

Microbiology and Tumor Biology Center,  
Karolinska Institutet, Stockholm, Sweden

**Mechanisms underlying impaired humoral immunity in  
primary and chronic HIV-1 infection**

**Kehmia Titanji**

Stockholm 2006

All previously published papers were reproduced with permission from the publishers.

Published and printed by Karolinska University Press

Box 200, SE-171 77 Stockholm, Sweden

© Kehmia Titanji, 2006

ISBN 91-7140-728-6

*To my beloved grandparents*

## ABSTRACT

B cells of HIV-1-infected patients have both phenotypic and functional dysfunctions which may be important in HIV-1 pathogenesis. Immune activation during HIV infection is an essential part of the body's defense against the virus, but may be the root cause of B cell dysfunctions. Evidence of B cell dysfunctions during HIV-1 infection include altered expression of activation markers, spontaneous apoptosis, and polyclonal activation. These may occur very early in infection, and may have important effects on the outcome of infection and treatment of the patient. Interaction between B cells and other cells of the immune system is essential to the proper functioning of the B cells and may also be altered during HIV-1 infection.

With this thesis I wanted to investigate if: (1) B cell dysfunctions are initiated during primary HIV-1 infection (2) Patients treated during primary infection may stand a better chance of controlling infection (3) Immune activation induces and enhances B cell dysfunctions in primary and chronic HIV infection; (4) Interactions between B cells and other lymphocytes may be altered in HIV infection, leading to malfunction of the B cells (5) HIV-infected patients respond poorly to vaccination and infections because they have impaired serological memory.

The results obtained indicate that:

Majority of B lymphocyte dysfunctions are initiated early in primary infection and persist throughout the course of the disease; Naive B lymphocytes are abnormally activated in HIV-1 infection and may contribute to excessive hypergammaglobulinemia; Loss of memory (CD27+) B cells is a feature of chronic infection, but is not observed in primary infection thus may be an effect of persistent immune activation; The Fas-FasL pathway may play a key role in the deletion of B cells in HIV infection; Antigen-specific humoral immunity is also impaired early during primary HIV-1 infection as shown by reduced levels of specific antibodies to HIV, and non-HIV antigens such as measles and pneumococcus polysaccharide antigens; Measles-specific memory B cells are deleted in HIV-1 infection; Immune activation, assessed by plasma sCD27, IgG and  $\beta$ -2 microglobulin, is lower in HIV-2 than in HIV-1 infection.

HIV infection leads to important B cell dysfunctions and impaired humoral immunity which in turn impair the ability of the patient to control the infection and/or mount an effective response to the virus. An ideal HIV vaccine would be one that is able to elicit strong humoral immune as well as cell-mediated responses and understanding the role of B cell dysfunctions in immunopathogenesis of HIV infection is important in refining strategies for effective therapies and vaccines.

**Keywords:** HIV-1 infection, B cells, primary HIV-1 infection, immune activation hypergammaglobulinemia, long-term humoral immunity, HIV-2 infection, antiretroviral therapy

## **LIST OF PUBLISHED PAPERS AND MANUSCRIPTS**

This thesis is based on the following papers, which are referred to in the text by their roman numerals:

**I** Mechanisms of hypergammaglobulinemia and impaired antigen-specific humoral immunity in HIV-1 infection

*Angelo De Milito, Anna Nilsson, Kehmia Titanji, Rigmor Thorstensson, Elisabet Reizenstein, Mitsuo Narita, Sven Grutzmeier, Anders Sönnnerborg, Francesca Chiodi*

Blood, 15 March 2004, Vol. 103, No. 6, pp. 2180-2186

**II** Primary HIV-1 infection sets the stage for important B lymphocyte dysfunctions

*Kehmia Titanji, Francesca Chiodi, Rino Bellocchio, Danika Schepis, Lyda Osorio, Chiara Tassandin, Giuseppe Tambussi, Sven Grutzmeier, Lucia Lopalco, Angelo De Milito*

AIDS(2005) Nov 18; 19(17): 1947-55

**III** Loss of memory B cells impairs maintenance of long-term serological memory during HIV-1 infection

*Kehmia Titanji, Angelo De Milito, Alberto Cagigi, Rigmor Thorstensson, Sven Grützmeier, Bo Hejdeman, Lucia Lopalco, Anna Nilsson, Francesca Chiodi*

Submitted manuscript, under review

**IV** Plasma levels of soluble CD27 and IgG indicate lower immune activation in HIV-2 compared to HIV-1 infection

*Kehmia Titanji, Sören Andersson, Ana E Sousa, Zacarias da Silva, Hans Norrgren, Ann Atlas, Francesca Chiodi, Angelo De Milito*

Manuscript

## **RELATED PUBLICATION**

Low frequency of plasma Nerve Growth Factor detection is associated with death of memory B cells in HIV-1 infection

*Kehmia Titanji, Anna Nilsson, Camilla Mörch, Astrid Samuelsson, Anders Sönnnerborg, Sven Grutzmeier, Maurizio Zazzi, Angelo De Milito*

Clin Exp Immunol 2003; 132: 297-303

## **TABLE OF CONTENTS**

### **1. INTRODUCTION**

#### **1-1 Human Immunodeficiency virus (HIV)**

1-1.1 Origin and types of HIV.....	1
1-1.2 Structure of HIV.....	2
1-1.3 HIV entry and infection course.....	2
1-1.4 HIV pathogenesis.....	6

#### **1-2 The Immune response to HIV**

1-2.1 Innate immune responses.....	8
1-2.2 Adaptive immune responses.....	9
1-2.3 Immune escape.....	11

#### **1-3 Therapy and Vaccination**

1-3.1 Current therapies.....	12
1-3.2 Vaccine prospects and trials.....	13

### **2. BACKGROUND AND RATIONALE .....** 17

### **3. AIM OF THIS THESIS.....** 23

### **4. MATERIALS AND METHODS.....** 24

### **5. RESULTS AND DISCUSSION**

5.1 Activation and Differentiation marker expression on B cells.....	29
5.2 Polyclonal activation and hypergammaglobulinemia.....	31
5.3 B cell depletion and memory B cell subpopulations.....	32
5.4 Specific antibody production.....	36
5.5 Memory B cells and long-term humoral immunity.....	36
5.6 Immune activation and sCD27.....	38
5.7 Antiretroviral therapy and B cell dysfunctions.....	40
<b>6. CONCLUSIONS.....</b>	44
<b>7. ACKNOWLEDGEMENTS.....</b>	45
<b>8. REFERENCES.....</b>	47

## **ABBREVIATIONS**

<b>AICD</b>	Activation-induced cell death
<b>AIDS</b>	Acquired Immunodeficiency Syndrome
<b>ARS</b>	Acute Retroviral Syndrome
<b>BAFF</b>	B cell activating factor from the TNF family
<b>BCMA</b>	B cell maturation antigen
<b>Blys</b>	B lymphocyte stimulator
<b>β<sub>2</sub>M</b>	Beta-2 microglobulin
<b>CHI</b>	Chronic HIV-1 infection
<b>CTL</b>	Cytotoxic T lymphocyte
<b>DC</b>	Dendritic cell
<b>ELISA</b>	Enzyme-linked immunosorbent assay
<b>ELISPOT</b>	Enzyme-linked immunospot
<b>FACS</b>	Fluorescence Activated Cell Sorter
<b>Fas(L)</b>	Fas (Ligand)
<b>HAART</b>	Highly Active Antiretroviral Therapy
<b>HIV</b>	Human Immunodeficiency Virus
<b>HLA</b>	Human Leukocyte Antigen
<b>HTLV</b>	Human T-cell lymphotropic virus
<b>IFN-γ</b>	Interferon-gamma
<b>Ig</b>	Immunoglobulin
<b>IL</b>	Interleukin
<b>KLH</b>	Keyhole Limpet Hemocyanin
<b>LAIR-1</b>	Leukocyte-Associated Immunoglobulin-like Receptor 1
<b>LTNP</b>	Long-Term Non-Progressor
<b>PHI</b>	Primary HIV-1 infection
<b>PSA</b>	Polyspecific Self-reactive Antibodies
<b>SIV</b>	Simian Immunodeficiency Virus
<b>STI</b>	Structured Treatment Interruption
<b>TACI</b>	Transmembrane activator and CAML interactor
<b>TNF (R)</b>	Tumor Necrosis Factor (Receptor)

# **1 INTRODUCTION**

Early in the 1980s clinicians in Los Angeles and Paris reported cases of *Pneumocystis carinii* pneumonia combined with severe depletion of CD4+ T cells in young homosexual men. It became evident that the disease was caused by a virus, but all attempts to link the disease to known viruses were fruitless. In 1983, the Human Immunodeficiency Virus (HIV) was discovered and identified as the causative agent of the Acquired Immunodeficiency Syndrome (AIDS). This discovery raised hopes that therapies and possibly an effective vaccine would soon be available to combat the disease. Twenty-three years on, AIDS remains one of the leading causes of death in the world, with an estimated 40 million people living with HIV. Arguably, more information has been generated on the breadth and specificity of immune responses to HIV than any other virus in history, but despite significant progress in understanding the biology of the virus, its modes of transmission and pathogenesis, many questions remain to be answered and an effective vaccine is yet to be developed.

Two main types of HIV, HIV-1 and HIV-2, are known to infect humans. HIV-1 is the more virulent virus type, affecting approximately 95% of people living with HIV today. This section outlines what is currently known about the origin, structure, pathogenesis and course of HIV infection, immune response to HIV and therapy and vaccination strategies.

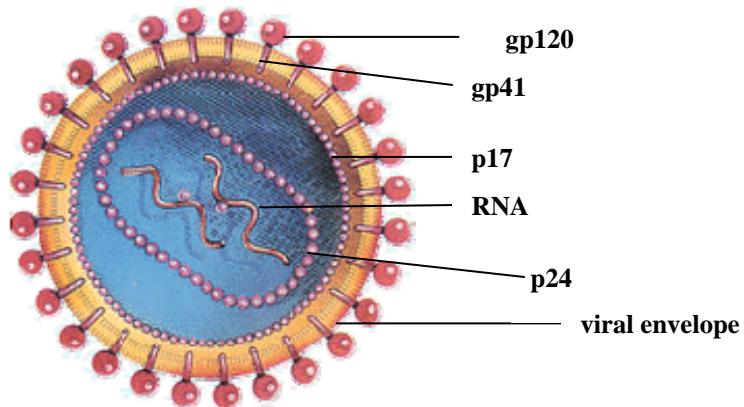
## **1-1 The Human Immunodeficiency Virus (HIV)**

### **1-1.1 Origin and types of HIV**

According to the UNAIDS/WHO, AIDS has killed about 25 million people since the beginning of the epidemic. In 2005 alone, it is estimated that 3.1 million people died from AIDS-related causes, 570 000 of whom were children. HIV-1 was initially named HTLV-III due to its similarities to the only other known human retrovirus, human T-cell lymphotropic virus I (HTLV-I) (Barre-Sinoussi et al. 1983; Gallo et al. 1983). HIV-1 has since been shown not to have any (evolutionary) genomic sequence relationships with HTLV-I. Another closely related retrovirus, HIV-2, was identified shortly after the discovery of HIV-1 (Clavel et al. 1986). HIV-2, which is endemic in certain parts of West Africa is more similar to the Simian Immunodeficiency virus (SIV) and is responsible for only approximately 5% of the AIDS cases worldwide.

Both HIV-1 and HIV-2 however are thought to have spread to humans from other non-human primates and it is now widely accepted that both viruses are of simian origin (Gao et al. 1992; Chen et al. 1996). Henceforth in this thesis, HIV will be used in reference to HIV-1.

### 1-1.2 Structure of HIV



**Figure 1. Structure of the HIV virion**

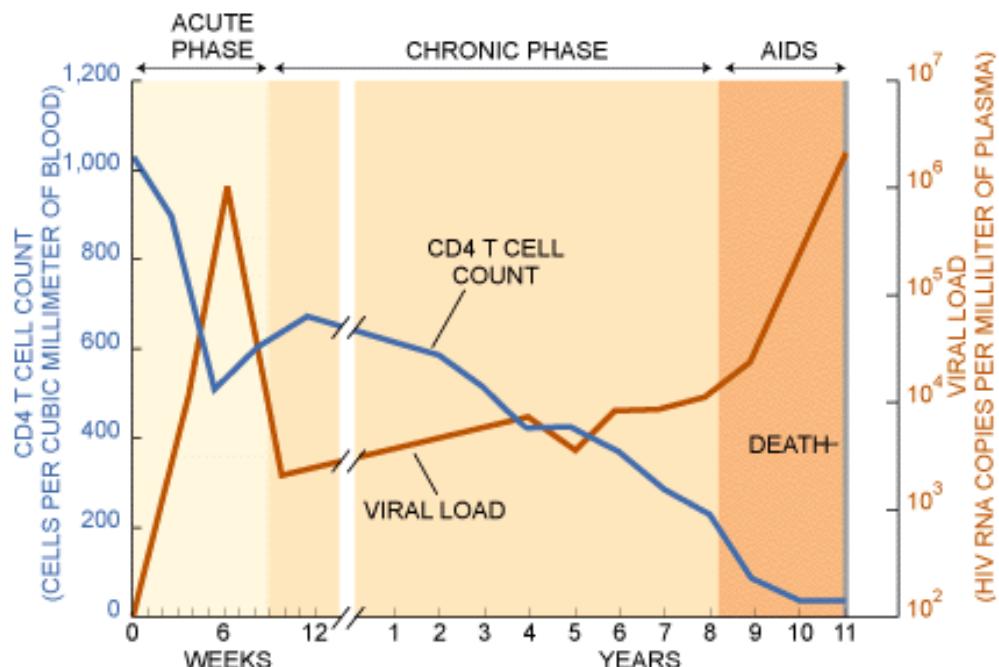
HIV is a retrovirus belonging to the lentivirus family. Each virion expresses 72 glycoprotein projections composed of gp120 and gp41, which are associated with each other, and serve as the viral receptor for CD4 on host cells (Coffin et al. 1986; Wyatt et al. 1998). The viral envelope derives from the host cell and contains some host-cell membrane proteins, including class I and class II MHC molecules. Within the envelope is the viral core, or nucleocapsid, which includes the matrix protein, p17 and an inner layer of a protein, p24 which makes up the capsid. The HIV genome consists of two copies of single-stranded RNA, which are associated with two molecules of reverse transcriptase (p64) and nucleoid proteins (p10), a protease and an integrase (p32).

