rev Immunol* 25:171-192.
87. Mescher, M.F., P. Agarwal, K.A. Casey, C.D. Hammerbeck, Z. Xiao, and J.M. Curtsinger. 2007. Molecular basis for checkpoints in the CD8 T cell response: tolerance versus activation. *Semin Immunol* 19:153-161.
88. Mescher, M.F., J.M. Curtsinger, P. Agarwal, K.A. Casey, M. Gerner, C.D. Hammerbeck, F. Popescu, and Z. Xiao. 2006. Signals required for programming effector and memory development by CD8+ T cells. *Immunol Rev* 211:81-92.
89. Germain, R.N., and I. Stefanová. 1999. The dynamics of T cell receptor signaling: complex orchestration and the key roles of tempo and cooperation. *Annu Rev Immunol* 17:467-522.
90. Bleackley, R.C. 2005. A molecular view of cytotoxic T lymphocyte induced killing. *Biochem. Cell Biol.* 83:747-751.
91. Andersen, M.H., D. Schrama, P. Thor Straten, and J. Becker. 2006. Cytotoxic T cells. *J Invest Dermatol* 126:32-41.
92. Barry, M., and R.C. Bleackley. 2002. Cytotoxic T lymphocytes: all roads lead to death. *Nat Rev Immunol* 2:401-409.
93. Sprent, J., and C.D. Surh. 2002. T cell memory. *Annu Rev Immunol* 20:551-579.
94. Hamann, D., P.A. Baars, M.H. Rep, B. Hooibrink, S.R. Kerkhof-Garde, M.R. Klein, and R.A. van Lier. 1997. Phenotypic and functional separation of memory and effector human CD8+ T cells. *J Exp Med* 186:1407-1418.
95. Sallusto, D. Lenig, R. Förster, M. Lipp, and Lanzavecchia. 1999. Two subsets of memory T lymphocytes with distinct homing potentials and effector functions. *Nature* 401:708-712.
96. Appay, V., P.R. Dunbar, M. Callan, P. Klenerman, G.M. Gillespie, L. Papagno, G.S. Ogg, A. King, F. Lechner, C.A. Spina, S. Little, D.V. Havlir, D.D. Richman, N. Gruener, G. Pape, A. Waters, P. Easterbrook, M. Salio, V. Cerundolo, A.J. McMichael, and S.L. Rowland-Jones. 2002. Memory CD8+ T cells vary in differentiation phenotype in different persistent virus infections. *Nat Med* 8:379-385.
97. Aandahl, E.M., M.F. Quigley, W.J. Moretto, Moll, Gonzalez, A. Sönnerborg, S. Lindbäck, F.M. Hecht, S.G. Deeks, M.G. Rosenberg, D.F. Nixon, and Sandberg. 2004. Expansion of CD7(low) and CD7(negative) CD8 T-cell effector subsets in HIV-1 infection: correlation with antigenic load and reversion by antiretroviral treatment. *Blood* 104:3672-3678.
98. Appay, V., and S.L. Rowland-Jones. 2004. Lessons from the study of T-cell differentiation in persistent human virus infection. *Semin Immunol* 16:205-212.
99. Papagno, L., C.A. Spina, A. Marchant, M. Salio, N. Rufer, S. Little, T. Dong, G. Chesney, A. Waters, P. Easterbrook, P.R. Dunbar, D. Shepherd, V. Cerundolo, V. Emery, P. Griffiths, C. Conlon, A.J. McMichael, D.D. Richman, S.L. Rowland-Jones, and V. Appay. 2004. Immune activation and CD8+ T-cell differentiation towards senescence in HIV-1 infection. *PLoS Biol* 2:E20.
100. Northfield, J.W., V. Kasprowicz, M. Lucas, N. Kersting, B. Bengsh, A. Kim, R. Phillips, B. Walker, R. Thimme, G. Lauer, and P. Klenerman. 2008. CD161 expression on hepatitis C virus-specific CD8+ T cells suggests a distinct pathway of T cell differentiation. *Hepatology* 47:396-406.
101. Bengsch, B., H.C. Spangenberg, N. Kersting, C. Neumann-Haefelin, E. Panther, F. von Weizsäcker, H.E. Blum, H. Pircher, and R. Thimme. 2007. Analysis of CD127 and KLRG1 expression on hepatitis C virus-specific CD8+ T cells reveals the existence of different memory T-cell subsets in the peripheral blood and liver. *J Virol* 81:945-953.
102. Aandahl, E.M., Sandberg, K.P. Beckerman, K. Taskén, W.J. Moretto, and D.F. Nixon. 2003. CD7 is a differentiation marker that identifies multiple CD8 T cell effector subsets. *J Immunol* 170:2349-2355.
103. Roederer, S. De Rosa, R. Gerstein, M. Anderson, M. Bigos, R. Stovel, T. Nozaki, D. Parks, L. Herzenberg, and L. Herzenberg. 1997. 8 color, 10-parameter flow cytometry to elucidate complex leukocyte heterogeneity. *Cytometry* 29:328-339.

104. De Rosa, S.C., L.A. Herzenberg, L.A. Herzenberg, and Roederer. 2001. 11-color, 13-parameter flow cytometry: identification of human naive T cells by phenotype, function, and T-cell receptor diversity. *Nat Med* 7:245-248.
105. Appay, v. Lier, Sallusto, and Roederer. 2008. Phenotype and function of human T lymphocyte subsets: Consensus and issues. *Cytometry A*
106. Betts, M.R., D.A. Price, J.M. Brenchley, K. Loré, F.J. Guenaga, A. Smed-Sorensen, D.R. Ambrozak, S.A. Migueles, M. Connors, M. Roederer, D.C. Douek, and R.A. Koup. 2004. The functional profile of primary human antiviral CD8+ T cell effector activity is dictated by cognate peptide concentration. *J Immunol* 172:6407-6417.
107. Casazza, J.P., M.R. Betts, D.A. Price, M.L. Precopio, L.E. Ruff, J.M. Brenchley, B.J. Hill, M. Roederer, D.C. Douek, and R.A. Koup. 2006. Acquisition of direct antiviral effector functions by CMV-specific CD4+ T lymphocytes with cellular maturation. *J Exp Med* 203:2865-2877.
108. Betts, M.R., B. Exley, D.A. Price, A. Bansal, Z.T. Camacho, V. Teaberry, S.M. West, D.R. Ambrozak, G. Tomaras, M. Roederer, J.M. Kilby, J. Tartaglia, R. Belshe, F. Gao, D.C. Douek, K.J. Weinhold, R.A. Koup, P. Goepfert, and G. Ferrari. 2005. Characterization of functional and phenotypic changes in anti-Gag vaccine-induced T cell responses and their role in protection after HIV-1 infection. *Proc Natl Acad Sci USA* 102:4512-4517.
109. Miller, J.D., D. Masopust, E.J. Wherry, S. Kaech, G. Silvestri, and R. Ahmed. 2005. Differentiation of CD8 T cells in response to acute and chronic viral infections: implications for HIV vaccine development. *Current drug targets Infectious disorders* 5:121-129.
110. Kaech, S.M., E.J. Wherry, and R. Ahmed. 2002. Effector and memory T-cell differentiation: implications for vaccine development. *Nat Rev Immunol* 2:251-262.
111. Rehermann, B., and M. Nascimbeni. 2005. Immunology of hepatitis B virus and hepatitis C virus infection. *Nat Rev Immunol* 5:215-229.
112. Dustin, L.B., and C.M. Rice. 2007. Flying under the radar: the immunobiology of hepatitis C. *Annu Rev Immunol* 25:71-99.
113. el-Awady, M.K., A.A. Tabll, e.l.R.M. Redwan, S. Youssef, M.H. Omran, F. Thakeb, and M. el-Demellawy. 2005. Flow cytometric detection of hepatitis C virus antigens in infected peripheral blood leukocytes: binding and entry. *World J Gastroenterol* 11:5203-5208.
114. Pham, T.N., D. King, S.A. Macparland, J.S. McGrath, S.B. Reddy, F.R. Bursey, and T.I. Michalak. 2008. Hepatitis C virus replicates in the same immune cell subsets in chronic hepatitis C and occult infection. *Gastroenterology* 134:812-822.
115. Lerat, H., S. Rumin, F. Habersetzer, F. Berby, M.A. Trabaud, C. Trépo, and G. Inchauspé. 1998. In vivo tropism of hepatitis C virus genomic sequences in hematopoietic cells: influence of viral load, viral genotype, and cell phenotype. *Blood* 91:3841-3849.
116. Webster, G., E. Barnes, D. Brown, and G.M. Dusheiko. 2000. HCV genotypes--role in pathogenesis of disease and response to therapy. *Baillière's best practice & research Clinical gastroenterology* 14:229-240.
117. Shepard, C.W., L. Finelli, and Alter. 2005. Global epidemiology of hepatitis C virus infection. *The Lancet infectious diseases* 5:558-567.
118. Fabris, P., V.M. Fleming, M.T. Giordani, and E. Barnes. 2008. Acute hepatitis C: clinical aspects, diagnosis, and outcome of acute HCV infection. *Curr Pharm Des* 14:1661-1665.
119. Bonino, F., F. Oliveri, P. Colombatto, P.L. Calvo, and M.R. Brunetto. 1995. Hepatitis C virus infection and liver disease. *Acta Gastroenterol Belg* 58:60-67.
120. Hoofnagle, J.H. 2002. Course and outcome of hepatitis C. *Hepatology* 36:S21-29.
121. Bruno, S., and C. Facciotto. 2008. The natural course of HCV infection and the need for treatment. *Annals of hepatology : official journal of the Mexican Association of Hepatology* 7:114-119.
122. Reddy, K.R., D.R. Nelson, and S. Zeuzem. 2008. Ribavirin: Current role in the optimal clinical management of chronic hepatitis C. *J Hepatol*
123. Chung, R.T., M. Gale, S.J. Polyak, S.M. Lemon, T.J. Liang, and J.H. Hoofnagle. 2008. Mechanisms of action of interferon and ribavirin in chronic hepatitis C: Summary of a workshop. *Hepatology* 47:306-320.