### 1-1.3 HIV entry and infection course

HIV primarily infects  $CD4^+$  T cells though certain HIV strains infect monocytes and other cells expressing CD4 on their surface. Gp120 binds with high affinity to CD4, thus enabling the virus to enter the host cell. In addition to CD4, HIV also requires the presence of co-receptors on the target cells-CCR5 on DCs and macrophages, and CXCR4 on activated T cells-for entry and productive infection. Once inside the cell,

the RNA genome of the virus is reverse transcribed and a cDNA copy integrates into the host genome. The integrated DNA (provirus) is transcribed and the various viral RNA messages spliced and translated into proteins, which along with a complete new copy of the RNA genome are used to form new viral particles. The gag proteins of the virus are cleaved by the viral protease into the forms that make up the nuclear capsid in a mature infectious viral particle.

The typical course of untreated HIV infection is made up of three main phases: the acute or primary phase, the clinically latent (chronic) phase, and the disease or AIDS phase (Figure 2). However, the course and the timing of onset of disease varies widely from patient to patient, and some patients, known as long-term non-progressors (LTNP), do not progress to disease after seroconversion, but are able to maintain normal CD4<sup>+</sup> T cell counts and low rates of viral replication in the absence of any therapy. Acute or Primary HIV infection (PHI) may be symptomatic and manifests as a mononucleosis-like syndrome, known as the acute retroviral syndrome (ARS), which appears about 2-4 weeks after initial exposure.



**Figure 2. Course of HIV infection**

The most common clinical manifestations of PHI include fever, malaise, lethargy, oral ulcers and lymphadenopathy and common laboratory abnormalities include anemia, leucopenia, thrombocytopenia and mild transaminase elevation. The non-specific

nature of the signs and symptoms, as well as the varying presentations of the patients makes diagnosing patients with PHI difficult. Soon after infection, viral RNA is detectable in the serum but antibodies against HIV proteins only become detectable in the serum of infected individuals approximately three months after infection has occurred (Gaines et al. 1987). Studies on the dynamics of HIV viremia and Ab seroconversion in PHI showed that the approximate time from exposure to detectable plasma HIV was 12 days, and 17 and 22 days to p24 antigenemia and first detectable Abs respectively (Fiebig et al. 2003). The most definitive method of detecting HIV infection at this early stage is therefore by measuring viral RNA levels using PCR. A significant decrease in CD4<sup>+</sup> T cells in the peripheral blood occurs 2-8 weeks after HIV infection (Gaines et al. 1990) and recently it has been shown that the gut may be the principal site of CD4<sup>+</sup> T cell depletion both in HIV and SIV infections (Lim et al. 1993; Li et al. 2005; Chase et al. 2006). Dissemination of virus to lymphoid organs results in a strong antiviral immune response, which though unable to eliminate the virus, is associated with a decline in plasma viremia and a transient stabilization of the CD4<sup>+</sup> T cell count and resolution of the clinical syndrome. The induction of an immune response to HIV is followed by a long period of relative clinical latency, also known as chronic HIV infection (CHI). The duration of this phase varies greatly but can be as long as 8-12 years. The time interval from infection to development of opportunistic disease varies form one individual to another, but according to the 1993 revised CDC definition, even if the patient is otherwise completely asymptomatic, a diagnosis of AIDS is made if the CD4<sup>+</sup> T cell count falls below 200 cells/ $\mu$ l blood. CD4<sup>+</sup> T lymphocyte counts consistently correlate with the development of HIV-related life-threatening opportunistic illnesses and disease progression. The absolute number or percentage of CD4<sup>+</sup> T cells and monitoring the evolution of CD4<sup>+</sup> T cell loss is an essential part of clinical monitoring and provides information to guide medical management of patients. This revised system of classification emphasizes the clinical importance of the CD4<sup>+</sup> T cell count in the categorization of HIV-related clinical conditions and replaces the previous system of classification published in 1986 which included only clinical disease criteria and was developed before the use of widespread CD4<sup>+</sup> T cell testing. The inclusion in the AIDS surveillance definition of persons with a CD4<sup>+</sup> T cell count of <200 cells/ $\mu$ l or a CD4<sup>+</sup> T cell percentage <14% will enable AIDS surveillance to reflect more accurately the number of HIV-

infected people at highest risk for severe HIV-related morbidity and immunosuppression.

The chronic phase is a period of rapid, continuous viral replication and a steady decline in CD4<sup>+</sup> T cell counts (Pantaleo et al. 1993; Perelson et al. 1993; Ho et al. 1995; Perelson et al. 1996). It is not completely understood how the balance between the production and clearance of virus and infected cells is maintained for such a long period of time. Two general mechanisms have been proposed: (1) the host immune response keeps the virus under control, but is unable to completely clear it and (2) viral replication is limited by the availability of suitable targets, particularly CD4<sup>+</sup> T cells. The apparent steady state gradually breaks down in the majority of patients, leading to the sharp decrease in numbers of CD4<sup>+</sup> T cells and high viral loads characteristic of AIDS. Disease progression has been shown to be clearly linked to viral load and extent of viral replication (Mellors et al. 1996), although other as yet unclear mechanisms may be important as well. Thus it is the level of replicating virus and the numbers of virus-infected cells that are critical for the development of immunodeficiency and the progression of disease. The mechanism by which efficient viral replication occurs in the face of an apparent strong host immune response is one of the great mysteries of HIV pathogenesis. A number of hypotheses have been put forward in an attempt to explain this: (1) the virus mutates rapidly and often and the resulting viral mutants can escape neutralizing Ab and cytotoxic T lymphocyte (CTL) responses; (2) a portion of the virus may replicate in immunologically privileged sites (Haase 1986); (3) special adaptations of the virus that make it ‘invisible’ to the immune system such as the large number of glycosylation sites on the Env proteins may serve as a shield against the host immune response (Botarelli et al. 1991; Benjouad et al. 1992). The complex replication cycle of the virus could also help avoid a CTL response by allowing an infected cell to shift rapidly from an antigen-negative state to one of high-level viral production and releasing a burst of virus before being detected as foreign; 4) Early damage to the immune system may preclude a completely protective immune response.

Once the CD4<sup>+</sup> T cell count drops below 200 cells/ $\mu$ l, when the total number of CD4<sup>+</sup> T cells in the body has been reduced by at least half (Haase 1999), the patient becomes susceptible to AIDS-defining opportunistic infections and tumors. Disease progression is however not an inevitable outcome of lentiviral infections, as is evidenced by SIV infection in monkeys. African green monkeys can be

persistently infected with SIV, apparently for life, but they do not appear to develop any disease. Although it has been suggested that long-term passage of HIV-1 in chimpanzees may result in the development of a more pathogenic virus (Novembre et al. 1997), most chimpanzees infected with wild-type HIV and macaques infected with mutants of SIV usually remain persistently infected but asymptomatic (Eichberg et al. 1987; Kestler et al. 1990; Johnson et al. 1993). This indicates that in the natural host, the virus and the host are well adapted to each other. The most important correlate of progression to AIDS is thought to be the steady-state viral load or set-point seen at the end of the primary phase of infection (Craib et al. 1997; Lefrere et al. 1998; Blattner et al. 2004). Prospective studies on a large cohort of men at high risk for HIV infection have shown that individuals with the highest viral loads have about a 3-fold shorter time of progression to disease than individuals whose viral loads lie within the lowest quartile (Mellors et al. 1996; Mellors et al. 1997).

Approximately 5% of patients, despite 10-15 years of documented HIV infection, do not progress to disease, and remain symptom-free with steady, reasonably normal CD4<sup>+</sup> T cell counts, and are thus known as LTNP (Cao et al. 1995; Kirchhoff et al. 1995; Pantaleo et al. 1995). It has been suggested that genetic defects in the infecting virus may be the reason why some patients do not progress to disease (Deacon et al. 1995; Kirchhoff et al. 1995). However, the LTNP are a heterogeneous group and the mechanisms underlying the lack of disease progression in these patients are not completely understood.

#### **1-1.4 HIV pathogenesis**

The hallmark of HIV infection is the destruction of CD4<sup>+</sup> T cells and the progressive loss of immune competence that results in profound immunodeficiency. Since these defects occur both in the presence and absence of direct infection of the CD4<sup>+</sup> T cells, it has been proposed that HIV not only causes disease by direct infection and killing of the CD4<sup>+</sup> T cells, but also by indirectly impairing immune cell function (Miura et al. 2005; Grossman et al. 2006). By virtue of the fact that CD4 is the main receptor for the virus, HIV is able to infect not only CD4<sup>+</sup> T cells, but also other cell types expressing this receptor, such as macrophages and dendritic cells. This gives rise to another unique characteristic of HIV i.e. the ability to exist in various replication states and tissue compartments, forming a viral reservoir, which allows it to withstand

host immune responses and antiretroviral therapy. All these properties are essential to the ability of the virus to persist and cause disease in the host.

### **CD4<sup>+</sup> T cell depletion**

Virally-mediated destruction of CD4<sup>+</sup> T cells has been clearly demonstrated *in vitro*, but the *in vivo* causes of CD4<sup>+</sup> T cell depletion during HIV infection are not completely known (McCune 2001; Douek et al. 2003). Three main mechanisms have been suggested to cause CD4<sup>+</sup> T cell depletion: (1) direct killing of infected cells by viral cytopathic effects or by CD8+ T cells (Wei et al. 1995; Lenardo et al. 2002); (2) immune activation-induced death of uninfected cells (Badley et al. 2000) and (3) virus-mediated killing of uninfected cells through binding and/or entry of viral proteins released by infected cells (Ledru et al. 1998; Badley et al. 2000; Lelievre et al. 2004). CD4<sup>+</sup> T cell depletion is also biphasic, with the acute phase of infection characterized by rapid, sharp drop in CD4<sup>+</sup> T cell numbers and the chronic phase of infection exhibiting a more progressive decline in CD4<sup>+</sup> T cell numbers. Direct killing of the cells through productive infection is responsible for the decline in primary infection while the high level of chronic immune activation seen in chronic infection probably accounts for most of the CD4<sup>+</sup> T cell depletion occurring at this stage. Though loss of CD4<sup>+</sup> T cells has mostly been studied in peripheral blood, it has recently been shown that CD4<sup>+</sup> T cell depletion during all stages of HIV-1 infection occurs predominantly in the gastrointestinal tract (Brenchley et al. 2004). The clues to our understanding of the mechanisms that lead to CD4<sup>+</sup> T cell depletion in HIV infection may lie in studies on lymphoid tissues of patients.

### **Cellular Reservoirs of HIV**

The establishment of a reservoir of latently infected cells is another key feature of HIV pathogenesis. Establishing a cellular reservoir of infected cells enables HIV to exist in different replication states and tissue compartments, allowing it to persist despite host immune responses and potent antiretroviral therapy. This reservoir is established during the acute phase of infection (Haase 1999), in cells located in the lymphatic tissue (Pantaleo et al. 1991) and is the principal site of virus storage and persistence (Pantaleo et al. 1993). CD4<sup>+</sup> T cells have been shown to serve as both active and latent reservoirs of infection (Ho et al. 1995; Wei et al. 1995; Chun et al. 1997; Wong et al. 1997). It has also been shown that antigen presenting cells such as

macrophages (Weinberg et al. 1991) and dendritic cells (Cameron et al. 1992), as well as follicular dendritic cells (Smith et al. 2001), may serve as latent reservoirs of infection.

## **1-2 The Immune Response to HIV infection**

A strong immune response to HIV infection is initiated in the host following acute infection. This immune response results in a decrease in plasma viral load and a corresponding increase in CD4<sup>+</sup> T cell counts, as well as resolution of the symptoms of the acute retroviral syndrome. The apparent control of HIV infection by the immune system, however, is only transient as these immune responses ultimately fail to eliminate the virus.

### **1-2.1 Innate Immune Responses**

Innate immune responses, an integral part of the immune response to viral infections, are also important in the host response to HIV infection, and consist of mucosal, soluble and cellular innate immune factors. Although most transmissions of HIV occur through mucosal sites, rates of productive infections at these sites are relatively low (Royce et al. 1997), indicating that innate local factors such as mucin, as well as natural antibodies to HIV found at mucosal sites, may provide an important first line of defense against HIV infection (Janoff et al. 1999).

Aside from the ability of innate immune cytokines such as IL-4, IL-6 and IL-12 to determine which type of helper T cell adaptive immune response predominates, various soluble components of innate immunity have been shown to have anti-HIV activity. TNF- $\alpha$  and interferons (Graziosi et al. 1996) have been shown to affect HIV replication and the presence or absence of certain chemokine receptors may affect the ability of HIV to infect cells (Berger 1997). Mannose-binding lectins and complement have also been shown to bind HIV and lyse the virus directly or induce phagocytosis of infected cells (Sullivan et al. 1996; Garred et al. 1997).

Neutrophils, DCs, NK cells and  $\gamma\delta$  T cells are among the innate immune cells important in HIV-1 infection and the anti-HIV response. It has been suggested that neutrophil function may be impaired in HIV infection (Szeli et al. 1992), and it was recently reported that peripheral blood neutrophils express CD4 and are likely infected by HIV *in vitro* (Biswas et al. 2003). DCs express CD4, CCR5 and DC-SIGN

and other C-type lectin receptors (CLRs) which facilitate infection of the DCs (Lee et al. 2001; Frank et al. 2002). DCs also capture virus through CLRs and such virus-carrying DCs help in disseminating the virus (Frank et al. 2002). Also plasmacytoid DCs, which have been identified as the major producer of type I IFN and IL-12 (Kadowaki et al. 2002), have been shown to be inversely correlated to HIV viremia (Donaghy et al. 2001). Proper NK cell function is associated with a relatively healthy clinical state in HIV-1 infection (Szalc et al. 1992) and ADCC may be important in fighting HIV infection (Biron et al. 1999); NK cells produce the HIV-suppressive C-C chemokines MIP-1 $\alpha$ , MIP-1 $\beta$  and CCL5 (RANTES) which have been shown to inhibit HIV replication *in vitro* (Fehniger et al. 1998). NK cells in HIV-infected patients however also exhibit several phenotypic and functional defects which are already evident in PHI (Alter et al. 2005). *In vitro* studies have also shown that  $\gamma\delta$  T cells, which are most commonly found at mucosal sites, are able to lyse HIV-infected cells (Wallace et al. 1996; Biswas et al. 2003).

### **1-2.2 Adaptive Immune Responses**

Adaptive immune responses, particularly neutralizing antibody and virus-specific CD8 $^{+}$  cytotoxic T lymphocyte (CTL) responses, are initiated early in the acute phase of infection and are associated with a 100- to 1000-fold fall in viral load, a partial increase in peripheral CD4 $^{+}$  T cell counts and resolution of the acute retroviral syndrome (Koup et al. 1994; Rosenberg et al. 1997).