124. Santantonio, T., and M. Fasano. 2008. Therapy of acute hepatitis C: a review of literature. *Curr Pharm Des* 14:1686-1689.
125. McKiernan, S.M., R. Hagan, M. Curry, G.S. McDonald, A. Kelly, N. Nolan, A. Walsh, J. Hegarty, E. Lawlor, and D. Kelleher. 2004. Distinct MHC class I and II alleles are associated with hepatitis C viral clearance, originating from a single source. *Hepatology* 40:108-114.
126. Neumann-Haefelin, C., S. McKiernan, S. Ward, S. Viazov, H.C. Spangenberg, T. Killinger, T.F. Baumert, N. Nazarova, I. Sheridan, O. Pybus, F. von Weizsäcker, M. Roggendorf, D. Kelleher, P. Klenerman, H.E. Blum, and R. Thimme. 2006. Dominant influence of an HLA-B27 restricted CD8+ T cell response in mediating HCV clearance and evolution. *Hepatology* 43:563-572.
127. Thursz, M., R. Yallop, R. Goldin, C. Trepo, and H.C. Thomas. 1999. Influence of MHC class II genotype on outcome of infection with hepatitis C virus. The HENCORE group. Hepatitis C European Network for Cooperative Research. *Lancet* 354:2119-2124.
128. Kamal, S.M., J. Fehr, B. Roesler, T. Peters, and J.W. Rasenack. 2002. Peginterferon alone or with ribavirin enhances HCV-specific CD4 T-helper 1 responses in patients with chronic hepatitis C. *Gastroenterology* 123:1070-1083.
129. Cramp, M.E., P. Carucci, S. Rossol, S. Chokshi, G. Maertens, R. Williams, and N.V. Naoumov. 1999. Hepatitis C virus (HCV) specific immune responses in anti-HCV positive patients without hepatitis C viraemia. *Gut* 44:424-429.
130. Shirakawa, H., A. Matsumoto, S. Joshita, M. Komatsu, N. Tanaka, T. Umemura, T. Ichijo, K. Yoshizawa, K. Kiyosawa, E. Tanaka, and N.I.T.R.G.i.a.l.i.A. A. 2008. Pretreatment prediction of virological response to peginterferon plus ribavirin therapy in chronic hepatitis C patients using viral and host factors. *Hepatology*
131. Backus, L.I., D.B. Boothroyd, B.R. Phillips, and L.A. Mole. 2007. Predictors of response of US veterans to treatment for the hepatitis C virus. *Hepatology* 46:37-47.
132. Loustaud-Ratti, V., S. Alain, A. Rousseau, I.F. Hubert, F.L. Sauvage, P. Marquet, F. Denis, F. Lunel, P. Calès, A. Lefebvre, A.L. Fauchais, E. Liozon, and E. Vidal. 2008. Ribavirin exposure after the first dose is predictive of sustained virological response in chronic hepatitis C. *Hepatology* 47:1453-1461.
133. Butera, D., S. Marukian, A.E. Iwamaye, E. Hembrador, T.J. Chambers, A.M. Di Bisceglie, E.D. Charles, A.H. Talal, I.M. Jacobson, C.M. Rice, and L.B. Dustin. 2005. Plasma chemokine levels correlate with the outcome of antiviral therapy in patients with hepatitis C. *Blood* 106:1175-1182.
134. Romero, A.I., M. Lagging, J. Westin, A.P. Dhillon, L.B. Dustin, J.M. Pawlotsky, A.U. Neumann, C. Ferrari, G. Missale, B.L. Haagmans, S.W. Schalm, S. Zeuzem, F. Negro, E. Verheij-Hart, K. Hellstrand, and D.-H.S. Group. 2006. Interferon (IFN)-gamma-inducible protein-10: association with histological results, viral kinetics, and outcome during treatment with pegylated IFN-alpha 2a and ribavirin for chronic hepatitis C virus infection. *J Infect Dis* 194:895-903.
135. Diago, M., G. Castellano, J. García-Samaniego, C. Pérez, I. Fernández, M. Romero, O.L. Iacono, and C. García-Monzón. 2006. Association of pretreatment serum interferon gamma inducible protein 10 levels with sustained virological response to peginterferon plus ribavirin therapy in genotype 1 infected patients with chronic hepatitis C. *Gut* 55:374-379.
136. Payan, C., A. Pivert, P. Morand, S. Fafi-Kremer, F. Carrat, S. Pol, P. Cacoub, C. Perronne, F. Lunel, and Unknown. 2007. Rapid and early virological response to chronic hepatitis C treatment with IFN alpha2b or PEG-IFN alpha2b plus ribavirin in HIV/HCV co-infected patients. *Gut* 56:1111-1116.
137. Sarasin-Filipowicz, M., E.J. Oakeley, F.H. Duong, V. Christen, L. Terracciano, W. Filipowicz, and M.H. Heim. 2008. Interferon signaling and treatment outcome in chronic hepatitis C. *Proc Natl Acad Sci USA* 105:7034-7039.
138. Lempicki, R.A., M.A. Polis, J. Yang, M. McLaughlin, C. Koratich, D.W. Huang, B. Fullmer, L. Wu, C.A. Rehm, H. Masur, H.C. Lane, K.E. Sherman, A.S. Fauci, and S. Kottilil. 2006. Gene expression profiles in hepatitis C virus (HCV) and HIV coinfection: class prediction analyses before treatment predict the outcome of anti-HCV therapy among HIV-coinfected persons. *J Infect Dis* 193:1172-1177.
139. Racanelli, V., and B. Rehermann. 2006. The liver as an immunological organ. *Hepatology* 43:S54-62.

140. Gale, M., and E.M. Foy. 2005. Evasion of intracellular host defence by hepatitis C virus. *Nature* 436:939-945.
141. García-Sastre, A., and C.A. Biron. 2006. Type 1 interferons and the virus-host relationship: a lesson in détente. *Science* 312:879-882.
142. Durante-Mangoni, E., R. Wang, A. Shaulov, Q. He, I. Nasser, N. Afdhal, M.J. Koziel, and M.A. Exley. 2004. Hepatic CD1d expression in hepatitis C virus infection and recognition by resident proinflammatory CD1d-reactive T cells. *J Immunol* 173:2159-2166.
143. van der Vliet, H.J., J.W. Molling, B.M. von Blomberg, W. Kölgen, A.G. Stam, T.D. de Gruijl, C.J. Mulder, H.L. Janssen, N. Nishi, A.J. van den Eertwegh, R.J. Scheper, and C.J. van Nieuwkerk. 2005. Circulating Valpha24+Vbeta11+ NKT cell numbers and dendritic cell CD1d expression in hepatitis C virus infected patients. *Clin Immunol* 114:183-189.
144. Lucas, S. Gadola, U. Meier, N.T. Young, G. Harcourt, A. Karadimitris, N. Coumi, D. Brown, G. Dusheiko, V. Cerundolo, and P. Klenerman. 2003. Frequency and phenotype of circulating Valpha24/Vbeta11 double-positive natural killer T cells during hepatitis C virus infection. *J Virol* 77:2251-2257.
145. Gonzalez, Falconer, Michaëlsson, Moll, Reichard, Alaeus, and Sandberg. 2008. Expansion of CD56- NK cells in chronic HCV/HIV-1 co-infection: reversion by antiviral treatment with pegylated IFNalpha and ribavirin. *Clin Immunol* 128:46-56.
146. Kanto, T. 2008. Virus associated innate immunity in liver. *Front Biosci* 13:6183-6192.
147. Wang, S.H., C.X. Huang, L. Ye, X. Wang, L. Song, Y.J. Wang, H. Liang, X.Y. Huang, and W.Z. Ho. 2008. Natural killer cells suppress full cycle HCV infection of human hepatocytes. *J Viral Hepat*
148. Lohmann, V., F. Körner, J. Koch, U. Herian, L. Theilmann, and R. Bartenschlager. 1999. Replication of subgenomic hepatitis C virus RNAs in a hepatoma cell line. *Science* 285:110-113.
149. Blight, K. 2000. Efficient Initiation of HCV RNA Replication in Cell Culture. *Science* 290:1972-1974.
150. Li, Y., T. Zhang, C. Ho, J.S. Orange, S.D. Douglas, and W.Z. Ho. 2004. Natural killer cells inhibit hepatitis C virus expression. *J Leukoc Biol* 76:1171-1179.
151. Crotta, S., A. Stilla, A. Wack, A. D'Andrea, S. Nuti, U. D'Oro, M. Mosca, F. Filliponi, R.M. Brunetto, F. Bonino, S. Abrignani, and N.M. Valiante. 2002. Inhibition of natural killer cells through engagement of CD81 by the major hepatitis C virus envelope protein. *J Exp Med* 195:35-41.
152. Tseng, C.T., and G.R. Klimpel. 2002. Binding of the hepatitis C virus envelope protein E2 to CD81 inhibits natural killer cell functions. *J Exp Med* 195:43-49.
153. Herzer, K., C.S. Falk, J. Encke, S.T. Eichhorst, A. Ulsenheimer, B. Seliger, and P.H. Krammer. 2003. Upregulation of major histocompatibility complex class I on liver cells by hepatitis C virus core protein via p53 and TAP1 impairs natural killer cell cytotoxicity. *J Virol* 77:8299-8309.
154. Van Thiel, D.H., X. Zhang, N. Baddour, H.I. Wright, L. Friedlander, and J.S. Gavaler. 1994. Intrahepatic mononuclear cell populations and MHC antigen expression in patients with chronic hepatitis C [correction of B]: effect of interferon-alpha. *Dig Dis Sci* 39:970-976.
155. Nattermann, J., Nischalke, V. Hofmeister, G. Ahlenstiel, H. Zimmermann, L. Leifeld, E.H. Weiss, T. Sauerbruch, and U. Spengler. 2005. The HLA-A2 restricted T cell epitope HCV core 35-44 stabilizes HLA-E expression and inhibits cytolysis mediated by natural killer cells. *Am J Pathol* 166:443-453.
156. Jinushi, M., T. Takehara, T. Tatsumi, T. Kanto, V. Groh, T. Spies, R. Kimura, T. Miyagi, K. Mochizuki, Y. Sasaki, and N. Hayashi. 2003. Expression and role of MICA and MICB in human hepatocellular carcinomas and their regulation by retinoic acid. *Int J Cancer* 104:354-361.
157. Jinushi, M., T. Takehara, T. Kanto, T. Tatsumi, V. Groh, T. Spies, T. Miyagi, T. Suzuki, Y. Sasaki, and N. Hayashi. 2003. Critical role of MHC class I-related chain A and B expression on IFN-alpha-stimulated dendritic cells in NK cell activation: impairment in chronic hepatitis C virus infection. *J Immunol* 170:1249-1256.
158. Gale, M.J., M.J. Korth, N.M. Tang, S.L. Tan, D.A. Hopkins, T.E. Dever, S.J. Polyak, D.R. Gretch, and M.G. Katze. 1997. Evidence that hepatitis C virus resistance to

interferon is mediated through repression of the PKR protein kinase by the nonstructural 5A protein. *Virology* 230:217-227.