#### **Humoral Immune responses**

Humoral immune responses, particularly neutralizing Ab (Nab) responses have an essential role in the clearance of many viral infections. Although Nabs are detectable in the early stages of HIV infection, sera of HIV infected individuals demonstrate limited neutralizing activity against primary HIV-1 isolates (Moore et al. 1995; Moog et al. 1997). Since antibodies are only detected in infected individuals well after the initial burst of viral replication has been contained early in infection (Pilgrim et al. 1997), the importance of Nab in early control of HIV replication and their efficiency in established HIV infection is not clear. Earlier studies in patients with acute HIV infection showed that the patients developed isolate-specific low titer Nab within 2-4 weeks after infection and shortly after that, virus variants resistant to autologous sera emerged (Albert et al. 1990). It has also been shown that Nab responses contribute to extensive variation in the envelope gene that is observed in the early months after PHI

(Richman et al. 2003). These observations suggest that the emergence of neutralization-resistant virus variants eventually contributes to disease progression.

Whereas neutralizing antibodies seem to have little effect on viral replication (Poignard et al. 1999), in non-human primate models, pre-existing circulating Nabs were able to block establishment of infection (Haigwood et al. 1996; Baba et al. 2000; Mascola et al. 2000). A number of human monoclonal Ab (mab) have been established that broadly neutralize primary isolates of different clades. Two Abs against gp120 (2G12, b12) and two others against gp41 (2F5, 4E10) were shown to be effective in vitro and also in animal models (Baba et al. 2000; Mascola et al. 2000). In vivo, these mabs conferred protection against intravenous, intravaginal, or oral challenge with simian human immunodeficiency virus in rhesus macaques indicating that they may prevent de novo infection in humans. In another study, a cocktail of 3 potent neutralizing mabs (b12, 2G12 and 2F5) was passively administered to hu-PBL-SCID mice infected with molecularly cloned virus in order to determine whether Nab can impact the course of established HIV infection. It was shown that (1) administration of the Nabs had minimal effect on viral replication, (2) neutralization escape mutants were rapidly selected and (3) the wild-type virus re-emerged as the Ab concentration waned (Poignard et al. 1999). Understanding the dynamics of Nab responses in HIV infection may be of use in the design of more effective therapies and vaccines.

### **HIV gp120 as superantigen**

The ability of gp120 to act as a superantigen by binding to a large number of immunoglobulins of the V<sub>H</sub>3 family has also been described as an important pathogenic mechanism (Berberian et al. 1993; Karray et al. 1997). This superantigen stimulation induces the synthesis of Abs to antigenic epitopes which lie in the mutable regions of gp120 and such Abs are largely ineffective against escape mutants which inevitably appear in the course of progressing infection (Moore et al. 1997).

### **Cellular Immune responses**

It has been shown that CD8<sup>+</sup> T cells can inhibit HIV replication *in vitro* (Walker et al. 1986) by lysing HIV-infected cells and blocking propagation of the infection (Tsubota et al. 1989). Certain soluble factors produced by CD8<sup>+</sup> T cells, such as the β-chemokines RANTES, MIP-1α and MIP-1β (Walker et al. 1986) and α-defensins (Zhang et al. 2002) have also been shown to exhibit anti-HIV activity. Decline in

plasma viremia during PHI was shown to be strongly associated with the initiation of an HIV-specific CTL response (Koup et al. 1994; Pantaleo et al. 1994; Borrow et al. 1997; Price et al. 1997), and studies in SIV-infected rhesus monkeys have demonstrated the importance of CD8<sup>+</sup> CTLs in controlling HIV-1 replication (Jin et al. 1999; Schmitz et al. 1999).

Although the role of virus-specific CD4<sup>+</sup> T lymphocytes in controlling HIV replication is not as well-defined as that of CTLs, it has been shown that vigorous HIV-specific CD4<sup>+</sup> T cell responses are also associated with control of viremia. Most HIV-infected individuals were indeed shown to have relatively high frequencies of circulating HIV-specific CD4<sup>+</sup> T cells and median CD4<sup>+</sup> T cell memory responses were lower in patients with progressive disease (Pitcher et al. 1999). Another study demonstrated that suppression of proliferation of HIV-specific CD4<sup>+</sup> T cells may be a mechanism used by HIV to impair the development of an effective virus-specific immune response (McNeil et al. 2001).

### **1-2.3 Immune escape**

The ability of HIV to continually replicate and survive in the face of the vigorous immune responses mounted by the host represents one of the best examples of immune escape known. Both humoral and cellular immune responses to HIV exert strong selection pressure on the virus, leading to immune evasion. The mechanisms through which this escape occurs include: CTL epitope escape, alterations in antigen processing, Nab epitope escape, dysfunction of T helper cells and CTLs and infection of T helper cells.

The clues to the ability of HIV to escape neutralizing antibodies lie in certain structural features of the virus. Nabs are directed against the viral envelope proteins, whose epitopes are ‘hidden’ in a variety of ways including extensive glycosylation (Johnson et al. 2002), which blocks access to the conserved protein core of the virus while stimulating little immune response (Reitter et al. 1998).

Viral escape from CTL responses has been shown in both acute and chronic infection (Phillips et al. 1991; Goulder et al. 1997; Price et al. 1997), and in SIV-infected monkeys (Allen et al. 2000) and mainly occurs as a result of HIV genetic variation (Phillips et al. 1991). Genetic variation often involves mutations within viral antigenic epitopes and can arise from single amino acid mutations at sites essential for MHC binding or TCR recognition. Epitope variants can interfere with CTL activity through

loss of binding to the presenting HLA I molecule or through lack of recognition of the altered peptide by the TCR (Milicic et al. 2005).

### **1-3. Therapy and Vaccination**

In 1986, the first antiretroviral therapy (ART) against HIV, Zidovudine (AZT), became available in the US, raising hopes about the possibility of curing AIDS (Yarchoan et al. 1986). In the 20 years since, several other drugs have been approved and are currently being used in various combinations to manage HIV infections all over the world. With the advent of Highly Active Antiretroviral Therapy (HAART) in the western world, HIV infection has come to be largely regarded as a chronic, manageable disease. However, drug resistance issues, cost, long-term toxicity issues and the inability of any of the drug combinations to completely clear viral reservoirs remain the main problems associated with antiretroviral therapy today. On the other hand, no reasonably successful vaccines, preventive or therapeutic, have so far been developed, despite intense research in the area. The following section outlines what is known about the various therapies currently available, and the problems associated with their distribution and use, as well as ongoing vaccine research and trials.

#### **1-3.1. Current therapies**

There are four main classes of antiretroviral drugs available for the clinical management of HIV infection today.

AZT, the first antiretroviral drug to be approved by the FDA, is a nucleoside analog reverse transcriptase inhibitor, NRTI (Ezzell 1987). NRTIs interfere with HIV replication by competitively inhibiting reverse transcriptase, thus leading to chain termination of HIV-1 proviral DNA (Richman 2001). The failure of monotherapy with AZT alone prompted the discovery of the next class of drugs, the protease inhibitors (PIs) (Dorsey et al. 1994; Vacca et al. 1994), which bind to the active site of HIV protease, thus preventing the maturation and infectivity of the virus (Richman 2001). The approval of more PIs and NRTIs led to the use of triple combination therapy (2NRTIs + 1PI) or HAART in the management of most HIV-infected patients (Hammer et al. 1997; Hirsch et al. 1999). The third class of drugs is the non-nucleoside reverse transcriptase inhibitors (NNRTIs), which were shown to be very effective in suppressing viral replication in combination with two NRTIs (Staszewski

et al. 1999). Recently, Enfuvirtide (T-20), a drug belonging to the fourth class of drugs known as fusion inhibitors, was approved by the FDA (Lalezari et al. 2003). T-20 prevents the conformational changes of gp41 required for the fusion of viral and cellular membranes (Kilby et al. 1998).

Many problems are however associated with the use of antiretroviral drugs, including metabolic disorders, difficulty for some patients to adhere to the complicated drug combinations they have to take daily for several years, toxicity of the drugs and viral resistance (Bangsberg et al. 2000; Richman 2001; Pomerantz et al. 2003). T-20, which showed a lot of promise also has several limitations including cost and difficulty to manufacture, and the fact that it has to be delivered by subcutaneous injection (Kilby et al. 1998). These problems and the fact that effective antiretroviral therapy is not readily available to the majority of people living with HIV infection in developing countries, make the investigation of other approaches for treating HIV infection necessary.

### **1-3.2. Structured treatment interruptions and vaccine prospects**

HIV-specific immunity has been shown to be protective so strategies to selectively induce or boost such immunity are an attractive alternative/supplement to current antiretroviral therapies. Structured treatment interruptions (STI) and therapeutic vaccination are two such strategies (Richman 2001; Pomerantz et al. 2003). STI involves cycles of ART alternated with periods without therapy during which released HIV antigen would ‘autoimmunize’ the body. Re-introduction of ART in the next treatment cycle would lead to better immune protection (Allen et al. 2002). So far, the results of STI in chronic infection have been disappointing (Garcia et al. 2001), in contrast to the acute phase of infection where the immune system is still relatively intact and may benefit from such an ‘autoimmunization’ strategy (Ortiz et al. 1999).

Despite its clinical benefits, HAART is not able to clear the reservoir of latently infected cells in patients and one possible explanation for this is the lack of antigenic stimulation in aviremic patients. The development of an effective HIV vaccine remains a primary goal of most of the HIV research in the world today and current vaccine research involves both therapeutic and prophylactic vaccines. In theory, therapeutic vaccines would involve the delivery of immunogens to an immunocompetent, aviremic individual under HAART in order to boost HIV-specific cell-mediated immunity, contain viral replication and delay viral rebound, thus

permitting a drug holiday. Preliminary results indicate that therapeutic vaccines are well-tolerated and can restore CD8<sup>+</sup> T cell responses in both newly and chronically infected individuals on HAART. However, despite the promising prospects of therapeutic vaccines, prophylactic vaccines would be the best strategy to control the spread of the HIV pandemic (Klein 2003). To date, more than 35 vaccine candidates have been tested in over 65 phase I/II clinical trials involving more than 10,000 volunteers (Girard et al. 2006). Using HIV immunogens in immunoprotection studies in macaques, it has been shown that cellular immune responses and protective immunity were best induced by immunizing the animals with a combination of vectors rather than with booster injections of a single vector (Hanke et al. 1999; Osterhaus et al. 1999; Shiver et al. 2002). The main limitation with the macaque models is that HIV does not productively infect the animals so while the models may be useful in establishing correlates of protection, their usefulness in determining the protective efficacy of human vaccines is doubtful. The consensus for many years has been that an effective HIV vaccine should elicit broadly cross-neutralizing antibodies and strong CTL and CD4<sup>+</sup> T cell responses, mucosal immunity and long-term immunity (Klein 2003). The candidate HIV vaccines currently under evaluation may be grouped into two main classes: envelope-based vaccines evoking humoral immune responses and those evoking cell-mediated responses. The former induce mainly neutralizing antibody and the latter induce mainly CD4<sup>+</sup> and CD8<sup>+</sup> T cell responses as well as cytokines. A lot of the initial most of the vaccine research was focused on envelope-based vaccines. One of such vaccines, a gp120-based vaccine made by VaxGen, became the first-ever AIDS vaccine to progress past phase III trials. It was put through 5 years of trials involving nearly 5500 HIV-negative people and proved to be ineffective. These results were quite disappointing to many scientists, but not totally unexpected (Watanabe 2003; Veljkovic et al. 2003). Even more serious than the disappointment to the scientific community, is the possibility that volunteers participating in trials of envelope-based vaccines may be at increased risk of infection (Veljkovic et al. 2003). It was shown that one of the ways HIV undermines the host immune response is through deceptive imprinting, where a bias towards antibodies specific for the initially infecting clonal virus population thwarts the attempts of the infected host to control viral variants that subsequently emerge (Kohler et al. 1994; Locher et al. 1999). It was postulated that gp120-based AIDS vaccines would only act as a decoy for the immune system and induce deceptive imprinting. Recently, it was

shown that Nab responses induced by gp120 account for the extensive variation in the envelope gene (Richman et al. 2003; Wei et al. 2003). Reports of fast breakthrough disease progression among volunteers participating in clinical trials seem to confirm the possible harmful effect of gp120-based vaccines. An outline of other vaccine concepts and vaccination strategies that have been tested is shown in Table 1.

The list in Table 1 is by no means exhaustive and new vaccine candidates and approaches are being introduced every day.

While 23 years may seem like a long time to wait for the discovery of an effective vaccine, it is nothing compared to how long it has taken for several effective vaccines to be developed, including vaccines against typhoid (105 years), *H. influenzae* (92 years), and measles (42 years). Hopefully a successful AIDS vaccine will not take as long to be developed, but meanwhile, the search goes on.

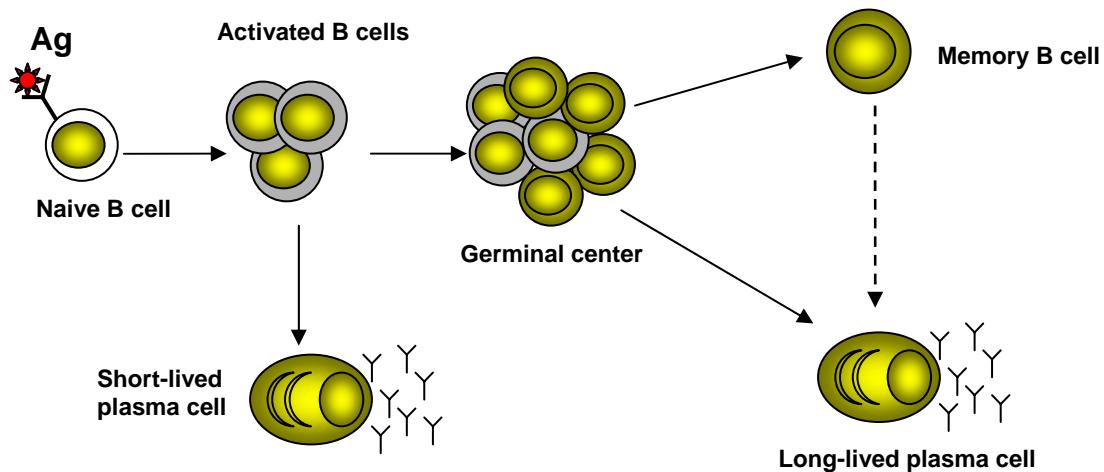
Vaccines	Examples	Strengths/Limitations/Problems
1. Live attenuated	SIV $\Delta$ <i>nef</i> mutant (Baba et al. 1999; Hofmann-Lehmann et al. 2003)	-Serious safety issues prohibit testing in humans
2. Inactivated	Formalin- and heat-inactivated HIV with modified <i>env</i> (Poon et al. 2005)	-Induced modest but significant titers of Nab against heterologous primary HIV isolates in animals
3. Virus-like particles (VLP) or pseudo-virions	Gag-Env pseudovirions (Hammonds et al. 2005)	-Induced Nab and CTL responses in animals
4. Subunit vaccines (Envelope-based and non-structural)	Gp120-based (Vaxgen), Tat-based (Cohen 2003; Voss et al. 2003)	-Some Tat-based vaccines induced strong CD4+ T cell and Ab responses in phase I trials -Some trials in animals showed protection against SHIV challenge
5. Naked DNA and live recombinant vaccines	Multigenic <i>env</i> , <i>gag</i> , <i>tat-rev</i> , <i>nef</i> MVA, Adenovirus-based e.g. Ad5- <i>gag</i> (Santra et al. 2005)	-Promising results, in terms of percent human responders and levels and duration of T cell responses obtained with Ad5 recombinant vaccines
6. Prime-boost combinations	DNA prime-live viral vector boost, Ad5 vector prime-poxvirus boost (Lemckert et al. 2005)	-In rhesus monkeys, responses arising from an Ad5-poxvirus prime-boost regimen were greater than those elicited by regimens with the individual vectors
7. Fusion proteins and peptides	Synthetic lipo-peptides containing MHC I restricted T cell epitopes (Pialoux et al. 2001)	-Promising results in terms of strong CTL responses in non-human primates and humans have been shown