159. Large, M.K., D.J. Kittlesen, and Y.S. Hahn. 1999. Suppression of host immune response by the core protein of hepatitis C virus: possible implications for hepatitis C virus persistence. *J Immunol* 162:931-938.
160. de Lucas, S., J. Bartolome, and V. Carreno. 2005. Hepatitis C virus core protein down-regulates transcription of interferon-induced antiviral genes. *J Infect Dis* 191:93-99.
161. Gerlach, J.T., H.M. Diepolder, M.C. Jung, N.H. Gruener, W.W. Schraut, R. Zachoval, R. Hoffmann, C.A. Schirren, T. Santantonio, and G.R. Pape. 1999. Recurrence of hepatitis C virus after loss of virus-specific CD4(+) T-cell response in acute hepatitis C. *Gastroenterology* 117:933-941.
162. Lechner, F., D.K. Wong, P.R. Dunbar, R. Chapman, R.T. Chung, P. Dohrenwend, G. Robbins, R. Phillips, P. Klenerman, and B.D. Walker. 2000. Analysis of successful immune responses in persons infected with hepatitis C virus. *J Exp Med* 191:1499-1512.
163. Thimme, R., D. Oldach, K.M. Chang, C. Steiger, S.C. Ray, and F.V. Chisari. 2001. Determinants of viral clearance and persistence during acute hepatitis C virus infection. *J Exp Med* 194:1395-1406.
164. Rosen, H.R., C. Miner, A.W. Sasaki, D.M. Lewinsohn, A.J. Conrad, A. Bakke, H.G. Bouwer, and D.J. Hinrichs. 2002. Frequencies of HCV-specific effector CD4+ T cells by flow cytometry: correlation with clinical disease stages. *Hepatology* 35:190-198.
165. Schirren, C.A., M.C. Jung, T. Worzfeld, M. Mamin, G. Baretton, J.T. Gerlach, N.H. Gruener, R. Zachoval, M. Houghton, H.G. Rau, and G.R. Pape. 2001. Hepatitis C virus-specific CD4+ T cell response after liver transplantation occurs early, is multispecific, compartmentalizes to the liver, and does not correlate with recurrent disease. *J Infect Dis* 183:1187-1194.
166. Thimme, R., J. Bukh, H.C. Spangenberg, S. Wieland, J. Pemberton, C. Steiger, S. Govindarajan, R.H. Purcell, and F.V. Chisari. 2002. Viral and immunological determinants of hepatitis C virus clearance, persistence, and disease. *Proc Natl Acad Sci USA* 99:15661-15668.
167. Schirren, C.A., R. Zachoval, J.T. Gerlach, A. Ulsenheimer, N.H. Gruener, H.M. Diepolder, G. Baretton, W. Schraut, H.G. Rau, H. Nitschko, G.R. Pape, and M.C. Jung. 2003. Antiviral treatment of recurrent hepatitis C virus (HCV) infection after liver transplantation: association of a strong, multispecific, and long-lasting CD4+ T cell response with HCV-elimination. *J Hepatol* 39:397-404.
168. Rosen, H., D.J. Hinrichs, D.R. Gretch, M.J. Koziel, S. Chou, M. Houghton, J. Rabkin, C.L. Corless, and H.G. Bouwer. 1999. Association of multispecific CD4(+) response to hepatitis C and severity of recurrence after liver transplantation. *Gastroenterology* 117:926-932.
169. Cramp, M.E., S. Rossol, S. Chokshi, P. Carucci, R. Williams, and N.V. Naoumov. 2000. Hepatitis C virus-specific T-cell reactivity during interferon and ribavirin treatment in chronic hepatitis C. *Gastroenterology* 118:346-355.
170. Poynard, T., P. Marcellin, S.S. Lee, C. Niederau, G.S. Minuk, G. Ideo, V. Bain, J. Heathcote, S. Zeuzem, C. Trepo, and J. Albrecht. 1998. Randomised trial of interferon alpha2b plus ribavirin for 48 weeks or for 24 weeks versus interferon alpha2b plus placebo for 48 weeks for treatment of chronic infection with hepatitis C virus. International Hepatitis Interventional Therapy Group (IHIT). *Lancet* 352:1426-1432.
171. McHutchison, J.G., S.C. Gordon, E.R. Schiff, M.L. Shiffman, W.M. Lee, V.K. Rustgi, Z.D. Goodman, M.H. Ling, S. Cort, and J.K. Albrecht. 1998. Interferon alfa-2b alone or in combination with ribavirin as initial treatment for chronic hepatitis C. Hepatitis Interventional Therapy Group. *N Engl J Med* 339:1485-1492.
172. Gruener, N.H., M.C. Jung, A. Ulsenheimer, J.T. Gerlach, R. Zachoval, H.M. Diepolder, G. Baretton, R. Schauer, G.R. Pape, and C.A. Schirren. 2004. Analysis of a successful HCV-specific CD8+ T cell response in patients with recurrent HCV-infection after orthotopic liver transplantation. *Liver Transpl* 10:1487-1496.
173. Chang, K.M., R. Thimme, J.J. Melpolder, D. Oldach, J. Pemberton, J. Moorhead-Loudis, J.G. McHutchison, H.J. Alter, and F.V. Chisari. 2001. Differential CD4(+) and CD8(+) T-cell responsiveness in hepatitis C virus infection. *Hepatology* 33:267-276.

174. Cooper, S., A.L. Erickson, E.J. Adams, J. Kansopon, A.J. Weiner, D.Y. Chien, M. Houghton, P. Parham, and C.M. Walker. 1999. Analysis of a successful immune response against hepatitis C virus. *Immunity* 10:439-449.
175. Shoukry, N.H., A. Grakoui, M. Houghton, D.Y. Chien, J. Ghrayeb, K.A. Reimann, and C.M. Walker. 2003. Memory CD8+ T cells are required for protection from persistent hepatitis C virus infection. *J Exp Med* 197:1645-1655.
176. Lanford, R.E., B. Guerra, H. Lee, D.R. Averett, B. Pfeiffer, D. Chavez, L. Notvall, and C. Bigger. 2003. Antiviral effect and virus-host interactions in response to alpha interferon, gamma interferon, poly(i)-poly(c), tumor necrosis factor alpha, and ribavirin in hepatitis C virus subgenomic replicons. *J Virol* 77:1092-1104.
177. Frese, M., V. Schwärzle, K. Barth, N. Krieger, V. Lohmann, S. Mihm, O. Haller, and R. Bartenschlager. 2002. Interferon-gamma inhibits replication of subgenomic and genomic hepatitis C virus RNAs. *Hepatology* 35:694-703.
178. Blight, K.J., A.A. Kolykhalov, and C.M. Rice. 2000. Efficient initiation of HCV RNA replication in cell culture. *Science* 290:1972-1974.
179. Gruener, N.H., F. Lechner, M.C. Jung, H. Diepolder, T. Gerlach, G. Lauer, B. Walker, J. Sullivan, R. Phillips, G.R. Pape, and P. Klenerman. 2001. Sustained dysfunction of antiviral CD8+ T lymphocytes after infection with hepatitis C virus. *J Virol* 75:5550-5558.
180. Urbani, S., C. Boni, G. Missale, G. Elia, C. Cavallo, M. Massari, G. Raimondo, and C. Ferrari. 2002. Virus-specific CD8+ lymphocytes share the same effector-memory phenotype but exhibit functional differences in acute hepatitis B and C. *J Virol* 76:12423-12434.
181. Lechner, F., N.H. Gruener, S. Urbani, J. Uggeri, T. Santantonio, A.R. Kammer, A. Cerny, R. Phillips, C. Ferrari, G.R. Pape, and P. Klenerman. 2000. CD8+ T lymphocyte responses are induced during acute hepatitis C virus infection but are not sustained. *Eur J Immunol* 30:2479-2487.
182. Grüner, N.H., T.J. Gerlach, M.C. Jung, H.M. Diepolder, C.A. Schirren, W.W. Schraut, R. Hoffmann, R. Zachoval, T. Santantonio, M. Cucchiarini, A. Cerny, and G.R. Pape. 2000. Association of hepatitis C virus-specific CD8+ T cells with viral clearance in acute hepatitis C. *J Infect Dis* 181:1528-1536.
183. Koziel, M.J., D.K. Wong, D. Dudley, M. Houghton, and B.D. Walker. 1997. Hepatitis C virus-specific cytolytic T lymphocyte and T helper cell responses in seronegative persons. *J Infect Dis* 176:859-866.
184. Koziel, M.J., D. Dudley, N. Afdhal, A. Grakoui, C.M. Rice, Q.L. Choo, M. Houghton, and B.D. Walker. 1995. HLA class I-restricted cytotoxic T lymphocytes specific for hepatitis C virus. Identification of multiple epitopes and characterization of patterns of cytokine release. *J Clin Invest* 96:2311-2321.
185. Koziel, M.J., D. Dudley, N. Afdhal, Q.L. Choo, M. Houghton, R. Ralston, and B.D. Walker. 1993. Hepatitis C virus (HCV)-specific cytotoxic T lymphocytes recognize epitopes in the core and envelope proteins of HCV. *J Virol* 67:7522-7532.
186. Koziel, M.J., D. Dudley, J.T. Wong, J. Dienstag, M. Houghton, R. Ralston, and B.D. Walker. 1992. Intrahepatic cytotoxic T lymphocytes specific for hepatitis C virus in persons with chronic hepatitis. *J Immunol* 149:3339-3344.
187. Wong, D.K., D.D. Dudley, N.H. Afdhal, J. Dienstag, C.M. Rice, L. Wang, M. Houghton, B.D. Walker, and M.J. Koziel. 1998. Liver-derived CTL in hepatitis C virus infection: breadth and specificity of responses in a cohort of persons with chronic infection. *J Immunol* 160:1479-1488.
188. Tsai, S.L., Y.M. Chen, M.H. Chen, C.Y. Huang, I.S. Sheen, C.T. Yeh, J.H. Huang, G.C. Kuo, and Y.F. Liaw. 1998. Hepatitis C virus variants circumventing cytotoxic T lymphocyte activity as a mechanism of chronicity. *Gastroenterology* 115:954-965.
189. Erickson, A.L., Y. Kimura, S. Igarashi, J. Eichelberger, M. Houghton, J. Sidney, D. McKinney, A. Sette, A.L. Hughes, and C.M. Walker. 2001. The outcome of hepatitis C virus infection is predicted by escape mutations in epitopes targeted by cytotoxic T lymphocytes. *Immunity* 15:883-895.
190. Weiner, A., A.L. Erickson, J. Kansopon, K. Crawford, E. Muchmore, A.L. Hughes, M. Houghton, and C.M. Walker. 1995. Persistent hepatitis C virus infection in a chimpanzee is associated with emergence of a cytotoxic T lymphocyte escape variant. *Proc Natl Acad Sci USA* 92:2755-2759.

191. Weiner, A.J., A.L. Erickson, J. Kansopon, K. Crawford, E. Muchmore, M. Houghton, and C.M. Walker. 1995. Association of cytotoxic T lymphocyte (CTL) escape mutations with persistent hepatitis C virus (HCV) infection. *Int Symp Princess Takamatsu Cancer Res Fund* 25:227-235.
192. Erickson, A.L., M. Houghton, Q.L. Choo, A.J. Weiner, R. Ralston, E. Muchmore, and C.M. Walker. 1993. Hepatitis C virus-specific CTL responses in the liver of chimpanzees with acute and chronic hepatitis C. *J Immunol* 151:4189-4199.
193. Shoukry, N.H., A.G. Cawthon, and C.M. Walker. 2004. Cell-mediated immunity and the outcome of hepatitis C virus infection. *Annu Rev Microbiol* 58:391-424.
194. Lucas, A.L. Vargas-Cuero, G.M. Lauer, E. Barnes, C.B. Willberg, N. Semmo, B.D. Walker, R. Phillips, and P. Klenerman. 2004. Pervasive influence of hepatitis C virus on the phenotype of antiviral CD8+ T cells. *J Immunol* 172:1744-1753.
195. Nelson, D.R., C.G. Marousis, G.L. Davis, C.M. Rice, J. Wong, M. Houghton, and J.Y. Lau. 1997. The role of hepatitis C virus-specific cytotoxic T lymphocytes in chronic hepatitis C. *J Immunol* 158:1473-1481.
196. Kovacs, A., L. Al-Harthi, S. Christensen, W. Mack, M. Cohen, and A. Landay. 2008. CD8+T cell Activation in Women Coinfected with Human Immunodeficiency Virus Type 1 and Hepatitis C Virus. *J Infect Dis* 0:080409094056215-080409094056000.
197. Grakoui, A., N.H. Shoukry, D.J. Woollard, J.H. Han, H.L. Hanson, J. Ghrayeb, K.K. Murthy, C.M. Rice, and C.M. Walker. 2003. HCV persistence and immune evasion in the absence of memory T cell help. *Science* 302:659-662.
198. Takaki, A., M. Wiese, G. Maertens, E. Depla, U. Seifert, A. Liebetrau, J.L. Miller, M.P. Manns, and B. Rehermann. 2000. Cellular immune responses persist and humoral responses decrease two decades after recovery from a single-source outbreak of hepatitis C. *Nat Med* 6:578-582.
199. Nascimbeni, M., E. Mizukoshi, M. Bosmann, M.E. Major, K. Mihalik, C.M. Rice, S.M. Feinstone, and B. Rehermann. 2003. Kinetics of CD4+ and CD8+ memory T-cell responses during hepatitis C virus rechallenge of previously recovered chimpanzees. *J Virol* 77:4781-4793.
200. Day, C.L., N.P. Seth, Lucas, H. Appel, L. Gauthier, G.M. Lauer, G.K. Robbins, Z.M. Szczepiorkowski, D.R. Casson, R.T. Chung, S. Bell, Harcourt, B.D. Walker, P. Klenerman, and K.W. Wucherpfennig. 2003. Ex vivo analysis of human memory CD4 T cells specific for hepatitis C virus using MHC class II tetramers. *J Clin Invest* 112:831-842.
201. Somsouk, M., G.M. Lauer, D. Casson, A. Terella, C.L. Day, B.D. Walker, and R.T. Chung. 2003. Spontaneous resolution of chronic hepatitis C virus disease after withdrawal of immunosuppression. *Gastroenterology* 124:1946-1949.
202. Day, C.L., G.M. Lauer, G.K. Robbins, B. McGovern, A.G. Wurcel, R.T. Gandhi, R.T. Chung, and B.D. Walker. 2002. Broad specificity of virus-specific CD4+ T-helper-cell responses in resolved hepatitis C virus infection. *J Virol* 76:12584-12595.
203. Barré-Sinoussi, F., J.C. Chermann, F. Rey, M.T. Nugeyre, S. Chamaret, J. Gruest, C. Dauguet, C. Axler-Blin, F. Vézinet-Brun, C. Rouzioux, W. Rozenbaum, and L. Montagnier. 1983. Isolation of a T-lymphotropic retrovirus from a patient at risk for acquired immune deficiency syndrome (AIDS). *Science* 220:868-871.
204. Gallo, R.C., S.Z. Salahuddin, M. Popovic, G.M. Shearer, M. Kaplan, B.F. Haynes, T.J. Palker, R. Redfield, J. Oleske, and B. Safai. 1984. Frequent detection and isolation of cytopathic retroviruses (HTLV-III) from patients with AIDS and at risk for AIDS. *Science* 224:500-503.
205. Frankel, A.D., and J.A. Young. 1998. HIV-1: fifteen proteins and an RNA. *Annu Rev Biochem* 67:1-25.
206. Montagnier, L., J.C. Chermann, F. Barré-Sinoussi, D. Klatzmann, S. Wain-Hobson, M. Alizon, F. Clavel, F. Brun-Vezinet, E. Vilmer, and C. Rouzioux. 1984. Lymphadenopathy associated virus and its etiological role in AIDS. *Int Symp Princess Takamatsu Cancer Res Fund* 15:319-331.
207. Melbye, M. 1986. The natural history of human T lymphotropic virus-III infection: the cause of AIDS. *British medical journal (Clinical research ed)* 292:5-12.
208. Dragic, T., V. Litwin, G.P. Allaway, S.R. Martin, Y. Huang, K.A. Nagashima, C. Cayanan, P.J. Maddon, R.A. Koup, J.P. Moore, and W.A. Paxton. 1996. HIV-1 entry into CD4+ cells is mediated by the chemokine receptor CC-CKR-5. *Nature* 381:667-673.