## **2 Background and rationale of the studies in this thesis**

The symptoms associated with acute HIV infection-fever, sore throat and swollen glands are not specific to HIV, but are rather a consequence of the high level of immune activation induced in the host to fight the infection. Although HIV selectively infects and replicates in activated CD4<sup>+</sup> T cells, immune activation is manifested not only in the activated CD4<sup>+</sup> T cells, but also in NK cells, B cells and macrophages. Unlike in most infections where the infection is cleared, immune activation in HIV infection becomes generalized and persists throughout the chronic phase of infection. Evidence of generalized immune activation include: increased frequency of cells expressing activation markers, increased production of pro-inflammatory cytokines, increased cell turnover, increased serum levels of neopterin and β-2 microglobulin (β-2 M) and polyclonal B cell activation (De Milito 2004; Appay et al. 2005; Chiodi 2006). Evidence obtained so far suggest that immune activation drives HIV pathogenesis: (1) In natural SIV infections of sooty mangabeys (natural hosts), despite high levels of viral replication, infected animals do not show any signs of increased immune activation and do not come down with disease. However, rhesus macaques (accidental hosts) experimentally infected with SIV rapidly develop immune activation similar to HIV-infected patients, and rapidly become immunodeficient (2) Controlling virus replication with effective ART dramatically reduces immune activation. It is proposed that immune activation in HIV infection drives accelerated proliferation and differentiation of central memory CD4<sup>+</sup> T cells, which are destined to die rapidly as a consequence of being infected by the virus and/or AICD. Eventually the naïve and central memory T cell pools become exhausted and unable to generate new primary responses and maintain the peripheral CD4<sup>+</sup> T cell pool (Grossman et al. 2002; Grossman et al. 2006). Thus, a stronger immune response to HIV might have the paradoxical effect of enhancing viral replication and accelerating lymphocyte dysfunctions and disease progression.

HIV-induced B cell hyperactivation manifests as hypergammaglobulinemia (Shirai et al. 1992), increased spontaneous secretion of immunoglobulin (Ig) *in vitro* (Amadori et al. 1988), and increased susceptibility to apoptosis (Samuelsson et al. 1997). B cells of HIV-infected patients respond poorly to various stimuli *in vitro* (Lane et al. 1983; Conge et al. 1998) and have poor *in vivo* responses to both T cell dependent and

independent antigens (Ags) (Opravil et al. 1991; Kroon et al. 1994; Kroon et al. 1999; Malaspina et al. 2005). The generation of an effective B cell response depends on the ability of B cells to interact with other cells of the immune system, particularly CD4<sup>+</sup> T cells ( $T_H$ ), combined with co-stimulation signals received from the  $T_H$  cells and subsequent differentiation into Ig-secreting (plasma) cells. A simplified scheme of B cell differentiation into memory B cells and plasma cells is shown below.



**Figure 3. Generation of memory B cells and plasma cells.**

Following Ag stimulation in secondary lymphoid tissues, naïve (CD19<sup>+</sup>CD27<sup>-</sup>) B cells undergo clonal expansion and form clusters of activated B cells (extrafollicular foci) which either (1) differentiate into short-lived plasma cells which produce low-affinity Abs or (2) migrate into a B cell follicle and with CD4<sup>+</sup> T cell help initiate a germinal center reaction. Following somatic hypermutation, Ig isotype switching and affinity maturation in the GC, Ag-selected GC B cells develop into memory B cells with high-affinity B cell receptors and terminally differentiated long-lived plasma cells which produce high affinity Abs and home to the bone marrow. Memory B cells can be maintained for several years and may periodically differentiate into plasma cells (Crotty et al. 2004).

CD4<sup>+</sup> T (T helper,  $T_H$ ) cells, co-stimulation provided by members of the TNF and TNFR families, as well as several cytokines are important in B cell responses to T cell-dependent (TD) Ags. Initial Ag binding provides the first activation signal to the naïve B cell which leads to increased expression of MHC II and B7 (CD80/CD86)

molecules on the B cells. The Ag-MHC II complex on the B cell is recognized by the T<sub>H</sub> cell and a co-stimulatory signal provided by B7 molecules through CD28/CTLA4 stimulates the T<sub>H</sub> cell which begins to express CD40L and secrete cytokines. CD40-CD154 interaction delivers the second signal to the B cell and once activated, B cells start to express membrane receptors for cytokines such as IL-2 and IL-4. Cytokine-cytokine receptor interactions generate signals required for proliferation and class switching during the differentiation of B cells into plasma cells. Signals received through CD27-CD70 and OX40-OX40 ligand also promote B cell survival, proliferation and Ab production (Agematsu et al. 1998; Walker et al. 2000; Frauwirth et al. 2002). Sustained Ab production long after initial infection or vaccination is an essential feature of host defense against pathogens, and constitutes long-term humoral/serological immunity (memory). Three main mechanisms have been proposed to maintain long-term humoral immunity: (1) Long-lived plasma cells in bone marrow secreting Ab over long periods of time, possibly throughout the lifetime of an individual (Manz et al. 1997; Slifka et al. 1998), (2) Periodic re-exposure to the pathogen resulting in differentiation of memory B cells into Ab-secreting plasma cells (Ochsenbein et al. 2000) and (3) Ag-independent constant differentiation of memory B cells into plasma cells following bystander or polyclonal activation (Bernasconi et al. 2002). As with most physiological processes, these mechanisms are probably not mutually exclusive but may act together to maintain long-term humoral immunity.

### **HIV induces phenotypic and functional abnormalities in B cells**

Several B cell abnormalities were identified in the very early cases of HIV infection (Lane et al. 1983), including elevated numbers of cells spontaneously secreting immunoglobulin, decreased B-cell proliferative responses to T-cell-independent B-cell mitogens and hypergammaglobulinemia. These observations indicated that B cells from patients were both hyperactivated and at the same time hyporeactive to stimulation. These earlier observations have been confirmed by other studies and the full scope of B cell dysfunctions in HIV infection is becoming clearer. B cells from HIV-infected patients have several phenotypic and functional abnormalities. The phenotypic abnormalities described include: increased expression of CD5 (Sampalo et al. 1993), Fas and FasL (Samuelsson et al. 1997) and decreased expression of CD21 (Moir et al. 2001) and CD27 (De Milito et al. 2001). Hence, the finding that loss of CD21 expression on B cells is reversed with decreasing viremia is consistent with the

overall concept that viral replication is responsible for numerous B cell abnormalities. CD21 has been shown to be an essential component of T-dependent Ab responses (Ahearn et al. 1996) and it was suggested that increased percentages of CD21<sup>low</sup> B cells that are engaged in a terminal differentiation pathway will ultimately die without further contribution to immune surveillance (Moir et al. 2001). CD27 is a marker of memory B cells (Klein et al. 1998; Tangye et al. 1998) and plays an important role in the differentiation of B cells into plasma cells. Most HIV-infected individuals lose up to half of their circulating memory B cells and it was suggested that persistent CD70-mediated T cell stimulation may induce differentiation of memory B cells into Ab-producing cells, leading to the reduction in memory B cell percentages and increased plasmacytosis observed in patients (De Milito et al. 2001; Nagase et al. 2001). Memory B cells from patients also express increased amounts of membrane-bound Fas and FasL, suggesting that memory B cells may be susceptible to Fas-mediated apoptosis (Samuelsson et al. 1997; Samuelsson et al. 1997; Titanji et al. 2003). Increased serum IgG and autoantibody production as well as reduced antigen-specific Abs have also been described in several studies (Lane et al. 1983; Shirai et al. 1992; De Milito 2004). These phenotypic alterations translate into, or are accompanied by several functional defects including poor response to common *in vitro* mitogens such as PWM and SAC and poor *in vivo* response to secondary infections and immunizations.

Several autoimmune phenomena are reported in HIV infection; these include the occurrence of autoantibodies against several self-Ags, the presence of CTLs with specificity for autologous non-infected CD4<sup>+</sup> T cells and the association of HIV infection with rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE) (Onlamoon et al. 2005). The autoimmune manifestations are not specific to HIV alone but are commonly associated with other human retroviruses such as HTLV-I and are also frequent in primates infected with lentiviruses (Ansari 2004). Interestingly most of the autoimmune manifestations improved with antiretroviral therapy, but others, such as SLE seem to arise following the initiation of HAART (Leder et al. 2001; Palacios et al. 2002). Molecular mimicry has also been proposed as an important mechanism for autoimmunity in HIV infection. According to the molecular mimicry theory, HIV may have molecular similarity to a self Ag(s) and may therefore induce an autoimmune response (Onlamoon et al. 2005). Autoimmunity during HIV-1 infection may contribute significantly to the immunopathogenesis of AIDS since titers

of autoantibodies to HLA molecules and other surface molecules of CD4<sup>+</sup> T cells appear to increase with the progression of disease.

Despite being identified early on in the pandemic, the mechanisms for these B cell dysfunctions are only now being elucidated partly due to the complexity of the mechanisms involved, but also due to less attention being paid to B lymphocyte dysfunctions compared to T lymphocyte dysfunctions in HIV infection.

The underlying feature of B cell dysfunctions in HIV infection thus seems to be hyperactivation. As with T cells, generalized immune activation resulting from persistent viral replication, may be the driving force behind these B cell perturbations.

### **HIV, Opportunistic infections and other infectious diseases**

Despite the fact that HAART has decreased the overall rates of opportunistic infections (OI) in countries with ready access to therapy, OIs remain the main cause of death in HIV-infected patients (Chaisson et al. 1998). Effective immune restoration through HAART-mediated control of viral replication is an important defense against OIs. However in patients without access to HAART or those in whom therapy has failed, prophylaxis against OIs is recommended. However, long-term prophylaxis for OIs also raises toxicity and drug interaction concerns. Due to their severely immunosuppressed status, vaccination against common infectious disease agents such as tetanus, influenza, and pneumococcus is recommended in HIV-infected patients with pronounced immunosuppression (Masur et al. 2002).

### **Pneumococcal Diseases**

Infection with *Streptococcus pneumoniae* is an important cause of invasive clinical manifestations such as septicemia and meningitis, and is the most common cause of bacterial pneumonia among most HIV-infected subjects (Kroon et al. 1999; Dworkin et al. 2001; French et al. 2004). Thus 23-valent pneumococcal vaccination is recommended for HIV-infected patients. The efficacy of pneumococcal vaccine in HIV patients was shown to vary with the time of administration, with patients with CD4<sup>+</sup> T cell counts  $\geq 500\text{cell}/\mu\text{l}$  responding best in terms of Ab titers and development of pneumococcal disease (Kroon et al. 1999; Dworkin et al. 2001). Re-immunization against pneumococcal Ags has been suggested as a possible strategy to boost protection in HIV-infected patients, but re-immunization has also been shown to produce modest benefits in terms of protective Ab titers (Tasker et al. 2002).

## **Measles**

Despite the availability of a safe and effective vaccine against measles, it remains a leading cause of childhood death. One of the potential obstacles to measles control and elimination is HIV (Moss et al. 1999), with almost half of all measles-related deaths occurring in sub-Saharan Africa. HIV infection may alter the communicability of measles by prolonging the infectious period and may result in high rates of measles vaccine failure, resulting in lower vaccine effectiveness (Helfand et al. 2005). It has been shown that many children with HIV infection lack measurable Abs to common vaccine Ags including measles but re-immunization led to development of detectable Abs to measles, tetanus and *Haemophilus influenzae type b* (Melvin et al. 2003). Limited data is available on measles in HIV-infected adults. Measles Abs were detectable in asymptomatic HIV-infected patients (Sha et al. 1991) and fatality rate from measles infection is estimated to be high in immunocompromised patients (Kaplan et al. 1992). Measles vaccination is recommended in HIV patients and vaccination seems to be safe (Sprauer et al. 1993), but measurable Ab response can be expected only in a minority of patients (Wallace et al. 1994).

### **3 Aim of this thesis**

**The main aim of this thesis was to characterize some of the mechanisms leading to defects in humoral immune responses in HIV-1 infection.**

**Brief background of thesis project:** Both the cellular and the humoral arms of the immune system are unable to control HIV infection, which ultimately results in severe exhaustion of several lymphocyte functions and increased susceptibility to secondary and opportunistic infections. Major immunological defects occur in the B cell compartment including polyclonal B cell activation as demonstrated by hypergammaglobulinemia and spontaneous immunoglobulin production by cultured peripheral lymphocytes, high incidence of B cell tumors, and the dysregulated expression of several surface molecules. **These observations formed the basis of the work performed in this thesis in which the following hypotheses were verified:**

- B cell dysfunctions are initiated during primary HIV infection
- Patients treated during primary HIV infection may stand a better chance of mounting an effective humoral immune response through recovered B cell functions
- Immune activation induces and enhances B cell dysfunctions in primary and chronic HIV infection
- Interactions between B cells and other immune cells may be altered in HIV infection, leading to malfunction of the B cells
- Maintenance of long-term humoral immunity in HIV-infected patients is impaired.

**Relevance of the thesis project:** The destruction and dysfunction of B lymphocytes during HIV infection may represent an important pathogenic mechanism of HIV infection. Characterization of the mechanisms leading to defects in humoral immune responses during HIV infection may be important in elucidating crucial aspects of immunopathogenesis relevant for immunotherapy and vaccine development. In addition, the similarity between HIV infection and certain autoimmune diseases opens the possibility of clarifying common pathogenic mechanisms leading to B cell defects.

## 4 Materials and Methods

### Patients and Samples

Whole blood samples were collected from HIV-infected individuals after informed consent, in collaboration with attending clinicians at various hospitals. Peripheral blood mononuclear cells (PBMCs) were isolated by density gradient centrifugation and immediately used in experiments, or stored in liquid nitrogen until required. Plasma samples were also collected and stored at -80°C until analysis. Only HIV-1-infected patients were studied in papers I, II and III while both HIV-1- and HIV-2-infected patients were studied in paper IV.

**Table 2. Patients and their disease stages, and specific B cell dysfunctions studied in the papers included in this thesis**

Paper	Patients (n)	HIV infection stage	Dysfunctions
I	72	CHI	Hypergammaglobulinemia, polyclonal B cell activation, Specific Ab production, Apoptosis
II	PHI (n=31) CHI (n=26)	PHI, CHI	B cell activation, Hypergammaglobulinemia, Apoptosis
III	CHI (n=41), PHI (n=40), LTNP (n=7))	PHI, CHI, LTNP	B cell activation, Specific Ab production, Long-term humoral immunity
IV	HIV-1 (n=24) HIV-2 (n=26)	CHI	Immune activation, Hypergammaglobulinemia

More details on the patients are included in the individual papers in this thesis.

Patients were enrolled at: Karolinska University Hospital (Stockholm, Sweden), San Raffaele Institute (Milan, Italy), National Public Health Laboratory (Bissau, Guinea Bissau) and at the Institute of Molecular Medicine, Faculty of Medicine (Lisbon, Portugal). Ethical permission was obtained from the ethical committee of Karolinska Institutet, as well as from the other participating institutions.

### **Isolation of total, naïve and memory B cells from PBMC**

Total B cells were isolated from PBMC by incubating the PBMC with CD19 MultiSort microbeads (Miltenyi), followed by positive selection on magnetic columns. Sorted cells were eluted from the column, treated with a release reagent to release the magnetic beads, incubated with CD27 microbeads and applied to a fresh column. Naïve B cells were defined as CD19+CD27- and memory B cells as CD19+CD27+ (Paper I).

### **Monoclonal Antibodies**

See individual papers

### **Immunofluorescence staining**

Cells were incubated with conjugated mAbs to the relevant surface antigens, and data was acquired using FACScan or FACSsort instruments and analyzed using Cellquest Pro software. For studying intracellular staining, cells were first permeabilized and fixed using a Dako Intrastain kit before incubation with the relevant antigens (Papers I-IV).

### **Total IgG quantification**

Plasma IgG was measured by nephelometry and secretion of IgG in vitro was measured by ELISA (Papers I-IV).

### **Detection of polyspecific self-reactive antibodies (PSA)**

Buffy coat preparations from healthy donors were used to obtain target lymphocytes. PBMC were incubated in RPMI medium for 30 minutes at 37°C, in order to remove the monocyte fraction by plastic adherence. The remaining lymphocytes were then washed with PBS and  $0.5 \times 10^6$  cells were incubated with 100µl plasma for 1 hour on ice. After washing twice, the cells were incubated with a FITC-conjugated mouse anti-human IgG mab for 30 minutes on ice, washed and fixed. Binding of PSA to lymphocytes was analyzed by flow cytometry, and reactivity expressed as MFI (Wang et al. 1999).

### **Specific antibody measurement**

**Tetanus toxoid (TT) antibodies.** Antibody levels were measured using an accredited modified Delfia test (Paper I).

**Anti-measles antibodies.** Anti-measles antibodies were measured using the Enzygnost® Anti-measles virus/IgG ELISA kit. Microtitration test plate wells were coated with inactivated measles antigen derived from permanent simian kidney cells infected with measles virus, and control wells were coated with control antigen (non-infected cells). The World Health Organization 2<sup>nd</sup> International Standard for anti-measles serum was used for quantification, and the cut-off for protective levels was set at > 0.2 IU/ml. (Papers I and III)

**Anti-pneumococcus antibodies.** Anti-pneumococcus polysaccharide (PPS) antibody titers were measured using the ELIZEN Pneumococcus IgG immunopotency level ELISA kit. Test plates provided in the kit were coated with polysaccharide capsules of the 23 most sero-prevalent pneumococcus strains (Paper III).

**Anti-HIV-1 gp41 antibodies.** Determination of anti-HIV-1 gp41 antibody titers was performed by using the Enzygnost® HIV ½ Plus ELISA following the manufacturers' instructions. Plasma was diluted in five-fold dilutions from 1/100 to 1/62500 and the antibody level for each sample was calculated at the fixed OD<sub>450</sub> value of 0.5 after interpolation (Papers I and III).

**sCD27.** sCD27 was quantified using the PeliKine Compact™ human soluble CD27 kit (CLB, Amsterdam, The Netherlands).

**β-2 microglobulin.** β<sub>2</sub>-M was quantified using a Quantikine® IVD® β2-microglobulin EIA kit (R&D Systems Minneapolis, USA).

## **Cell culture**

Cells were cultured in several different conditions as underlined below. The headings indicate what each cell culture condition was used to investigate.

***Spontaneous IgG secretion.*** PBMC or purified B cells were cultured in RPMI medium at a concentration of  $0.5 \times 10^6$  cells/ml for 7 days. To determine the role of cell-cell contact in polyclonal activation (reflected by spontaneous IgG secretion), the following cell culture conditions were used: (1) B cell-depleted PBMC were seeded in the bottom chamber of a transwell plate with purified B cells seeded in the top chamber, (2) purified B cells alone, and (3) total PBMC. Cells were cultured at concentrations of  $10^6$  cells/ml in RPMI medium for 7 days. Cell culture supernatants were collected on day 7 for IgG measurement by ELISA (Papers I-IV).

***Apoptosis.*** PBMC or purified B cells were cultured for 18 hours with or without a Fas agonistic Ab (clones CH-11 and UB-2) or FasL in order to evaluate spontaneous and Fas-mediated apoptosis. After 18 hours, the cells were stained for Annexin-V and apoptosis was analyzed by FACS (Papers I and II).

***Activation of B cells in PBMC cultures prior to Elispot assay.*** Cryopreserved PBMC were quickly thawed, washed in sterile phosphate-buffered saline (PBS) and resuspended in X-Vivo serum-free culture medium. Cells were cultured in 48-well plates at a concentration of  $0.5 \times 10^6$  cells/well with: 10 µg/ml CpG ODN 2006, 1 µg/ml anti-CD40 mAb, 100 ng/ml IL-2 and 50 ng/ml IL-10. Cells were cultured for 6 days at 37°C, 5% CO<sub>2</sub> (Paper III).

## **Elispot assay of IgG- and measles-specific antibody-secreting cells**

On day 5 of the 6-day PBMC activation period (alternatively: 1 day before the cell activation cultures were terminated), separate wells of 96-well filtration plates were

coated overnight at 4°C with 0.5 µg/well affinity-purified goat anti-human IgG, 0.5 µg/well Keyhole Limpet Hemocyanin (KLH) or 5 µg/well Measles grade 2 antigen, with 0.05 M Na<sub>2</sub>CO<sub>3</sub> coating buffer. Optimal antigen concentrations were determined after multiple titration experiments and samples were run in triplicate for each test antigen. Plates were washed and blocked with 5% FCS/RPMI-1640 for at least 1.5 hours at 37°C, 7% CO<sub>2</sub>. Stimulated PBMC were washed and 3 x 10<sup>5</sup> cells/well were added to the coated plates and incubated overnight at 37°C, 7% CO<sub>2</sub>. Plates were washed 3 times after overnight incubation: twice with PBS only and once with PBS containing 0.05% Tween-20. For detection, 1 µg/ml biotinylated goat anti-human IgG detection antibody was added to the wells and the plate incubated for 1.5 hours at room temperature (RT). After another wash with PBS-Tween, Alkaline Phosphatase-Conjugated Avidin (AAF) was added to the plates followed by 1.5 hours of incubation at RT. The plates were then washed with distilled water and developed with 5-bromo-4-chloro-3-indolyl phosphate (BCIP) in the dark at RT for 10 minutes. Plates were washed 6 times with distilled water and air-dried overnight. Spots were enumerated with an AID ELISPOT reader using AID software version 3.2.3 (Paper III).

### **Statistical analyses**

Data was analyzed using parametric and non-parametric tests as appropriate, using SigmaStat (Papers I-IV) and Stata (Paper II) statistical software programs.

## 5 RESULTS AND DISCUSSION

### 5.1 Expression of activation and differentiation markers on B cells

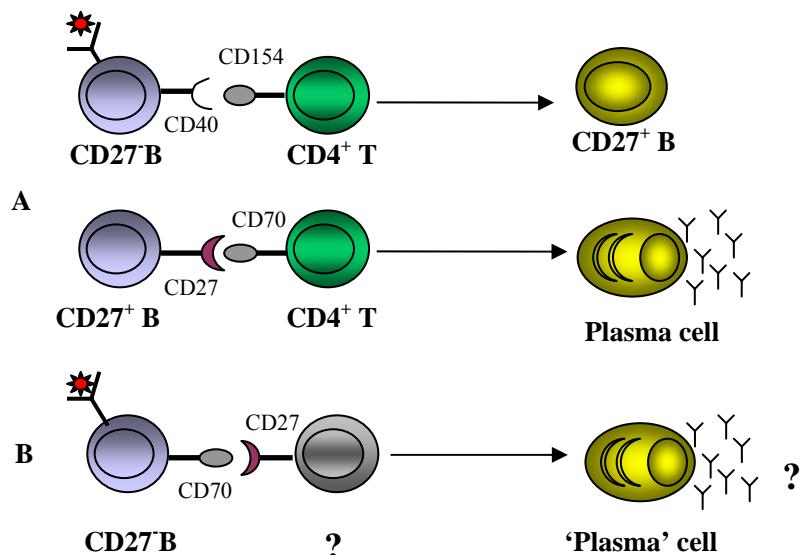
Phenotypic changes indicative of *in vivo* activation of B cells were among the first described B cell dysfunctions in HIV infection (Martinez-Maza et al. 1987; De Milito 2004). The expression of certain activation markers including the TNFR family members (and their ligands) Fas, FasL, CD27 and CD70 on B cells was previously shown to be dysregulated and associated to functional B cell defects (Samuelsson et al. 1997; De Milito et al. 2001). This knowledge served as a starting point for activation marker expression analyses aimed at identifying subpopulations of B cells contributing to B cell dysfunctions (Papers I and II).

CD70 (CD27L) expression on naïve B cells is normally tightly regulated and transient (Lens et al. 1996) and reflects recent antigenic stimulation, whereas leukocyte-associated inhibitory receptor 1 (LAIR-1) initially described as an inhibitory receptor on lymphocytes represents a differentiation marker on B cells (van der Vuurst de Vries et al. 1999). Fas, as well as being one of the best known pro-apoptotic death receptors, is also an activation marker (Miyawaki et al. 1992).

In Paper I, we studied the expression of the activation markers CD70 and LAIR-1 on naïve ( $CD19^+CD27^-$ ) B cells in CHI. We found a three-fold higher CD70 expression and a four-fold decrease of LAIR-1 expression on naïve B cells from patients compared to controls. Whereas the percentage of  $CD70^+$  naïve B cells was positively correlated with plasma IgG, neither CD70 nor LAIR-1 expression was correlated to  $CD4^+$  T cell count or viral load. We further purified the naïve B cells from 3 untreated patients and 3 donors and stained the cells for intracellular IgG expression. We found that the  $CD70^+$  naïve B cells from the patients had significantly higher proportions of intracellular IgG than the controls.

CD27/CD70 CD40/CD154 and interactions have different and sequential functions in T cell-dependent B cell responses (Jacquot et al. 1997). Following binding of antigen to the BCR, CD40/CD154 interaction is required for germinal center formation, induction of isotype switching and memory B cell generation. There are at least two possible functional outcomes of subsequent encounters between B cells and  $T_H$  cells: if the  $T_H$  cell expresses CD154, the B cell will differentiate into a memory B cell; if

the T<sub>H</sub> cell expresses CD70 instead, the B cell will differentiate into a plasma cell (Figure 4A).



**Figure 4. Possible effect of CD70 upregulation on naïve (CD27-) B cells in HIV-1**  
(A) CD40/CD154 and CD27/CD70 in B cell activation and (B) upregulation of CD70 on naïve B cells in HIV-1 infection may lead to Ab production

Our results suggest a differentiation ‘short-cut’ may be taken by naïve B cells of HIV-infected patients, where antigen stimulation leads to CD70 upregulation on naïve B cells leading to differentiation into Ab-secreting ‘plasma’ cells. This may contribute to hypergammaglobulinemia (Figure 4B).

In Paper II, we studied the expression of CD70, Fas and LAIR-1 on naïve and memory (CD19<sup>+</sup>CD27<sup>+</sup>) B cells in PHI. We found that CD70 expression on naïve B cells in PHI was comparable to controls and over 2-fold lower than levels in CHI. CD70 expression on memory B cells in PHI was comparable with expression in CHI and controls.

Fas expression was significantly upregulated on both naïve and memory B cells in PHI and was comparable to expression levels in CHI. LAIR-1 expression was significantly decreased on both naïve and memory B cells in PHI and levels were comparable to those found in CHI.

Our data indicate that B cells from HIV-infected patients are hyperactivated and differentiated, as shown by the expression of certain activation markers in both PHI and CHI. It has been shown that a distinct population of immature/transitional population of B cells, distinguished by the expression of CD10, can be detected in AIDS patients (Martinez-Maza et al. 1987; Malaspina et al. 2006). Some of these transitional cells were shown to be restricted to the CD27<sup>-</sup> B cell subset and their transitional status was reflected by their unresponsiveness to proliferation induced by BCR triggering, less diversified V<sub>H</sub>3 sequence genes and low expression of activation markers. It was also recently shown that in other human immunodeficient states characterized by defective humoral immunity, such as X-linked lymphoproliferative disease (XLP) and common variable immunodeficiency (CVID), there is an expansion of a subset of transitional B cells (CD24<sup>high</sup>CD38<sup>high</sup>) displaying several characteristics of immature B cells (Cuss et al. 2006). Like the transitional B cells identified in HIV-infected patients, these transitional B cells were also CD10<sup>+</sup> and it was demonstrated that these cells also exhibit less proliferation, differentiation and chemotaxis in vitro than mature B cells. The hyperactivation and differentiation seen in PHI and CHI may eventually be outweighed by the immune system's attempts to renew its B cell pool, leading to reversion to an immature/transitional state.

## 5.2 Polyclonal activation and hypergammaglobulinemia

Polyclonal activation reflected by hypergammaglobulinemia is a major feature of B cell dysfunction in HIV infection (Morris et al. 1998; Moir et al. 2001; De Milito 2004) and was examined in all the papers in this thesis.