209. Choe, H., M. Farzan, Y. Sun, N. Sullivan, B. Rollins, P.D. Ponath, L. Wu, C.R. Mackay, G. LaRosa, W. Newman, N. Gerard, C. Gerard, and J. Sodroski. 1996. The beta-chemokine receptors CCR3 and CCR5 facilitate infection by primary HIV-1 isolates. *Cell* 85:1135-1148.
210. Louwagie, J., W. Janssens, J. Mascola, L. Heyndrickx, P. Hegerich, G. van der Groen, F.E. McCutchan, and D.S. Burke. 1995. Genetic diversity of the envelope glycoprotein from human immunodeficiency virus type 1 isolates of African origin. *J Virol* 69:263-271.
211. Gottlieb, M.S., R. Schroff, H.M. Schanker, J.D. Weisman, P.T. Fan, R.A. Wolf, and A. Saxon. 1981. Pneumocystis carinii pneumonia and mucosal candidiasis in previously healthy homosexual men: evidence of a new acquired cellular immunodeficiency. *N Engl J Med* 305:1425-1431.
212. Friedman-Kien, A.E., L.J. Laubenstein, P. Rubinstein, E. Buimovici-Klein, M. Marmor, R. Stahl, I. Spigland, K.S. Kim, and S. Zolla-Pazner. 1982. Disseminated Kaposi's sarcoma in homosexual men. *Ann Intern Med* 96:693-700.
213. (CDC), C.f.D.C. 1981. Kaposi's sarcoma and Pneumocystis pneumonia among homosexual men--New York City and California. *MMWR Morb Mortal Wkly Rep* 30:305-308.
214. (CDC), C.f.D.C. 1981. Pneumocystis pneumonia--Los Angeles. *MMWR Morb Mortal Wkly Rep* 30:250-252.
215. UNAIDS. 2006. EPI Update 2006 - Full report in english.
216. Cranage, M., S. Sharpe, C. Herrera, A. Cope, M. Dennis, N. Berry, C. Ham, J. Heeney, N. Rezk, A. Kashuba, P. Anton, I. McGowan, and R. Shattock. 2008. Prevention of SIV rectal transmission and priming of T cell responses in macaques after local pre-exposure application of tenofovir gel. *PLoS Med* 5:e157; discussion e157.
217. Tévi-Benissan, C., M. Makuva, MorelliA, M.C. Georges-Courbot, M. Matta, A. Georges, and L. Bélec. 2000. Protection of cynomolgus macaque against cervicovaginal transmission of SIVmac251 by the spermicide benzalkonium chloride. *J Acquir Immune Defic Syndr* 24:147-153.
218. Williams, B.G., J.O. Lloyd-Smith, E. Gouws, C. Hankins, W.M. Getz, J. Hargrove, I. de Zoysa, C. Dye, and B. Auvert. 2006. The potential impact of male circumcision on HIV in Sub-Saharan Africa. *PLoS Med* 3:e262.
219. Gray, R.H., G. Kigozi, D. Serwadda, F. Makumbi, S. Watya, F. Nalugoda, N. Kiwanuka, L.H. Moulton, M.A. Chaudhary, M.Z. Chen, N.K. Sewankambo, F. Wabwire-Mangen, M.C. Bacon, C.F. Williams, P. Opendi, S.J. Reynolds, O. Laeyendecker, T.C. Quinn, and M.J. Wawer. 2007. Male circumcision for HIV prevention in men in Rakai, Uganda: a randomised trial. *Lancet* 369:657-666.
220. Bailey, R.C., S. Moses, C.B. Parker, K. Agot, I. Maclean, J.N. Krieger, C.F. Williams, R.T. Campbell, and J.O. Ndinya-Achola. 2007. Male circumcision for HIV prevention in young men in Kisumu, Kenya: a randomised controlled trial. *Lancet* 369:643-656.
221. Lehman, D.A., and C. Farquhar. 2007. Biological mechanisms of vertical human immunodeficiency virus (HIV-1) transmission. *Rev Med Virol* 17:381-403.
222. Taiwo, B.O., and C.B. Hicks. 2002. Primary human immunodeficiency virus. *South Med J* 95:1312-1317.
223. Weber, J. 2001. The pathogenesis of HIV-1 infection. *Br Med Bull* 58:61-72.
224. Koup, R.A., J.T. Safrit, Y. Cao, C.A. Andrews, G. McLeod, W. Borkowsky, C. Farthing, and D.D. Ho. 1994. Temporal association of cellular immune responses with the initial control of viremia in primary human immunodeficiency virus type 1 syndrome. *J Virol* 68:4650-4655.
225. McMichael, A.J. 1996. HIV. The immune response. *Curr Opin Immunol* 8:537-539.
226. Williams, A.B. 1997. New horizons: antiretroviral therapy in 1997. *The Journal of the Association of Nurses in AIDS Care : JANAC* 8:26-38.
227. Richman, D.D. 2001. HIV chemotherapy. *Nature* 410:995-1001.
228. Hammer, S.M., J.J. Eron, P. Reiss, Schooley, M.A. Thompson, S. Walmsley, P. Cahn, M.A. Fischl, J.M. Gatell, M.S. Hirsch, D.M. Jacobsen, J.S. Montaner, D.D. Richman, P.G. Yeni, P.A. Volberding, and I.A. Society-USA. 2008. Antiretroviral treatment of adult HIV infection: 2008 recommendations of the International AIDS Society-USA panel. *JAMA* 300:555-570.
229. Qian, K., S.L. Morris-Natschke, and K.H. Lee. 2008. HIV entry inhibitors and their potential in HIV therapy. *Med Res Rev*

230. Flowers, C.C., C. Woffendin, J. Petryniak, S. Yang, and G.J. Nabel. 1997. Inhibition of recombinant human immunodeficiency virus type 1 replication by a site-specific recombinase. *J Virol* 71:2685-2692.
231. Sarkar, I., I. Hauber, J. Hauber, and F. Buchholz. 2007. HIV-1 proviral DNA excision using an evolved recombinase. *Science* 316:1912-1915.
232. Scott-Algara, D., L.X. Truong, P. Versmisse, A. David, T.T. Luong, N.V. Nguyen, I. Theodorou, F. Barré-Sinoussi, and G. Pancino. 2003. Cutting edge: increased NK cell activity in HIV-1-exposed but uninfected Vietnamese intravascular drug users. *J Immunol* 171:5663-5667.
233. Ravet, S., D. Scott-Algara, E. Bonnet, H.K. Tran, T. Tran, N. Nguyen, L.X. Truong, I. Theodorou, F. Barré-Sinoussi, G. Pancino, and P. Paul. 2007. Distinctive NK-cell receptor repertoires sustain high-level constitutive NK-cell activation in HIV-exposed uninfected individuals. *Blood* 109:4296-4305.
234. Fehniger, T.A., G. Herbein, H. Yu, M.I. Para, Z.P. Bernstein, W.A. O'Brien, and M.A. Caligiuri. 1998. Natural killer cells from HIV-1+ patients produce C-C chemokines and inhibit HIV-1 infection. *J Immunol* 161:6433-6438.
235. Alkhatib, G., C. Combadiere, C.C. Broder, Y. Feng, P.E. Kennedy, P.M. Murphy, and E.A. Berger. 1996. CC CKR5: a RANTES, MIP-1alpha, MIP-1beta receptor as a fusion cofactor for macrophage-tropic HIV-1. *Science* 272:1955-1958.
236. Kottilil, S., T.W. Chun, S. Moir, S. Liu, M. McLaughlin, C.W. Hallahan, F. Maldarelli, L. Corey, and A.S. Fauci. 2003. Innate immunity in human immunodeficiency virus infection: effect of viremia on natural killer cell function. *J Infect Dis* 187:1038-1045.
237. Oliva, A., A.L. Kinter, M. Vaccarezza, A. Rubbert, A. Catanzaro, S. Moir, J. Monaco, L. Ehler, S. Mizell, R. Jackson, Y. Li, J.W. Romano, and A.S. Fauci. 1998. Natural killer cells from human immunodeficiency virus (HIV)-infected individuals are an important source of CC-chemokines and suppress HIV-1 entry and replication in vitro. *J Clin Invest* 102:223-231.
238. Bluman, E.M., K.J. Bartynski, B.R. Avalos, and M.A. Caligiuri. 1996. Human natural killer cells produce abundant macrophage inflammatory protein-1 alpha in response to monocyte-derived cytokines. *J Clin Invest* 97:2722-2727.
239. Alter, G., T.J. Suscovich, N. Teigen, A. Meier, H. Streeck, C. Brander, and M. Altfeld. 2007. Single-stranded RNA derived from HIV-1 serves as a potent activator of NK cells. *J Immunol* 178:7658-7666.
240. Iannello, A., O. Debbeche, S. Samarani, and A. Ahmad. 2008. Antiviral NK cell responses in HIV infection: I. NK cell receptor genes as determinants of HIV resistance and progression to AIDS. *J Leukoc Biol*
241. Martin, M.P., X. Gao, J.H. Lee, G.W. Nelson, R. Detels, J.J. Goedert, S. Buchbinder, K. Hoots, D. Vlahov, J. Trowsdale, M. Wilson, S.J. O'Brien, and M. Carrington. 2002. Epistatic interaction between KIR3DS1 and HLA-B delays the progression to AIDS. *Nat Genet* 31:429-434.
242. Flores-Villanueva, P.O., E.J. Yunis, J.C. Delgado, E. Vittinghoff, S. Buchbinder, J.Y. Leung, A.M. Uglialoro, O.P. Clavijo, E.S. Rosenberg, S.A. Kalams, J.D. Braun, S.L. Boswell, B.D. Walker, and A.E. Goldfeld. 2001. Control of HIV-1 viremia and protection from AIDS are associated with HLA-Bw4 homozygosity. *Proc Natl Acad Sci USA* 98:5140-5145.
243. Thomas, R., E. Yamada, G. Alter, M.P. Martin, A.A. Bashirova, P.J. Norman, M. Altfeld, P. Parham, S.K. Anderson, D.W. McVicar, and M. Carrington. 2008. Novel KIR3DL1 alleles and their expression levels on NK cells: convergent evolution of KIR3DL1 phenotype variation? *J Immunol* 180:6743-6750.
244. Fortis, C., P. Biswas, L. Soldini, F. Veglia, A.M. Careddu, F. Delfanti, B. Mantelli, M. Murone, A. Lazzarin, and G. Poli. 1999. Dual role of TNF-alpha in NK/LAK cell-mediated lysis of chronically HIV-infected U1 cells. Concomitant enhancement of HIV expression and sensitization of cell-mediated lysis. *Eur J Immunol* 29:3654-3662.
245. Sirianni, M.C., G. De Sanctis, B. Macchi, S. Soddu, F. Ensoli, F. Aiuti, and L. Fontana. 1988. Natural killer activity from normal peripheral blood lymphocytes against a human T lymphotropic retrovirus type III (HTLV-III)-infected cell line. *Diagnostic and clinical immunology* 5:297-303.
246. Bandyopadhyay, S., U. Ziegner, D.E. Campbell, D.S. Miller, J.A. Hoxie, and S.E. Starr. 1990. Natural killer cell-mediated lysis of T cell lines chronically infected with HIV-1. *Clin Exp Immunol* 79:430-435.