In Paper I, we found that by simply culturing PBMC from patients, one could detect up to four-fold higher IgG titers in cell culture supernatants compared to supernatants from healthy donor PBMC cultures. We found a positive correlation between the amounts of IgG secreted in vitro and patients' CD4<sup>+</sup> T cell counts, indicating a possible role of CD4<sup>+</sup> T cells in hypergammaglobulinemia. Cell cultures using transwell culture plates also indicated that hypergammaglobulinemia was dependent on B cell-non-B cell interactions. We also wanted to find out which B cell population could contribute to hypergammaglobulinemia in CHI. Shortly before we started the study, evidence in a recent paper indicated that activated naïve B cells may have a role in the hypergammaglobulinemia observed in chronic viral infections (Hunziker et al. 2003). Purified naïve B cells from HIV patients spontaneously secreted twice as

much IgG as controls when placed in culture. Autoantibody production is another feature of B cell dysfunction in HIV-1 infection and it has been shown that amounts of lymphocyte-binding Ab known as polyspecific self-reactive Ab (PSA) are elevated in patients' plasma (Wang et al. 1999). We found higher PSA reactivity in CHI compared to controls, and a correlation between PSA reactivity and CD70+ naïve B cells.

In our study the level of plasma IgG in PHI was comparable to the amounts in CHI and significantly higher than amounts in healthy subjects (paper II), indicating that hypergammaglobulinemia is also readily detectable during primary infection.

Interestingly, we found that similar to PHI and CHI, the LTNP patients also had significantly higher amounts of plasma IgG than healthy controls (paper III).

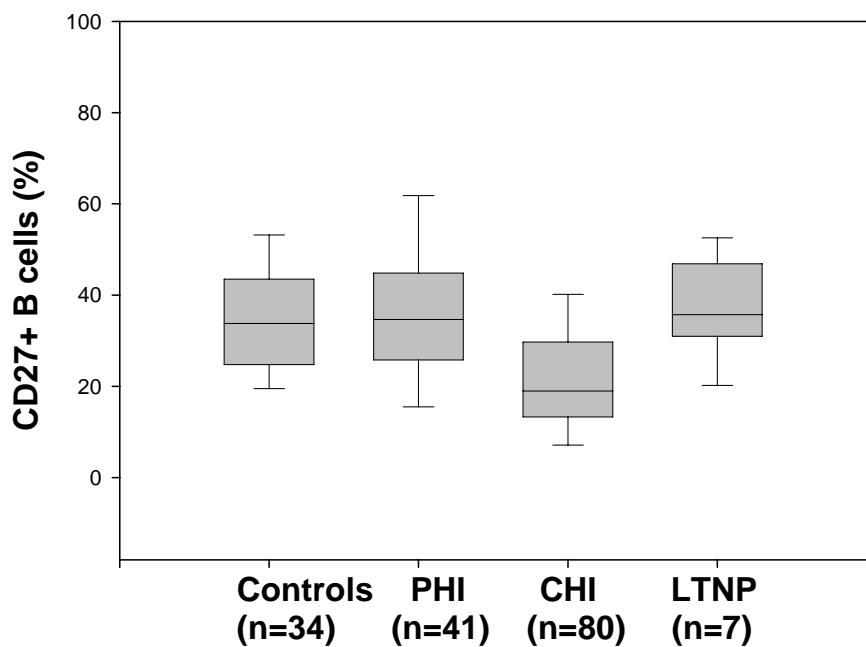
In paper IV, we showed that in a cohort of patients from Guinea Bissau, plasma IgG amounts in HIV-2-infected patients were comparable to healthy controls but significantly lower than amounts in HIV-1-infected patients. We also showed that background immune activation is an important factor to consider when evaluating plasma IgG in HIV-infected patients, since the uninfected subjects from Guinea Bissau had significantly higher amounts of plasma IgG than European controls.

### **5.3 B cell depletion and memory B cell subpopulations**

Although not as intensely studied as CD4<sup>+</sup> T cell depletion, B cell depletion is an important pathogenic feature of HIV infection. A reduction in circulating memory B cells, associated with a significant increase in spontaneous *in vitro* cell death was previously reported in CHI (De Milito et al. 2001; Titanji et al. 2003).

We reported that memory B cells from patients with CHI undergo high rates of spontaneous apoptosis (paper I). In paper II, we set out to investigate if such cell death was also present in PHI, and to find some clues to what mechanisms may be involved. We found that both naïve and memory B cells of patients with PHI had high rates of spontaneous apoptosis when placed in short-term cultures. We went on to investigate whether alterations in intracellular expression of bcl-2 may play a role in this spontaneous apoptosis. Bcl-2 expression was increased in naïve B cells from patients with PHI, but expression in memory B cells was comparable to expression in controls. Our cumulative data on memory B cell percentages in HIV-infected patients at various stages of infection show that the patients with CHI have the most profound

depletion of memory B cells and LTNP patients are characterized by a normal amount of circulating memory B cells (Figure 5).

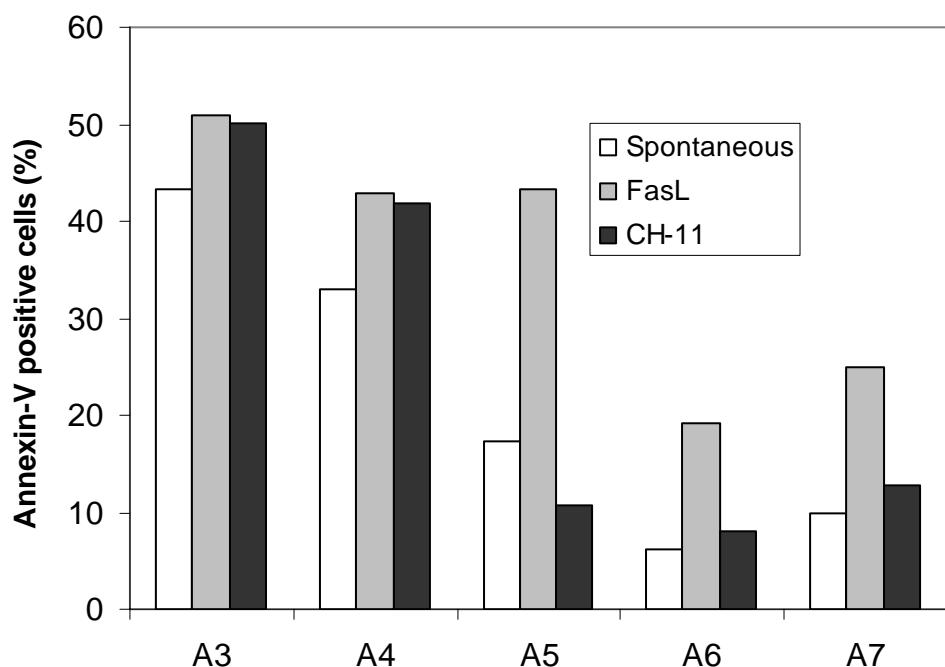


**Figure 5. Memory (CD19+CD27+) B cell percentages in HIV-1 infection**

We have previously shown that death of memory B cells may be due to limited availability of Nerve Growth Factor (NGF) (Titanji et al. 2003). Human B cells proliferate and differentiate into IgM and IgA secreting cells in the presence of NGF (Otten et al. 1989) and NGF was shown to be an autocrine survival factor for B cells (Torcia et al. 1996). We showed that culturing B cells in the presence of recombinant NGF induced up to 20% reduction in memory cell death (Titanji et al. 2003). In our initial attempts to investigate whether Fas-mediated apoptosis was a pathway involved in the depletion of memory B cells, we cultured B cells with agonistic Fas mAb (CH-11) but did not observe any induction of apoptosis in this system (Titanji et al. 2003). Recently, it was shown that Fas-mediated apoptosis could be induced in a particular ( $CD21^{\text{low}}$ ) subset of B cells obtained from viremic CHI patients (Moir et al. 2004). The authors showed that: (1) genes associated with IFN stimulation and terminal differentiation are upregulated in viremic patients, (2)  $CD21^{\text{low}}$  B cells had high levels of CD95 and were more susceptible to Fas-mediated apoptosis induced by incubation with soluble FasL and (3) increased expression of B cell maturation Ag (BCMA) on

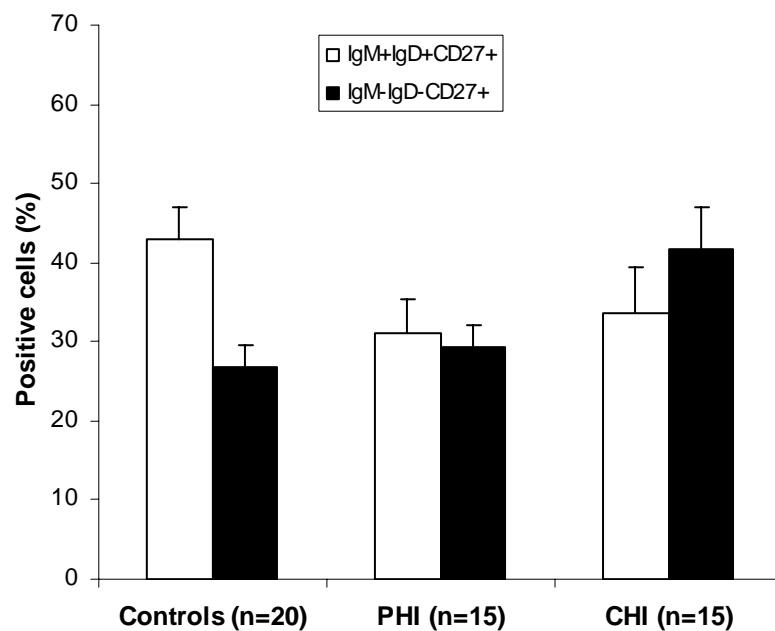
$\text{CD21}^{\text{low}}$  B cells was associated with reduction of the more potent B lymphocyte stimulating factor (Blys) receptor BAFF-R resulting in reduced Blys binding and Blys-mediated survival. Blys, also known as B cell activation factor belonging to the TNF family (BAFF), is a B cell survival and maturation factor (Mackay et al. 2003) and is known to bind to three receptors: TACI, BCMA and BAFF-R (Gross et al. 2000; Thompson et al. 2000; Thompson et al. 2001), though BAFF-R is the principal BAFF receptor (Ng et al. 2004). It was recently shown that BAFF may enhance the survival of plasmablasts generated in secondary lymphoid tissues (Avery et al. 2003). The role of B cell survival factors in HIV infection has however not been well characterized.

We have since confirmed the susceptibility of patients' B cells to Fas-mediated apoptosis in a small group of patients. We cultured purified B cells in the presence of 2  $\mu\text{g/ml}$  His-tagged FasL or 1  $\mu\text{g/ml}$  CH-11 and found that FasL but not CH-11 induced higher apoptosis compared to control cultures. Interestingly, we observed that in the patients with spontaneous apoptosis higher than 30%, Fas ligation did not significantly increase B cell apoptosis (Figure 6).



**Figure 6. Apoptosis of B cells in CHI**

Based on the expression of IgM and IgD, the human memory (CD27+) B cell compartment can be divided into several subsets (Tangye et al. 1998; Weller et al. 2004). IgM memory B cells, so called because they possess somatically hypermutated genes and express CD27 just like conventional memory B cells, make up about 50% of the circulating memory B cell population (Klein et al. 1997; Kruetzmann et al. 2003). It was interesting to note that while we did not find any significant loss of total memory (CD27+) B cells in PHI, we found that IgM memory B cells were lower in PHI compared to controls and CHI, but this decrease did not reach statistical significance. The percentage of IgM<sup>-</sup>IgD<sup>+</sup> B cells was higher in the patients compared to controls indicating that a higher degree of isotype switch occurs in HIV infection (Figure 7).



**Figure 7. Percentages of IgM+IgD+ and IgM-IgD- B cells in controls and HIV-infected patients**

We also found an increased fraction of IgM<sup>-</sup>IgD<sup>+</sup> memory B cells, which is a very small subpopulation in healthy controls. This is in line with a previous study where it was shown that HIV-1-infected patients with AIDS-related complex (ARC) early in infection had an 8-fold increase in serum levels of IgD (Mizuma et al. 1987). Taken together, depletion of B cells in HIV-1 infection occurs as a result of death ligand-

mediated apoptosis and possibly through altered amounts of B cell survival factors as well as altered expression of receptors to survival factors.

#### **5.4 Specific antibody production**

The observed depletion of circulating memory B cells prompted us to investigate whether there would be a corresponding decrease in levels of antigen-specific antibodies to HIV antigens as well as non-HIV antigens.

In Paper I, we found that both anti-measles and anti-tetanus toxoid (TT) Ab titers were significantly lower in CHI compared to healthy controls and there was a positive correlation between the anti-measles Ab levels and percentage of memory B cells in the patients. We also arbitrarily divided the patients into those with normal (NM) and those with low (LM) percentages of memory B cells based on the median percentages of memory B cells found in the patients. Titers of anti-gp41, anti-measles and anti-TT Abs were higher in patients with normal percentages of memory B cells compared to those with low percentages of memory B cells. In contrast, plasma IgG and PSA reactivity was similar in the NM and LM groups.

In paper III we evaluated antibody titers against measles, pneumococcus and gp41 in PHI, CHI and LTNP compared to controls. Both anti-measles and anti-pneumococcus Ab titers were significantly decreased in PHI and CHI compared to controls. In the LTNP patients, Ab titers against both antigens were comparable to controls.

Due to the low amounts of pre-existing antibodies against several antigens in patients with HIV-1 infection, patients with chronic infection may be particularly vulnerable to common infectious agents and it is recommended that patients receive vaccination against pathogens such as influenza, tetanus and pneumococcus (Kroon et al. 1994; Kroon et al. 1999; Zanetti et al. 2002). However, the benefits of such vaccinations as assessed by levels of antibody titers generated, are usually modest and seem to be linked to the disease stage and progression (Kroon et al. 1994; Malaspina et al. 2005).

#### **5.5 Memory B cells and long-term humoral immunity**

The finding that the titers of specific Ab are severely decreased in patients with PHI and CHI indicated that long-term humoral immunity in these patients is significantly impaired. We hypothesized that the reduction of specific Ab titers in patients was a direct consequence of the loss of memory B cells. We therefore set out to verify this (Paper III), using measles as our model antigen in elispot assays where we counted

total IgG- and measles antibody secreting cells (ASC) in patients with PHI, CHI and LTNP. We found significantly fewer numbers of both IgG and measles ASC in PHI and CHI, but not in LTNP where the numbers of ASC were comparable to those found in healthy controls. When we translated the number of ASC into number of memory B cells, we found that the percentage of measles-specific memory B cells in PHI and CHI were lower than in healthy controls. The number of anti-measles ASC was positively correlated to anti-measles Ab titers in CHI.

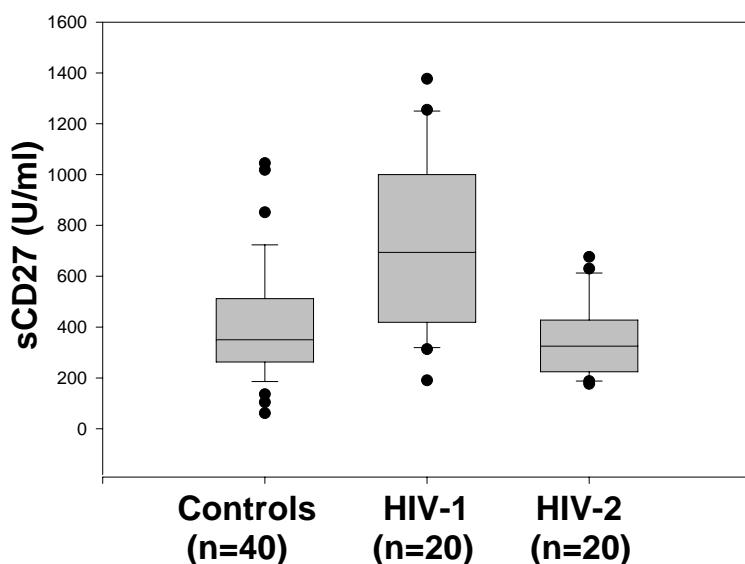
The ability to mount an accelerated humoral immune response to a previously encountered antigen depends on the presence of memory B cells, and is the basis of vaccination (Zinkernagel 2003; Crotty et al. 2004). The loss of antigen (measles)-specific memory B cells would explain the decline in the anti-measles antibody titers, but also may have important consequences for measles infection of HIV-infected patients. Studies show that maternally acquired immunity in children born to HIV-infected women is poorer than that in newborns from healthy women, either because of low maternal serum antibody levels or deficient transplacental transfer. (de Moraes-Pinto et al. 1993). Neonatal antibody levels to tetanus toxoid, measles, and S. pneumoniae were also shown to be significantly lower in the HIV group. Both maternal hypergammaglobulinemia and maternal HIV infection may contribute to these low antibody levels at birth and thus lead to early infection in this high-risk population (de Moraes-Pinto et al. 1996). As a result, it was suggested that the measles vaccine schedule should be revised for these children and the same should be done for future passive immunization regarding fetus protection in pregnant HIV-seropositive women. It has been shown that HIV-infected children have poorer antibody responses to measles vaccination, and the antibody titers induced by vaccination decline faster than in healthy children (Helfand et al. 2005).

Thus HIV-infected children are likely to have higher rates of both primary and secondary vaccine failure (al-Attar et al. 1995; Arpadi et al. 1996), and HIV-infected children who do not develop protective Ab titers after initial measles vaccination will not develop protective titers after re-vaccination (Helfand et al. 2005). Our data and others' indicate that HIV-1 infection may interfere with long-term humoral immunity through: (1) Deletion of CD4<sup>+</sup> T cells, leading to defective help, and (2) deletion of memory B cells.

## 5.6 Immune activation and sCD27

The profound immune activation associated with HIV-1 infection is thought to be one of the driving forces behind several of the immune disorders, including the depletion of CD4<sup>+</sup> T cells. A lower degree of immune activation has also been proposed as an explanation for the lower pathogenicity of HIV-2 infection. Soluble CD27 (sCD27) is released through proteolytic cleavage of surface CD27 following T cell activation (Hintzen et al. 1991) and elevated amounts of plasma sCD27 are thus a good indicator of immune activation (Loenen et al. 1992; van Oers et al. 1993). High titers of sCD27 have also been found in the sera of patients with B cell tumors with a strong correlation between serum sCD27 levels and tumor load (van Oers et al. 1993; Nilsson et al. 2005). Plasma cells also express high levels of CD27 (Jung et al. 2000; Avery et al. 2005) and serum sCD27 levels may be of significance in diseases characterized by dysfunctions of B cell differentiation and activation such as SLE and primary Sjögren's syndrome (Font et al. 1996; Bohnhorst et al. 2002).

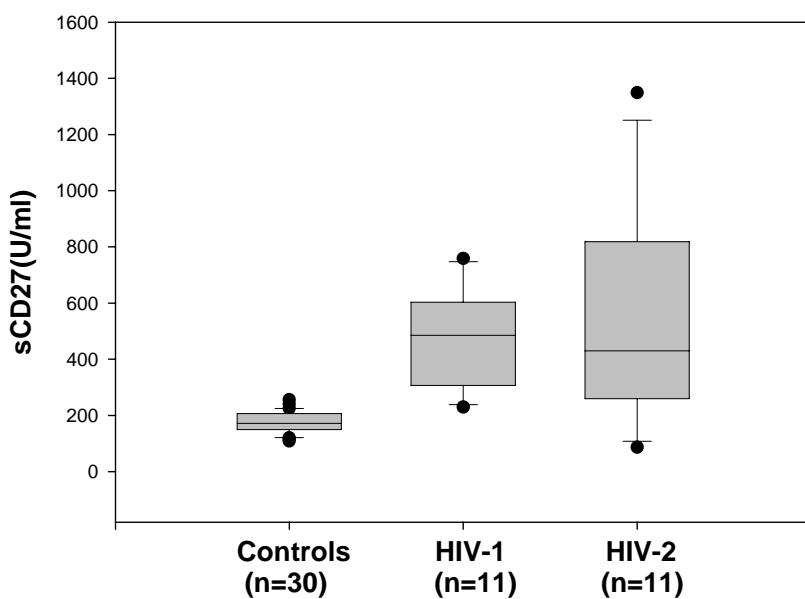
In paper IV, we found that the amount of sCD27 in the plasma of HIV-2-infected patients was lower than the amount in HIV-1-infected patients from Guinea Bissau (Figure 8A). sCD27 was also negatively correlated with CD4<sup>+</sup> T cell count in the HIV-1-infected. Unlike sCD27 however, the amount of  $\beta$ -2 microglobulin was comparable in the two patient groups.



**Figure 8A. Levels of sCD27 in HIV-1 and HIV-2 in patients from Guinea Bissau**

We also compared levels of plasma IgG in HIV-1 and HIV-2 infections. Plasma IgG in the HIV-1-infected patients was significantly higher than that in controls and HIV-2-infected patients from Guinea Bissau, and we observed a strong positive correlation between sCD27 and IgG in HIV-1 but not in HIV-2. The amounts of plasma IgG in HIV-2 were comparable to those in healthy controls. Unlike  $\beta$ -2M sCD27 seemed to be a more discriminatory marker of immune activation. The correlation between sCD27 and plasma IgG suggest a possible role of sCD27 in hypergammaglobulinemia.

It has been suggested that infectious diseases like malaria and helminth infections prevalent in developing parts of the world, e.g. in sub-saharan Africa may induce a high background of immune activation and make the host more susceptible to HIV infection and faster disease progression (Bentwich et al. 1995). In an attempt to determine how much background immune activation may have contributed to the levels of immune activation, we analyzed groups of patients living in Europe. Unlike in the Guinea Bissau cohort, levels of sCD27 were comparable in HIV-1 and HIV-2 but significantly higher than levels in the corresponding European controls (Figure 8B).



**Figure 8B. Levels of sCD27 in European patients**

The levels of both sCD27 and IgG were significantly lower in the European controls compared to the Guinea Bissau controls.

We recently measured sCD27 in European PHI and LTNP patients as well (not included in Paper IV,) and found that the PHI patients had the highest levels of sCD27 [533.7 U/ml (378-809)] compared to controls [172 U/ml (152-183)], CHI [485 U/ml (335.3-592)], HIV-2 [430 U/ml (288.8-764)] and LTNP [362 U/ml (251-472.4)]. Interestingly, while sCD27 in LTNPs was lower compared to the other patient groups, it was still significantly higher than in controls. The situation of the LTNP is far from straightforward. It is reported that LTNPs do have increased activation of the immune system (Franceschi et al. 1997; Rodes et al. 2004) and show biological signs of disease progression after 10 years of HIV infection (Lefrere et al. 1997). This suggests that though LTNP may have levels of immune activation comparable to patients with progressive infection, other mechanisms may be at play in these LTNP, helping them maintain a relatively healthy state.

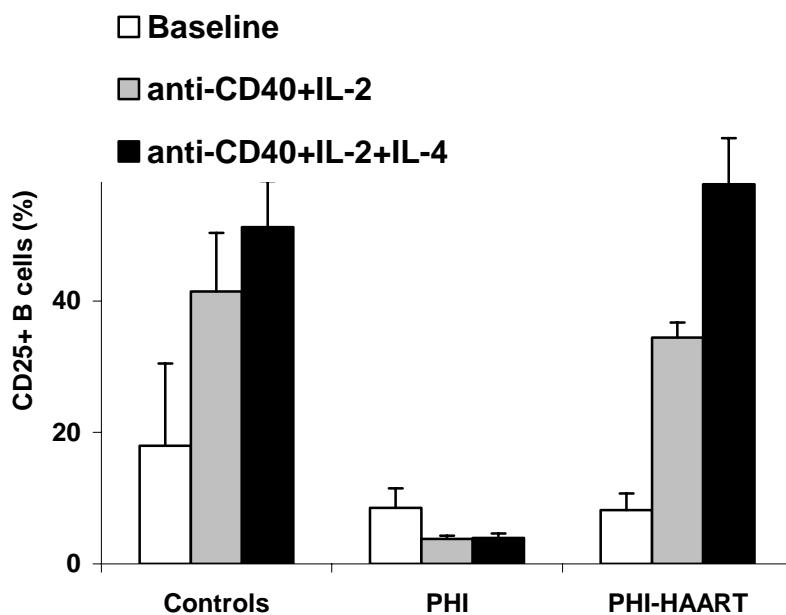
Evidence suggests that HIV is a relatively new human pathogen and may not yet have reached a stable non-lethal co-existence with its host, as has SIV in sooty mangabeys and African green monkeys (Broussard et al. 2001; Silvestri et al. 2003). It has been shown that HIV-2 is more closely related to SIV than to HIV-1 and similar to SIV, the relatively milder course of infection may be explained by a lower degree of immune activation than HIV-1. One may also speculate that HIV-2 entered the human population earlier than HIV-1 and may therefore be more adapted to the human host than HIV-1. Homozygosity for a 32bp deletion in CCR5 has been associated with protection against primary HIV-1 infection (Samson et al. 1996) and it has recently been shown that the lower pathogenicity of HIV-2 infection may be due to down-regulated expression of CCR5 in HIV-2-infected individuals (Shea et al. 2004). These results highlight the importance of chronic immune activation in the pathogenesis of HIV-1 disease and suggest a possible role of genetic factors in modulating the course of HIV disease.

## **5.7 Antiretroviral therapy and B cell dysfunctions**

The most obvious effects of successful antiretroviral therapy (ART) in HIV infection are decreased plasma viral load and increased CD4<sup>+</sup> T cell counts. However, the effects of ART on B cell dysfunctions are not completely clear.

In Paper II, we investigated the effects of short-term (6 months) ART on B cell activation and dysfunctions in PHI, reasoning that PHI would represent the appropriate phase to study B cell dynamics. We found that ART produced

heterogeneous results in terms of restoring activation marker expression to normal. CD70 and LAIR-1 expression were largely unaffected while Fas expression on both naïve and memory B cells was significantly decreased. Bcl-2 expression was also unaffected by therapy. Spontaneous apoptosis of both naïve and memory B cells was also reduced by ART, but was only normalized in the naïve B cell subset. Plasma IgG levels were also reduced compared to baseline levels, but were not normalized either. We also determined whether therapy regimens containing PI (HAART) had any advantage over non-PI-containing regimens (NRTI), in terms of improving the state of B cells in patients. We found there was a two-fold increase in B cell percentages in patients on HAART, but HAART and NRTI had similar effects on most of the other parameters studied. We also found that the B cells of HAART-treated PHI patients recovered the ability to respond to *in vitro* T cell stimulation. It was recently reported that B cells from HIV-infected patients are unable to upregulate CD25 in response to activated CD4<sup>+</sup> T cells (Moir et al. 2003). We therefore evaluated the induction of CD25 (IL-2 receptor) on B cells following T-cell dependent activation *in vitro*, a parameter of functional B-T cell interaction.



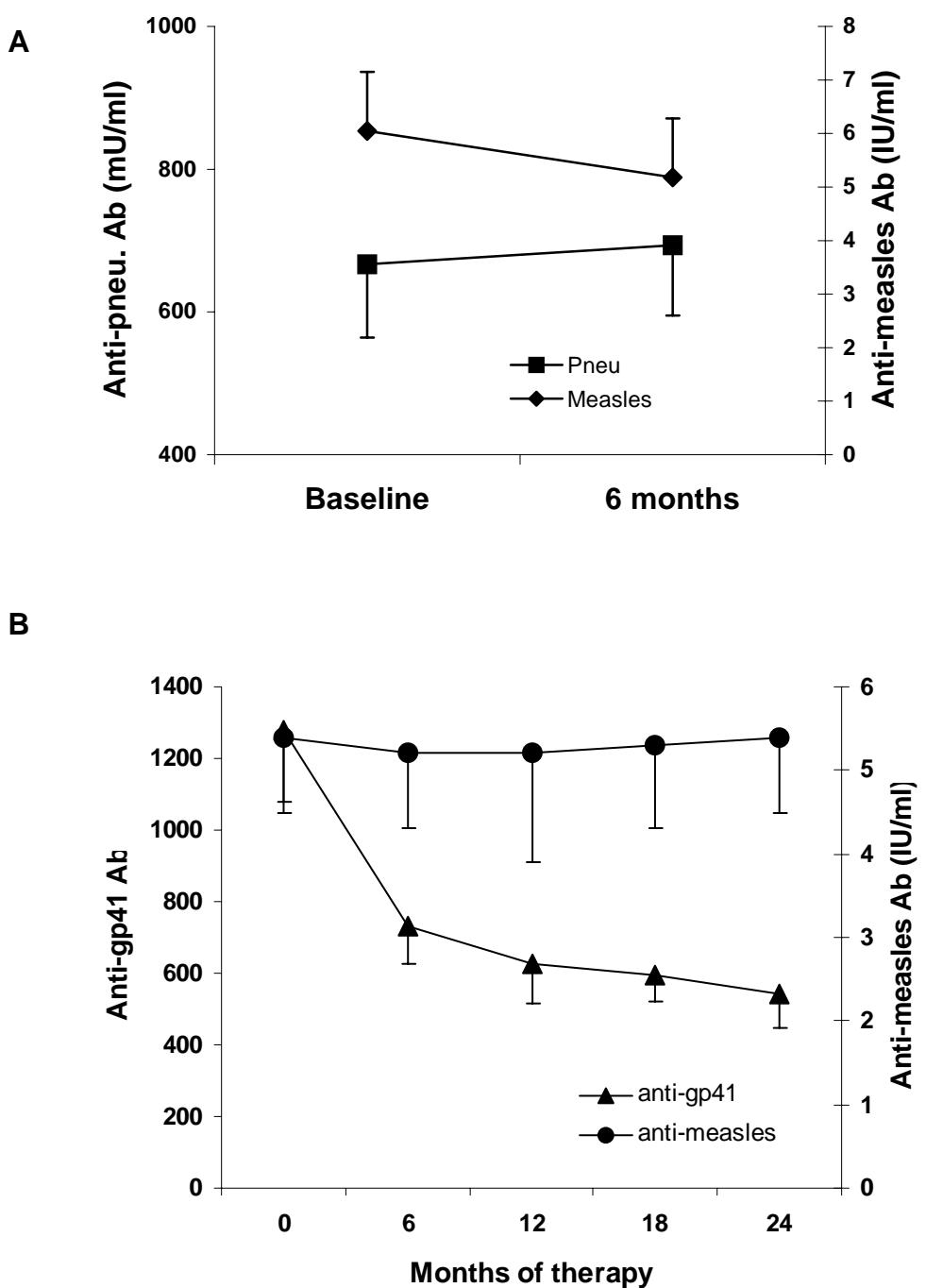
**Figure 9. Induction of CD25 expression on activated B cells in PHI.**

We observed that CD25 expression on B cells analyzed *ex vivo* was very low in patients with PHI as compared to healthy persons. In addition, B cells from patients at diagnosis of PHI did not upregulate CD25 after activation through anti-CD40 + IL-2

(and IL-4) while B cells from normal subjects promptly increased CD25 expression. However, after 6 months of therapy, B cells from PHI patients recovered the ability to upregulate CD25 expression after T-cell dependent stimulation to levels comparable to healthy controls (Figure 9).