247. Cohen, G.B., R.T. Gandhi, D.M. Davis, O. Mandelboim, B.K. Chen, J.L. Strominger, and D. Baltimore. 1999. The selective downregulation of class I major histocompatibility complex proteins by HIV-1 protects HIV-infected cells from NK cells. *Immunity* 10:661-671.
248. Bonaparte, M.I., and E. Barker. 2004. Killing of human immunodeficiency virus-infected primary T-cell blasts by autologous natural killer cells is dependent on the ability of the virus to alter the expression of major histocompatibility complex class I molecules. *Blood* 104:2087-2094.
249. Bonaparte, M.I., and E. Barker. 2003. Inability of natural killer cells to destroy autologous HIV-infected T lymphocytes. *AIDS* 17:487-494.
250. Ward, J.P., M.I. Bonaparte, and E. Barker. 2004. HLA-C and HLA-E reduce antibody-dependent natural killer cell-mediated cytotoxicity of HIV-infected primary T cell blasts. *AIDS* 18:1769-1779.
251. Mavilio, D., J. Benjamin, M. Daucher, G. Lombardo, S. Kottilil, M.A. Planta, E. Marcenaro, C. Bottino, L. Moretta, A. Moretta, and A.S. Fauci. 2003. Natural killer cells in HIV-1 infection: dichotomous effects of viremia on inhibitory and activating receptors and their functional correlates. *Proc Natl Acad Sci USA* 100:15011-15016.
252. Kottilil, S. 2003. Natural killer cells in HIV-1 infection: role of NK cell-mediated non-cytolytic mechanisms in pathogenesis of HIV-1 infection. *Indian J Exp Biol* 41:1219-1225.
253. Fauci, A.S., D. Mavilio, and S. Kottilil. 2005. NK cells in HIV infection: paradigm for protection or targets for ambush. *Nat Rev Immunol* 5:835-843.
254. Iannello, A., O. Debbeche, S. Samarani, and A. Ahmad. 2008. Antiviral NK cell responses in HIV infection: II. viral strategies for evasion and lessons for immunotherapy and vaccination. *J Leukoc Biol*
255. Gumá, M., C. Cabrera, I. Erkizia, M. Bofill, B. Clotet, L. Ruiz, and M. López-Botet. 2006. Human cytomegalovirus infection is associated with increased proportions of NK cells that express the CD94/NKG2C receptor in aviremic HIV-1-positive patients. *J Infect Dis* 194:38-41.
256. Fogli, M., P. Costa, G. Murdaca, M. Setti, M.C. Mingari, L. Moretta, A. Moretta, and A. De Maria. 2004. Significant NK cell activation associated with decreased cytolytic function in peripheral blood of HIV-1-infected patients. *Eur J Immunol* 34:2313-2321.
257. Ward, J., M. Bonaparte, J. Sacks, J. Guterman, M. Fogli, D. Mavilio, and E. Barker. 2007. HIV modulates the expression of ligands important in triggering natural killer cell cytotoxic responses on infected primary T-cell blasts. *Blood*
258. De Maria, A., M. Fogli, P. Costa, G. Murdaca, F. Puppo, D. Mavilio, A. Moretta, and L. Moretta. 2003. The impaired NK cell cytolytic function in viremic HIV-1 infection is associated with a reduced surface expression of natural cytotoxicity receptors (NKp46, NKp30 and NKp44). *Eur J Immunol* 33:2410-2418.
259. De Maria, A., R. Biassoni, M. Fogli, M. Rizzi, C. Cantoni, P. Costa, R. Conte, D. Mavilio, B. Ensoli, A. Cafaro, A. Moretta, and L. Moretta. 2001. Identification, molecular cloning and functional characterization of NKp46 and NKp30 natural cytotoxicity receptors in Macaca fascicularis NK cells. *Eur J Immunol* 31:3546-3556.
260. Kottilil, S., K. Shin, M. Planta, M. McLaughlin, C.W. Hallahan, M. Ghany, T.W. Chun, M.C. Sneller, and A.S. Fauci. 2004. Expression of chemokine and inhibitory receptors on natural killer cells: effect of immune activation and HIV viremia. *J Infect Dis* 189:1193-1198.
261. Ahmad, R., S.T. Sindhu, P. Tran, E. Toma, R. Morisset, J. Menezes, and A. Ahmad. 2001. Modulation of expression of the MHC class I-binding natural killer cell receptors, and NK activity in relation to viral load in HIV-infected/AIDS patients. *J Med Virol* 65:431-440.
262. Søndergaard, S.R., H. Aladdin, H. Ullum, J. Gerstoft, P. Skinhøj, and B.K. Pedersen. 1999. Immune function and phenotype before and after highly active antiretroviral therapy. *J Acquir Immune Defic Syndr* 21:376-383.
263. Parato, K.G., A. Kumar, A.D. Badley, J.L. Sanchez-Dardon, K.A. Chambers, C.D. Young, W.T. Lim, S. Kravcik, D.W. Cameron, and J.B. Angel. 2002. Normalization of natural killer cell function and phenotype with effective anti-HIV therapy and the role of IL-10. *AIDS* 16:1251-1256.
264. Costa, P., S. Rusconi, D. Mavilio, M. Fogli, G. Murdaca, D. Pende, M.C. Mingari, M. Galli, L. Moretta, and A. De Maria. 2001. Differential disappearance of inhibitory natural

killer cell receptors during HAART and possible impairment of HIV-1-specific CD8 cytotoxic T lymphocytes. *AIDS* 15:965-974.
265. Mattapallil, J.J., D.C. Douek, B. Hill, Y. Nishimura, M. Martin, and M. Roederer. 2005. Massive infection and loss of memory CD4+ T cells in multiple tissues during acute SIV infection. *Nature* 434:1093-1097.
266. Brenchley, J.M., D.A. Price, and D.C. Douek. 2006. HIV disease: fallout from a mucosal catastrophe? *Nat Immunol* 7:235-239.
267. Brenchley, J.M., T.W. Schacker, L.E. Ruff, D.A. Price, J.H. Taylor, G.J. Beilman, P.L. Nguyen, A. Khoruts, M. Larson, A.T. Haase, and D.C. Douek. 2004. CD4+ T cell depletion during all stages of HIV disease occurs predominantly in the gastrointestinal tract. *J Exp Med* 200:749-759.
268. Douek, D.C., J.M. Brenchley, M.R. Betts, D.R. Ambrozak, B.J. Hill, Y. Okamoto, J.P. Casazza, J. Kuruppu, K. Kunstman, S. Wolinsky, Z. Grossman, M. Dybul, A. Oxenius, D.A. Price, M. Connors, and R.A. Koup. 2002. HIV preferentially infects HIV-specific CD4+ T cells. *Nature* 417:95-98.
269. Anderson, R.W., M.S. Ascher, and H.W. Sheppard. 1998. Direct HIV cytopathicity cannot account for CD4 decline in AIDS in the presence of homeostasis: a worst-case dynamic analysis. *J Acquir Immune Defic Syndr Hum Retrovirol* 17:245-252.
270. Douek, D.C., L.J. Picker, and R.A. Koup. 2003. T cell dynamics in HIV-1 infection. *Annu Rev Immunol* 21:265-304.
271. Hazenberg, M.D., J.W. Stuart, S.A. Otto, J.C. Borleffs, C.A. Boucher, R.J. de Boer, F. Miedema, and D. Hamann. 2000. T-cell division in human immunodeficiency virus (HIV)-1 infection is mainly due to immune activation: a longitudinal analysis in patients before and during highly active antiretroviral therapy (HAART). *Blood* 95:249-255.
272. McCune, J.M. 2001. The dynamics of CD4+ T-cell depletion in HIV disease. *Nature* 410:974-979.
273. Grossman, Z., M. Meier-Schellersheim, A.E. Sousa, R.M. Victorino, and W.E. Paul. 2002. CD4+ T-cell depletion in HIV infection: are we closer to understanding the cause? *Nat Med* 8:319-323.
274. Douek, D.C., M.R. Betts, B.J. Hill, S.J. Little, R. Lempicki, J.A. Metcalf, J. Casazza, C. Yoder, J.W. Adelsberger, R.A. Stevens, M.W. Baseler, P. Keiser, D.D. Richman, R.T. Davey, and R.A. Koup. 2001. Evidence for increased T cell turnover and decreased thymic output in HIV infection. *J Immunol* 167:6663-6668.
275. Lempicki, R.A., J.A. Kovacs, M.W. Baseler, J.W. Adelsberger, R.L. Dewar, V. Natarajan, M.C. Bosche, J.A. Metcalf, R.A. Stevens, L.A. Lambert, W.G. Alvord, M.A. Polis, R.T. Davey, D.S. Dimitrov, and H.C. Lane. 2000. Impact of HIV-1 infection and highly active antiretroviral therapy on the kinetics of CD4+ and CD8+ T cell turnover in HIV-infected patients. *Proc Natl Acad Sci USA* 97:13778-13783.
276. Palmer, B.E., N. Blyveis, A.P. Fontenot, and C.C. Wilson. 2005. Functional and phenotypic characterization of CD57+CD4+ T cells and their association with HIV-1-induced T cell dysfunction. *J Immunol* 175:8415-8423.
277. Boritz, E., B.E. Palmer, and C.C. Wilson. 2004. Human immunodeficiency virus type 1 (HIV-1)-specific CD4+ T cells that proliferate in vitro detected in samples from most viremic subjects and inversely associated with plasma HIV-1 levels. *J Virol* 78:12638-12646.
278. Younes, S.A., B. Yassine-Diab, A.R. Dumont, M.R. Boulassel, Z. Grossman, J.P. Routy, and R.P. Sekaly. 2003. HIV-1 viremia prevents the establishment of interleukin 2-producing HIV-specific memory CD4+ T cells endowed with proliferative capacity. *J Exp Med* 198:1909-1922.
279. Harari, A., S. Petitpierre, F. Vallelian, and G. Pantaleo. 2004. Skewed representation of functionally distinct populations of virus-specific CD4 T cells in HIV-1-infected subjects with progressive disease: changes after antiretroviral therapy. *Blood* 103:966-972.
280. Iyasere, C., J.C. Tilton, A.J. Johnson, S. Younes, B. Yassine-Diab, R.P. Sekaly, W.W. Kwok, S.A. Migueles, A.C. Laborico, W.L. Shupert, C.W. Hallahan, R.T. Davey, M. Dybul, S. Vogel, J. Metcalf, and M. Connors. 2003. Diminished proliferation of human immunodeficiency virus-specific CD4+ T cells is associated with diminished interleukin-2 (IL-2) production and is recovered by exogenous IL-2. *J Virol* 77:10900-10909.
281. Imami, N., A. Pires, G. Hardy, J. Wilson, B. Gazzard, and F. Gotch. 2002. A balanced type 1/type 2 response is associated with long-term nonprogressive human immunodeficiency virus type 1 infection. *J Virol* 76:9011-9023.

282. Rosenberg, E.S., J.M. Billingsley, A.M. Caliendo, S.L. Boswell, P.E. Sax, S.A. Kalams, and B.D. Walker. 1997. Vigorous HIV-1-specific CD4+ T cell responses associated with control of viremia. *Science* 278:1447-1450.
283. Kalams, S.A., and B.D. Walker. 1998. The critical need for CD4 help in maintaining effective cytotoxic T lymphocyte responses. *J Exp Med* 188:2199-2204.
284. Borrow, P., H. Lewicki, B.H. Hahn, G.M. Shaw, and M.B. Oldstone. 1994. Virus-specific CD8+ cytotoxic T-lymphocyte activity associated with control of viremia in primary human immunodeficiency virus type 1 infection. *J Virol* 68:6103-6110.
285. Jin, X., D.E. Bauer, S.E. Tuttleton, S. Lewin, A. Gettie, J. Blanchard, C.E. Irwin, J.T. Safrit, J. Mittler, L. Weinberger, L.G. Kostrikis, Zhang, A.S. Perelson, and D.D. Ho. 1999. Dramatic rise in plasma viremia after CD8(+) T cell depletion in simian immunodeficiency virus-infected macaques. *J Exp Med* 189:991-998.
286. Schmitz, J.E., M.J. Kuroda, S. Santra, V.G. Sasseville, M.A. Simon, M.A. Lifton, P. Racz, K. Tenner-Racz, M. Dalesandro, B.J. Scallon, J. Ghrayeb, M.A. Forman, D.C. Montefiori, E.P. Rieber, N.L. Letvin, and K.A. Reimann. 1999. Control of viremia in simian immunodeficiency virus infection by CD8+ lymphocytes. *Science* 283:857-860.
287. Yang, O.O., S.A. Kalams, M. Rosenzweig, A. Trocha, N. Jones, M. Koziel, B.D. Walker, and R.P. Johnson. 1996. Efficient lysis of human immunodeficiency virus type 1-infected cells by cytotoxic T lymphocytes. *J Virol* 70:5799-5806.
288. Kaslow, R.A., M. Carrington, R. Apple, L. Park, A. Muñoz, A.J. Saah, J.J. Goedert, C. Winkler, S.J. O'Brien, C. Rinaldo, R. Detels, W. Blattner, J. Phair, H. Erlich, and D.L. Mann. 1996. Influence of combinations of human major histocompatibility complex genes on the course of HIV-1 infection. *Nat Med* 2:405-411.
289. Carrington, M., G.W. Nelson, M.P. Martin, T. Kissner, D. Vlahov, J.J. Goedert, R. Kaslow, S. Buchbinder, K. Hoots, and S.J. O'Brien. 1999. HLA and HIV-1: heterozygote advantage and B*35-Cw*04 disadvantage. *Science* 283:1748-1752.
290. Wang, Y.J., B. Li, J.M. Carlson, H. Streeck, A.D. Gladden, R. Goodman, A. Schneidewind, K.A. Power, I. Toth, N. Frahm, G. Alter, C. Brander, M. Carrington, B. Walker, M. Altfeld, D. Heckerman, and T.M. Allen. 2008. Protective HLA Class I Alleles Restricting Acute-Phase CD8+ T Cell Responses are Associated with Viral Escape Mutations Located in Highly Conserved Regions of HIV-1. *J Virol*
291. Duda, A., L. Turner, J. Fox, N. Robinson, S. Dustan, S. Kaye, H. Fryer, M. Carrington, M. McClure, A.R. McLean, S. Fidler, J. Weber, R. Phillips, A.J. Frater, and t.S.T. Investigators. 2008. HLA-ASSOCIATED CLINICAL PROGRESSION CORRELATES WITH EPITOPE REVERSION RATES IN EARLY HIV INFECTION. *J Virol*
292. Altman, J.D., P.A. Moss, P.J. Goulder, D.H. Barouch, M.G. McHeyzer-Williams, J.I. Bell, A.J. McMichael, and M.M. Davis. 1996. Phenotypic analysis of antigen-specific T lymphocytes. *Science* 274:94-96.
293. Ogg, G.S., X. Jin, S. Bonhoeffer, P.R. Dunbar, M.A. Nowak, S. Monard, J.P. Segal, Y. Cao, S.L. Rowland-Jones, V. Cerundolo, A. Hurley, M. Markowitz, D.D. Ho, D.F. Nixon, and A.J. McMichael. 1998. Quantitation of HIV-1-specific cytotoxic T lymphocytes and plasma load of viral RNA. *Science* 279:2103-2106.
294. Collins, K.L., B.K. Chen, S.A. Kalams, B.D. Walker, and D. Baltimore. 1998. HIV-1 Nef protein protects infected primary cells against killing by cytotoxic T lymphocytes. *Nature* 391:397-401.
295. Johnson, W.E., and R.C. Desrosiers. 2002. Viral persistance: HIV's strategies of immune system evasion. *Annu Rev Med* 53:499-518.
296. McMichael, A.J., and R.E. Phillips. 1997. Escape of human immunodeficiency virus from immune control. *Annu Rev Immunol* 15:271-296.
297. Allen, T.M., D.H. O'Connor, P. Jing, J.L. Dzuris, B.R. Mothé, T.U. Vogel, E. Dunphy, M.E. Liebl, C. Emerson, N. Wilson, K.J. Kunstman, X. Wang, D.B. Allison, A.L. Hughes, R.C. Desrosiers, J.D. Altman, S.M. Wolinsky, A. Sette, and D.I. Watkins. 2000. Tat-specific cytotoxic T lymphocytes select for SIV escape variants during resolution of primary viraemia. *Nature* 407:386-390.
298. Zajac, A.J., J.N. Blattman, K. Murali-Krishna, D.J. Sourdive, M. Suresh, J.D. Altman, and R. Ahmed. 1998. Viral immune evasion due to persistence of activated T cells without effector function. *J Exp Med* 188:2205-2213.
299. Trimble, L.A., and Lieberman. 1998. Circulating CD8 T lymphocytes in human immunodeficiency virus-infected individuals have impaired function and downmodulate CD3 zeta, the signaling chain of the T-cell receptor complex. *Blood* 91:585-594.

300. Appay, V., D.F. Nixon, S.M. Donahoe, G.M. Gillespie, T. Dong, A. King, G.S. Ogg, H.M. Spiegel, C. Conlon, C.A. Spina, D.V. Havlir, D.D. Richman, A. Waters, P. Easterbrook, A.J. McMichael, and S.L. Rowland-Jones. 2000. HIV-specific CD8(+) T cells produce antiviral cytokines but are impaired in cytolytic function. *J Exp Med* 192:63-75.
301. Andersson, Behbahani, Lieberman, Connick, Landay, Patterson, Sönnerborg, Loré, Uccini, and Fehniger. 1999. Perforin is not co-expressed with granzyme A within cytotoxic granules in CD8 T lymphocytes present in lymphoid tissue during chronic HIV infection. *AIDS* 13:1295-1303.
302. Champagne, P., G.S. Ogg, A.S. King, C. Knabenhans, K. Ellefsen, M. Nobile, V. Appay, G.P. Rizzardi, S. Fleury, M. Lipp, R. Förster, S. Rowland-Jones, R.P. Sékaly, A.J. McMichael, and G. Pantaleo. 2001. Skewed maturation of memory HIV-specific CD8 T lymphocytes. *Nature* 410:106-111.
303. Day, C.L., D.E. Kaufmann, P. Kiepiela, J.A. Brown, E.S. Moodley, S. Reddy, E.W. Mackey, J.D. Miller, A.J. Leslie, C. DePierres, Z. Mncube, J. Duraiswamy, B. Zhu, Q. Eichbaum, M. Altfeld, E.J. Wherry, H.M. Coovadia, P.J. Goulder, P. Klenerman, R. Ahmed, G.J. Freeman, and B.D. Walker. 2006. PD-1 expression on HIV-specific T cells is associated with T-cell exhaustion and disease progression. *Nature* 443:350-354.
304. Shin, H., and E.J. Wherry. 2007. CD8 T cell dysfunction during chronic viral infection. *Curr Opin Immunol*
305. Maier, H., M. Isogawa, G.J. Freeman, and F.V. Chisari. 2007. PD-1:PD-L1 interactions contribute to the functional suppression of virus-specific CD8+ T lymphocytes in the liver. *J Immunol* 178:2714-2720.
306. Brenchley, J.M., D.A. Price, T.W. Schacker, T.E. Asher, G. Silvestri, S. Rao, Z. Kazzaz, E. Bornstein, O. Lambotte, D. Altmann, B.R. Blazar, B. Rodriguez, L. Teixeira-Johnson, A. Landay, J.N. Martin, F.M. Hecht, L.J. Picker, M.M. Lederman, S.G. Deeks, and D.C. Douek. 2006. Microbial translocation is a cause of systemic immune activation in chronic HIV infection. *Nat Med* 12:1365-1371.
307. Zhang, J.Y., Z. Zhang, X. Wang, J.L. Fu, J. Yao, Y. Jiao, L. Chen, H. Zhang, J. Wei, L. Jin, M. Shi, G.F. Gao, H. Wu, and F.S. Wang. 2007. PD-1 up-regulation is correlated with HIV-specific memory CD8+ T-cell exhaustion in typical progressors but not in long-term nonprogressors. *Blood* 109:4671-4678.
308. Sherman, K.E., S.D. Rouster, R.T. Chung, and N. Rajicic. 2002. Hepatitis C Virus prevalence among patients infected with Human Immunodeficiency Virus: a cross-sectional analysis of the US adult AIDS Clinical Trials Group. *Clin Infect Dis* 34:831-837.
309. Monforte, A., D. Abrams, C. Pradier, R. Weber, P. Reiss, F. Bonnet, O. Kirk, M. Law, S. De Wit, N. Friis-Møller, A.N. Phillips, C.A. Sabin, J.D. Lundgren, and D.C.o.A.E.o.A.-H.D.D.A.D.S. Group. 2008. HIV-induced immunodeficiency and mortality from AIDS-defining and non-AIDS-defining malignancies. *AIDS* 22:2143-2153.
310. Bica, I., B. McGovern, R. Dhar, D. Stone, K. McGowan, R. Scheib, and D.R. Snydman. 2001. Increasing mortality due to end-stage liver disease in patients with human immunodeficiency virus infection. *Clin Infect Dis* 32:492-497.
311. Daar, E.S., H. Lynn, S. Donfield, E. Gomperts, M.W. Hilgartner, W.K. Hoots, D. Chernoff, S. Arkin, W.Y. Wong, C.A. Winkler, and H.G.a.D. Study. 2001. Relation between HIV-1 and hepatitis C viral load in patients with hemophilia. *J Acquir Immune Defic Syndr* 26:466-472.
312. Daar, E.S., H. Lynn, S. Donfield, E. Gomperts, S.J. O'Brien, M.W. Hilgartner, W.K. Hoots, D. Chernoff, S. Arkin, W.Y. Wong, C.A. Winkler, and H.G.a.D. Study. 2001. Hepatitis C virus load is associated with human immunodeficiency virus type 1 disease progression in hemophiliacs. *J Infect Dis* 183:589-595.
313. Thomas, D.L., J. Astemborski, D. Vlahov, S.A. Strathdee, S.C. Ray, K.E. Nelson, N. Galai, K.R. Nolt, O. Laeyendecker, and J.A. Todd. 2000. Determinants of the quantity of hepatitis C virus RNA. *J Infect Dis* 181:844-851.
314. Harcourt, G., E. Gomperts, S. Donfield, and P. Klenerman. 2006. Diminished frequency of hepatitis C virus specific interferon gamma secreting CD4+ T cells in human immunodeficiency virus/hepatitis C virus coinfected patients. *Gut* 55:1484-1487.
315. Kim, A.Y., G.M. Lauer, K. Ouchi, M.M. Addo, Lucas, J. Schulze zur Wiesch, J. Timm, M. Boczanowski, J.E. Duncan, A.G. Wurcel, D. Casson, R.T. Chung, R. Draenert, P. Klenerman, and B.D. Walker. 2005. The magnitude and breadth of hepatitis C virus-

specific CD8+ T cells depend on absolute CD4+ T-cell count in individuals coinfected with HIV-1. *Blood* 105:1170-1178.

316. Capa, L., V. Soriano, J. García-Samaniego, M. Nuñez, M. Romero, C. de Mendoza, A. Cascajero, F. Muñoz, J. González-Lahoz, and J.M. Benito. 2007. Evolution of T-cell responses to hepatitis C virus (HCV) during pegylated interferon plus ribavirin treatment in HCV-monoinfected and in HCV/HIV-coinfected patients. *Antivir Ther (Lond)* 12:459-468.
317. Kim, A.Y., J. Schulze zur Wiesch, T. Kuntzen, J. Timm, D.E. Kaufmann, J.E. Duncan, A.M. Jones, A.G. Wurcel, B.T. Davis, R.T. Gandhi, G.K. Robbins, T.M. Allen, R.T. Chung, G.M. Lauer, and B.D. Walker. 2006. Impaired hepatitis C virus-specific T cell responses and recurrent hepatitis C virus in HIV coinfection. *PLoS Med* 3:e492.
318. Dutoit, V., D. Ciuffreda, D. Comte, J. Gonvers, and Pantaleo. 2005. Differences in HCV-specific T cell responses between chronic HCV infection and HIV/HCV co-infection. *Eur J Immunol* 35:3493-3504.
319. Ciuffreda, D., D. Comte, M. Cavassini, E. Giostra, L. Bühler, M. Perruchoud, M.H. Heim, M. Battegay, D. Genné, B. Mulhaupt, R. Malinverni, C. Oneta, E. Bernasconi, M. Monnat, A. Cerny, C. Chuard, J. Borovicka, G. Mentha, M. Pascual, J. Gonvers, G. Pantaleo, and V. Dutoit. 2008. Polyfunctional HCV-specific T-cell responses are associated with effective control of HCV replication. *Eur J Immunol* 38:2665-2677.
320. Miller, M.F., C. Haley, M.J. Koziel, and C.F. Rowley. 2005. Impact of hepatitis C virus on immune restoration in HIV-infected patients who start highly active antiretroviral therapy: a meta-analysis. *Clin Infect Dis* 41:713-720.
321. Kottilil, S., S. Jagannatha, A. Lu, S. Liu, M. McLaughlin, J.A. Metcalf, R. Dewar, C. Campbell, C. Koratich, F. Maldarelli, H. Masur, and M.A. Polis. 2004. Changes in hepatitis C viral response after initiation of highly active antiretroviral therapy and control of HIV viremia in chronically co-infected individuals. *HIV clinical trials* 5:25-32.
322. Chung, R.T., S.R. Evans, Y. Yang, D. Theodore, H. Valdez, R. Clark, C. Shikuma, T. Nevin, K.E. Sherman, and A.C.T.G.S. Team. 2002. Immune recovery is associated with persistent rise in hepatitis C virus RNA, infrequent liver test flares, and is not impaired by hepatitis C virus in co-infected subjects. *AIDS* 16:1915-1923.
323. Gucker, F.T., and C.T. O'Konski. 1949. Electronic methods of counting aerosol particles. *Chem Rev* 44:373-388.
324. Hulett, H.R., W.A. Bonner, J. Barrett, and L.A. Herzenberg. 1969. Cell sorting: automated separation of mammalian cells as a function of intracellular fluorescence. *Science* 166:747-749.
325. Herzenberg, L.A., D. Parks, B. Sahaf, O. Perez, M. Roederer, and L.A. Herzenberg. 2002. The history and future of the fluorescence activated cell sorter and flow cytometry: a view from Stanford. *Clin Chem* 48:1819-1827.
326. Valet, G. 2003. Past and present concepts in flow cytometry: a European perspective. *J Biol Regul Homeost Agents* 17:213-222.
327. Julius, M.H., E. Simpson, and L.A. Herzenberg. 1973. A rapid method for the isolation of functional thymus-derived murine lymphocytes. *Eur J Immunol* 3:645-649.
328. Julius, M.H., T. Masuda, and L.A. Herzenberg. 1972. Demonstration that antigen-binding cells are precursors of antibody-producing cells after purification with a fluorescence-activated cell sorter. *Proc Natl Acad Sci USA* 69:1934-1938.
329. Loken, M.R., D.R. Parks, and L.A. Herzenberg. 1977. Identification of cell asymmetry and orientation by light scattering. *J Histochem Cytochem* 25:790-795.
330. Julius, M.H., and L.A. Herzenberg. 1974. Isolation of antigen-binding cells from unprimed mice: demonstration of antibody-forming cell precursor activity and correlation between precursor and secreted antibody avidities. *J Exp Med* 140:904-920.
331. Loken, M.R., D.R. Parks, and L.A. Herzenberg. 1977. Two-color immunofluorescence using a fluorescence-activated cell sorter. *J Histochem Cytochem* 25:899-907.
332. Parks, D.R., R.R. Hardy, and L.A. Herzenberg. 1984. Three-color immunofluorescence analysis of mouse B-lymphocyte subpopulations. *Cytometry* 5:159-168.
333. Hardy, R.R., K. Hayakawa, D.R. Parks, and L.A. Herzenberg. 1983. Demonstration of B-cell maturation in X-linked immunodeficient mice by simultaneous three-colour immunofluorescence. *Nature* 306:270-272.

334. Bigos, M., N. Baumgarth, G.C. Jager, O.C. Herman, T. Nozaki, R.T. Stovel, D.R. Parks, and L.A. Herzenberg. 1999. Nine color eleven parameter immunophenotyping using three laser flow cytometry. *Cytometry* 36:36-45.
335. Chattopadhyay, P.K., C. Hogerkorp, and M. Roederer. 2008. A chromatic explosion: the development and future of multiparameter flow cytometry. *Immunology* 125:441-449.
336. Roederer, M., J.M. Brenchley, M.R. Betts, and S.C. De Rosa. 2004. Flow cytometric analysis of vaccine responses: how many colors are enough? *Clin Immunol* 110:199-205.
337. De Rosa, S.C., J.M. Brenchley, and M. Roederer. 2003. Beyond six colors: a new era in flow cytometry. *Nat Med* 9:112-117.
338. Betts, M.R., J.M. Brenchley, D.A. Price, S.C. De Rosa, D.C. Douek, M. Roederer, and R.A. Koup. 2003. Sensitive and viable identification of antigen-specific CD8+ T cells by a flow cytometric assay for degranulation. *J Immunol Methods* 281:65-78.
339. Perfetto, S., P.K. Chattopadhyay, L. Lamoreaux, R. Nguyen, D. Ambrozak, R.A. Koup, and M. Roederer. 2006. Amine reactive dyes: an effective tool to discriminate live and dead cells in polychromatic flow cytometry. *J Immunol Methods* 313:199-208.
340. Vermes, I., C. Haanen, H. Steffens-Nakken, and C. Reutelingsperger. 1995. A novel assay for apoptosis. Flow cytometric detection of phosphatidylserine expression on early apoptotic cells using fluorescein labelled Annexin V. *J Immunol Methods* 184:39-51.
341. Lyons, A.B., and C.R. Parish. 1994. Determination of lymphocyte division by flow cytometry. *J Immunol Methods* 171:131-137.
342. Al-Harthi, L., G. Marchetti, C.M. Steffens, J. Poulin, R. Sékaly, and Landay. 2000. Detection of T cell receptor circles (TRECs) as biomarkers for de novo T cell synthesis using a quantitative polymerase chain reaction-enzyme linked immunosorbent assay (PCR-ELISA). *J Immunol Methods* 237:187-197.
343. Perfetto, S., P.K. Chattopadhyay, and M. Roederer. 2004. Seventeen-colour flow cytometry: unravelling the immune system. *Nat Rev Immunol* 4:648-655.
344. Bonetta, L. 2005. Flow cytometry smaller and better. *Nat Meth* 2:11.
345. Rahman, M. 2006. Introduction to Flow Cytometry. 36.
346. Chattopadhyay, P.K., D.A. Price, T.F. Harper, M.R. Betts, J. Yu, E. Gostick, S. Perfetto, P. Goepfert, R.A. Koup, S.C. De Rosa, M.P. Bruchez, and M. Roederer. 2006. Quantum dot semiconductor nanocrystals for immunophenotyping by polychromatic flow cytometry. *Nat Med* 12:972-977.
347. Resch-Genger, Grabolle, Cavaliere-Jaricot, Nitschke, and Nann. 2008. Quantum dots versus organic dyes as fluorescent labels. *Nat Meth* 5:763-775.
348. Krutzik, P.O., M.R. Clutter, and G.P. Nolan. 2005. Coordinate analysis of murine immune cell surface markers and intracellular phosphoproteins by flow cytometry. *J Immunol* 175:2357-2365.
349. Krutzik, P.O., M.B. Hale, and G.P. Nolan. 2005. Characterization of the murine immunological signaling network with phosphospecific flow cytometry. *J Immunol* 175:2366-2373.
350. Tung, J.W., D.R. Parks, W.A. Moore, L.A. Herzenberg, and L.A. Herzenberg. 2004. New approaches to fluorescence compensation and visualization of FACS data. *Clin Immunol* 110:277-283.
351. Roederer, M. 2001. Spectral Compensation for Flow Cytometry: Visualization Artifacts, Limitations, and Caveats. *Cytometry* 12.
352. Bagwell, C.B., and E.G. Adams. 1993. Fluorescence spectral overlap compensation for any number of flow cytometry parameters. *Ann N Y Acad Sci* 677:167-184.
353. Maecker, H.T., A. Rinfret, P. D'Souza, J. Darden, E. Roig, C. Landry, P. Hayes, J. Birungi, O. Anzala, M. Garcia, A. Harari, I. Frank, R. Baydo, M. Baker, J. Holbrook, J. Ottinger, L. Lamoreaux, C.L. Epling, E. Sinclair, M.A. Suni, K. Punt, S. Calarota, S. El-Bahi, G. Alter, H. Maila, E. Kuta, J. Cox, C. Gray, M. Altfeld, N. Nougarede, J. Boyer, L. Tussey, T. Tobery, B. Bredt, M. Roederer, R. Koup, V.C. Maino, K. Weinhold, G. Pantaleo, J. Gilmour, H. Horton, and R.P. Sekaly. 2005. Standardization of cytokine flow cytometry assays. *BMC Immunol* 6:13.
354. Homburger, H.A., W. Rosenstock, H. Paxton, M.L. Paton, and A.L. Landay. 1993. Assessment of interlaboratory variability of immunophenotyping. Results of the College of American Pathologists Flow Cytometry Survey. *Ann N Y Acad Sci* 677:43-49.

355. Baumgarth, N., and Roederer. 2000. A practical approach to multicolor flow cytometry for immunophenotyping. *J Immunol Methods* 243:77-97.
356. Parks, D.R., M. Roederer, and W.A. Moore. 2006. A new "Logicle" display method avoids deceptive effects of logarithmic scaling for low signals and compensated data. *Cytometry A* 69:541-551.
357. Herzenberg, L.A., J. Tung, W.A. Moore, L.A. Herzenberg, and D.R. Parks. 2006. Interpreting flow cytometry data: a guide for the perplexed. *Nat Immunol* 7:681-685.
358. Maecker, H.T., T. Frey, L.E. Nomura, and J. Trotter. 2004. Selecting fluorochrome conjugates for maximum sensitivity. *Cytometry A* 62:169-173.
359. Moll, M., C. Kuylenstierna, V.D. Gonzalez, S.K. Andersson, L. Bosnjak, A. Sönnerborg, M.F. Quigley, and Sandberg. 2009. Severe functional impairment and elevated PD-1 expression in the CD1d-restricted NKT cells retained in chronic HIV-1 infection *Eur J Immunol* In press.
360. Nakagawa, R., I. Serizawa, K. Motoki, M. Sato, H. Ueno, R. Iijima, H. Nakamura, A. Shimosaka, and Y. Koezuka. 2000. Antitumor activity of alpha-galactosylceramide, KRN7000, in mice with the melanoma B16 hepatic metastasis and immunohistological study of tumor infiltrating cells. *Oncol Res* 12:51-58.
361. Huang, Y., A. Chen, X. Li, Z. Chen, W. Zhang, Y. Song, D. Gurner, D. Gardiner, S. Basu, D.D. Ho, and M. Tsuji. 2008. Enhancement of HIV DNA vaccine immunogenicity by the NKT cell ligand, alpha-galactosylceramide. *Vaccine* 26:1807-1816.
362. Poli, A., T. Michel, M. Thérésine, E. Andrès, F. Hentges, and J. Zimmer. 2008. CD56 brightnatural killer (NK) cells: an important NK cell subset. *Immunology* in press:24.
363. Golden-Mason, L., L. Madrigal-Estebas, E. McGrath, M.J. Conroy, E.J. Ryan, J.E. Hegarty, C. O'Farrelly, and D.G. Doherty. 2008. Altered natural killer cell subset distributions in resolved and persistent hepatitis C virus infection following single source exposure. *Gut* 57:1121-1128.
364. De Maria, A., M. Fogli, S. Mazza, M. Basso, A. Picciotto, P. Costa, S. Congia, M.C. Mingari, and L. Moretta. 2007. Increased natural cytotoxicity receptor expression and relevant IL-10 production in NK cells from chronically infected viremic HCV patients. *Eur J Immunol* 37:445-455.
365. Deniz, G., G. Erten, U.C. Kücüksezer, D. Kocacik, C. Karagiannidis, E. Aktas, C.A. Akdis, and M. Akdis. 2008. Regulatory NK cells suppress antigen-specific T cell responses. *J Immunol* 180:850-857.
366. Graham, Wells, Liu, Sherman, Peters, Chung, Bhan, Andersen, Koziel, and A.S. Team. 2006. Relationships between cellular immune responses and treatment outcomes with interferon and ribavirin in HIV/hepatitis C virus co-infection. *AIDS* 20:345-351.
367. Biswas, P.S., V. Pedicord, A. Ploss, E. Menet, I. Leiner, and E.G. Pamer. 2007. Pathogen-specific CD8 T cell responses are directly inhibited by IL-10. *J Immunol* 179:4520-4528.
368. McCloskey, T.W., N. Oyaizu, M. Kaplan, and S. Pahwa. 1995. Expression of the Fas antigen in patients infected with human immunodeficiency virus. *Cytometry* 22:111-114.
369. Groux, H., G. Torpier, D. Monté, Y. Mouton, A. Capron, and J.C. Ameisen. 1992. Activation-induced death by apoptosis in CD4+ T cells from human immunodeficiency virus-infected asymptomatic individuals. *J Exp Med* 175:331-340.
370. Giorgi, J.V., Z. Liu, L.E. Hultin, W.G. Cumberland, K. Hennessey, and R. Detels. 1993. Elevated levels of CD38+ CD8+ T cells in HIV infection add to the prognostic value of low CD4+ T cell levels: results of 6 years of follow-up. The Los Angeles Center, Multicenter AIDS Cohort Study. *J Acquir Immune Defic Syndr* 6:904-912.
371. Giorgi, J.V., L.E. Hultin, J.A. McKeating, T.D. Johnson, B. Owens, L.P. Jacobson, R. Shih, J. Lewis, D.J. Wiley, J.P. Phair, S.M. Wolinsky, and R. Detels. 1999. Shorter survival in advanced human immunodeficiency virus type 1 infection is more closely associated with T lymphocyte activation than with plasma virus burden or virus chemokine coreceptor usage. *J Infect Dis* 179:859-870.
372. Hazenberg, M.D., S.A. Otto, B.H. van Benthem, M.T. Roos, R.A. Coutinho, J.M. Lange, D. Hamann, M. Prins, and F. Miedema. 2003. Persistent immune activation in HIV-1 infection is associated with progression to AIDS. *AIDS* 17:1881-1888.
373. Kestens, L., G. Vanham, C. Vereecken, M. Vandenbruaene, G. Vercauteren, R.L. Colebunders, and P.L. Gigase. 1994. Selective increase of activation antigens HLA-DR

and CD38 on CD4+ CD45RO+ T lymphocytes during HIV-1 infection. *Clin Exp Immunol* 95:436-441.

374. Lange, C.G., M.M. Lederman, J.S. Madero, K. Medvik, R. Asaad, C. Pacheko, C. Carranza, and H. Valdez. 2002. Impact of suppression of viral replication by highly active antiretroviral therapy on immune function and phenotype in chronic HIV-1 infection. *J Acquir Immune Defic Syndr* 30:33-40.
375. Kaufmann, G.R., J.J. Zaunders, P. Cunningham, and D.A. Cooper. 1999. Phenotypic analysis of CD8+ T lymphocytes in a cohort of HIV type 1-infected patients treated with saquinavir, ritonavir, and two nucleoside analogs for 1 year, and association with plasma HIV type 1 RNA. *AIDS Res Hum Retroviruses* 15:963-972.
376. Tilling, R., S. Kinloch, L.E. Goh, D. Cooper, L. Perrin, F. Lampe, J. Zaunders, B. Hoen, C. Tsoukas, J. Andersson, G. Janossy, and Q.S. Group. 2002. Parallel decline of CD8+/CD38++ T cells and viraemia in response to quadruple highly active antiretroviral therapy in primary HIV infection. *AIDS* 16:589-596.
377. Perrella, A., Perrella, P. Conca, and G. Tarantino. 2000. CD8+/CD38+: immune activity and clinical significance in HCV patients with and without interferon therapy. *European review for medical and pharmacological sciences* 4:127-131.
378. Savarino, A., F. Bottarel, F. Malavasi, and U. Dianzani. 2000. Role of CD38 in HIV-1 infection: an epiphenomenon of T-cell activation or an active player in virus/host interactions? *AIDS* 14:1079-1089.
379. Barber, D.L., E.J. Wherry, D. Masopust, B. Zhu, J.P. Allison, A.H. Sharpe, G.J. Freeman, and R. Ahmed. 2006. Restoring function in exhausted CD8 T cells during chronic viral infection. *Nature* 439:682-687.
380. Kaufmann, D.E., D.G. Kavanagh, F. Pereyra, J.J. Zaunders, E.W. Mackey, T. Miura, S. Palmer, M. Brockman, A. Rathod, A. Piechocka-Trocha, B. Baker, B. Zhu, S. Le Gall, M.T. Waring, R. Ahern, K. Moss, A.D. Kelleher, J.M. Coffin, G.J. Freeman, E.S. Rosenberg, and B.D. Walker. 2007. Upregulation of CTLA-4 by HIV-specific CD4(+) T cells correlates with disease progression and defines a reversible immune dysfunction. *Nat Immunol* 8:1246-1254.
381. Lanier, L.L., T.J. Kipps, and J.H. Phillips. 1985. Functional properties of a unique subset of cytotoxic CD3+ T lymphocytes that express Fc receptors for IgG (CD16/Leu-11 antigen). *J Exp Med* 162:2089-2106.
382. Sandor, M., and R.G. Lynch. 1993. Lymphocyte Fc receptors: the special case of T cells. *Immunol Today* 14:227-231.
383. Groh, V., S. Porcelli, M. Fabbi, L.L. Lanier, L.J. Picker, T. Anderson, R.A. Warnke, A.K. Bhan, J.L. Strominger, and M.B. Brenner. 1989. Human lymphocytes bearing T cell receptor gamma/delta are phenotypically diverse and evenly distributed throughout the lymphoid system. *J Exp Med* 169:1277-1294.
384. Angelini, D.F., G. Borsellino, M. Poupot, A. Diamantini, R. Poupot, G. Bernardi, F. Poccia, J.J. Fournié, and L. Battistini. 2004. FcgammaRIII discriminates between 2 subsets of Vgamma9Vdelta2 effector cells with different responses and activation pathways. *Blood* 104:1801-1807.
385. Lafont, V., J. Liautard, J.P. Liautard, and J. Favero. 2001. Production of TNF-alpha by human V gamma 9V delta 2 T cells via engagement of Fc gamma RIIIA, the low affinity type 3 receptor for the Fc portion of IgG, expressed upon TCR activation by nonpeptidic antigen. *J Immunol* 166:7190-7199.
386. Clémenceau, B., N. Congy-Jolivet, G. Gallot, R. Vivien, J. Gaschet, G. Thibault, and H. Vié. 2006. Antibody-dependent cellular cytotoxicity (ADCC) is mediated by genetically modified antigen-specific human T lymphocytes. *Blood* 107:4669-4677.
387. Deeks, S.G., C.M. Kitchen, L. Liu, H. Guo, R. Gascon, A.B. Narváez, P. Hunt, J.N. Martin, J.O. Kahn, J. Levy, M.S. McGrath, and F.M. Hecht. 2004. Immune activation set point during early HIV infection predicts subsequent CD4+ T-cell changes independent of viral load. *Blood* 104:942-947.
388. Ancuta, P., A. Kamat, K. Kunstman, E. Kim, P. Autissier, A. Wurcel, T. Zaman, D. Stone, M. Mefford, S. Morgello, E. Singer, S. Wolinsky, and D. Gabuzda. 2008. Microbial translocation is associated with increased monocyte activation and dementia in AIDS patients. *PLoS ONE* 3:e2516.
389. Jirillo, E., D. Caccavo, T. Magrone, E. Piccigallo, L. Amati, A. Lembo, C. Kalis, and M. Gumenscheimer. 2002. The role of the liver in the response to LPS: experimental and clinical findings. *J Endotoxin Res* 8:319-327.

390. Dolganiuc, A., O. Norkina, K. Kodys, D. Catalano, G. Bakis, C. Marshall, P. Mandrekar, and G. Szabo. 2007. Viral and host factors induce macrophage activation and loss of toll-like receptor tolerance in chronic HCV infection. *Gastroenterology* 133:1627-1636.
391. Balagopal, A., F.H. Philp, J. Astemborski, T.M. Block, A. Mehta, R. Long, G.D. Kirk, S.H. Mehta, A.L. Cox, D.L. Thomas, and S.C. Ray. 2008. Human immunodeficiency virus-related microbial translocation and progression of hepatitis C. *Gastroenterology* 135:226-233.