We also investigated the effect of ART on titers of antigen-specific Ab in PHI and CHI (Figure 10). ART did not restore the titers of anti-measles or anti-pneumococcus Ab in PHI, or the titers of anti-measles Ab in CHI. Conversely, titers of anti-gp41 Ab steadily declined with plasma viral load during follow-up in both PHI and CHI. These (except for the pneumococcus) data are presented in paper III.

In attempt to cover the spectrum of the proposed mechanisms for maintenance of long-term humoral immunity, we studied a variety of Ags in paper III: Pneumococcus (TI Ag), measles (TD Ag) both assumed not to be circulating in the patients at the time of this study, as well as HIV gp41 which represented an Ag that was currently circulating in the patients. The picture presented by our results is the following: Maintenance of humoral immunity (as assessed by plasma Ab levels) to both TI and TD Ags is impaired in HIV infection and the maintenance of Ab titers against the HIV-specific Ag gp41 seems to require a high level of viral replication since reducing viral replication with ART led to a decrease in anti-gp41 Ab titers.



**Figure 10. Effect of therapy on Ag-specific Abs in (A) PHI and (B) CHI**

## **6 CONCLUSIONS**

The following conclusions can be drawn from the studies presented in this thesis:

- Memory B cells of patients with chronic HIV infection undergo massive spontaneous apoptosis.
- Loss of memory B cells is a feature of chronic infection, but is not observed in primary infection thus may be an effect of persistent immune activation
- The Fas-FasL pathway may contribute to the deletion of B cells in HIV infection
- Antigen-specific humoral immunity is also impaired early during primary HIV-1 infection as shown by reduction in levels of specific antibodies to HIV as well as to measles and pneumococcal antigens and tetanus toxoid
- Measles-specific memory B cells are deleted in HIV-1 infection
- Naive B lymphocytes are abnormally activated in HIV-1 infection and may contribute to hypergammaglobulinemia
- The majority of B lymphocyte dysfunctions is initiated early in primary infection and persists throughout the course of the disease.
- Immune activation, as assessed by sCD27 in plasma, is lower in HIV-2 infection than in HIV-1 infection, and this lower immune activation may explain the slower rate of disease progression in HIV-2 infection
- Antiretroviral therapy is able to correct only a small portion of the B cell dysfunctions observed in HIV-1-infected patients.

## 7 ACKNOWLEDGEMENTS

The work presented in this thesis was performed at MTC, Karolinska Institutet and was sponsored by grants from: SIDA/SAREC, Vetenskapsrådet (VR), Swedish Physicians against AIDS (Svenska Läkare mot AIDS), Petrus och Augusta Hedlunds Stiftelse and Karolinska Institutet.

I would like to acknowledge all the patients and volunteers who have selflessly and generously participated in the studies by donating their samples and time.

*Francesca Chiodi*- for the chance to work in your lab, your passion for science, constant support and encouragement (even when I thought it was no fun dealing with reviewers!) and for putting together such a wonderful group of people to work with. Thank you for understanding my independent streak and helping me grow as a scientist-I'm so glad I came to that first interview 4 years ago (though to be honest I don't remember a word of what I said that day☺)

*Angelo De Milito*- supervisor extraordinaire! For teaching me so much, trusting me with valuable experiments and letting me find my feet and an unforgettable week in Barcelona; for your attention to detail, enthusiasm and interest in my projects and for invaluable input, even when it had to be over the phone from Rome the past 2 years. Thanks for sharing your passion for Italian food and for introducing me to your *Susanne* thanks to whom I've spent a lovely couple of years in Bromma!

All the collaborators and physicians who provided invaluable help: *Ann Atlas, Sven Grützmeier, Bo Hejdeman, Anna Nilsson, Lucia Lopalco, Giuseppe Tambussi and Rigmor Thorstensson*.

*Eva Severinson*- for that phone interview and for a great learning experience during the year I spent at Biomedicinska Forskaskolan.

*Camilla Ahlqvist*- for your time and patience and help from the very first day I arrived at Forskaskolan, and for always answering my questions (with a smile☺) and helping me out whenever I bugged you about one thing or the other.

*Ingrid Faye*- for taking me in on my first project rotation and *Olle Terenius* for patiently teaching me so much in the lab when I was still green and for your refreshing passion for bugs!

All my co-authors: *Sören Andersson, Ann Atlas, Rino Bellocco, Alberto Cagigi, Zacarias da Silva, Sven Grützmeier, Bo Hejdeman, Lucia Lopalco, Mitsuo Narita, Anna Nilsson, Hans Norrgren, Lyda Osorio, Elisabet Reizenstein, Danika Schepis, Ana E Sousa, Anders Sönderborg, Chiara Tassandin, Giuseppe Tambussi, Rigmor Thorstensson*.

My partners in crime-the members of the Chiodi group, for being so much fun to work with and for all the fun we have in the lab, writing room and on outings and conferences:

*Ann Atlas*- for generosity with your time and your way with people; *Alberto Cagigi*- for keeping the muppets alive and for understanding when I changed radio stations in the lab ☺; *Liv Eidsmo*- for all the interesting conversations, scientific and otherwise; *Caroline Fluur*-for your sense of humor and that week in Baltimore; *Frida Mowafi*- for starting the PhD with me and for sharing the girls too; *Anna Nilsson*- for brainstorming sessions and for that week in Colorado; *Bence Rethi*- for your special brand of humor and for letting me pick your brains so often; *Stefano Sammicheli*-(‘79 really was a good year!) for being so much fun to be around; *Nancy Vivar*- for being so understanding and compassionate and for all the lunches; *Farideh Sabri*- for your gentleness and for bringing Nik around; *Malgosha Kryzowska and Tran thi thanh ha*

*Kerstin Andreasson and Lena Norrenius* for invaluable help in the P3 lab

*Anneka Erhnst*-for the early journal club days and for introducing me to Kungsholmen!

*IMED- Maorong Ruan*-for your wealth of knowledge and for letting me tap it so often; *Lyda Osorio* - for help with my apoptosis experiments; *Vanoohi Fredriksson*-for your energy and help in the lab; *Nicolas Ruffin*-c'était un plaisir de pouvoir parler français avec toi

All the people at MTC who helped me out along the way: *Mats Wahlgren, Mikael Jondal; Anna Lödberg and Eva Pijnenburg* for invaluable help with administrative issues; *Sàndor Feldoto and Alex Candia* for answering my frantic calls for help when my computer turned against me; *Birgitta Wester* for help with the FACS and conversations about Sweden; *Greger Blomquist, Torbjörn Laggar, Håkan Wikland and Birgitta Karlsson* for help with all things practical; *Danika*-for all those control experiments and fun company; *György, Lori, Anquan, Ami*, for interesting lunch company.

*Malin Åkerblom*-for being so nice and helpful in so many ways.

*Helena Aineskog* for your encouraging words and upbeat attitude; *Zongpei Chen* for sharing that first forskaskolan year with me and company at the movies; *Alexandra 'Sasha' Selivanova* for your positive attitude and laughter; *Maria Bengtsson* and *Lena Lövkist* for always taking the time to talk whenever we met; *Jenny Karlsson* for your interesting mails och din underbara skånska, and for all the 'lunch träffar'.

*Johanna Gardeström, Hanna Brunzell and Stina Johansson* for introducing me to the concept of 'tjejer middagar', for all the fun times and for really making me feel like I belonged.

*Monika Berge-* for lovely dinners, for sharing my love for Afrika, for fun conversations and the Richard Bona concert.

*Nicky, Danni et Titi Nkoundjo* pour votre amitié et soutien, malgré tout, toutes ces années; *Ako (Lalas), Nate, Honorie, Ngozi, Ekwe*, for phone calls and laughter and for being such good friends even when I was too tied up in the lab to be a good friend myself.

*Marilyn, Efeti and Christie-* for being my friends all these years.

*Anders & Ewa Liljas-* för att ni har funnits där för mig från den allra första början och har givit mig ett hem att komma till när jag höll på att stressa ijhäl mig i Stockholm; för hemlagad mat och alla mysiga middagar; stort tack också till *Per* som bidrog till att göra julen 2005 så oförglömligt! Och för *Tumrus* så klart!

*Ebob-* for being my 'sista', the best friend any one could ask for and for supporting me in everything I do. I'm so proud of you!

My very own support squad-my wonderful family! *Ba & Mamie-* the best parents a girl could ever hope for, for too many things to name but mostly for the support and love I've counted on all my life and for being such role models. *Mamie*, for taking it in stride when it became clear you would have another absent-minded scientist on your hands; *Papa, Mamie, Ma Kah & the Lima family; Tangwi Anna and Tangwi Be*-for encouraging me in all I do; *Bambot & Mamie Jubsia and the Galloping Circle crew*-for that Christmas and for the Baltimore home base; *the grandes-soeurs and their families-Ma Ju, Ma Nah, Ma Ethel* for being true big sisters and helping me out whenever I needed you-from desperate airport phone calls to discussions on love and guys; the *grands-frères -Ni Ernest* for support when I was in 'chaumencam' and *Ni Wilson (Papa Wi)* for being such a good example and for bringing *Marion* into the family, *Ni Bidla* for being just a phone call away, interesting stories and for bringing *Kuna* into the family; my little sisters *Loema* and *Boghuma*-you can't believe how proud I am of you girls! For loving your slightly distracted big sister, for all the phone calls reminding me to eat and for making me laugh when I felt blue; the *petits frères-Da & Legima* for always cheering me up with your weird stories and view of the world. Njika! I love you guys so much and I could never, ever have done this without you.

*Mommy T, Feyi* and the rest of the *Thompson family*-for love and support; thanks for welcoming me into your family.

*Yemi, loml*, for unconditional love, laughter, introducing me to Kennesaw, chauffeur duties (☺), those phone calls-the last few years have been easier because of you. Thanks for being my very own tough guy with a soft heart. Mo ni ife e. It only gets better!

## 8 REFERENCES

- Agematsu, K., H. Nagumo, Y. Oguchi, T. Nakazawa, K. Fukushima, K. Yasui, S. Ito, T. Kobata, C. Morimoto and A. Komiyama (1998). "Generation of plasma cells from peripheral blood memory B cells: synergistic effect of interleukin-10 and CD27/CD70 interaction." *Blood* 91(1): 173-80.
- Ahearn, J. M., M. B. Fischer, D. Croix, S. Goerg, M. Ma, J. Xia, X. Zhou, R. G. Howard, T. L. Rothstein and M. C. Carroll (1996). "Disruption of the Cr2 locus results in a reduction in B-1a cells and in an impaired B cell response to T-dependent antigen." *Immunity* 4(3): 251-62.
- al-Attar, I., J. Reisman, M. Muehlmann and K. McIntosh (1995). "Decline of measles antibody titers after immunization in human immunodeficiency virus-infected children." *Pediatr Infect Dis J* 14(2): 149-51.
- Albert, J., B. Abrahamsson, K. Nagy, E. Aurelius, H. Gaines, G. Nystrom and E. M. Fenyo (1990). "Rapid development of isolate-specific neutralizing antibodies after primary HIV-1 infection and consequent emergence of virus variants which resist neutralization by autologous sera." *Aids* 4(2): 107-12.
- Allen, T. M., A. D. Kelleher, J. Zaunders and B. D. Walker (2002). "STI and beyond: the prospects of boosting anti-HIV immune responses." *Trends Immunol* 23(9): 456-60.
- Allen, T. M., D. H. O'Connor, P. Jing, J. L. Dzuris, B. R. Mothe, 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(6802): 386-90.
- 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(10): 3366-9.
- Amadori, A., A. De Rossi, G. P. Faulkner-Valle and L. Chieco-Bianchi (1988). "Spontaneous in vitro production of virus-specific antibody by lymphocytes from HIV-infected subjects." *Clin Immunol Immunopathol* 46(3): 342-51.
- Ansari, A. A. (2004). "Autoimmunity, anergy, lentiviral immunity and disease." *Autoimmun Rev* 3(7-8): 530-40.
- Appay, V., F. Boutboul and B. Autran (2005). "The HIV infection and immune activation: "to fight and burn"." *Curr Infect Dis Rep* 7(6): 473-9.
- Arpadi, S. M., L. E. Markowitz, A. L. Baughman, K. Shah, H. Adam, A. Wiznia, G. Lambert, J. Dobroszycki, J. L. Heath and W. J. Bellini (1996). "Measles antibody in vaccinated human immunodeficiency virus type 1-infected children." *Pediatrics* 97(5): 653-7.
- Avery, D. T., J. I. Ellyard, F. Mackay, L. M. Corcoran, P. D. Hodgkin and S. G. Tangye (2005). "Increased expression of CD27 on activated human memory B cells correlates with their commitment to the plasma cell lineage." *J Immunol* 174(7): 4034-42.
- Avery, D. T., S. L. Kalled, J. I. Ellyard, C. Ambrose, S. A. Bixler, M. Thien, R. Brink, F. Mackay, P. D. Hodgkin and S. G. Tangye (2003). "BAFF selectively enhances the survival of plasmablasts generated from human memory B cells." *J Clin Invest* 112(2): 286-97.
- Baba, T. W., V. Liska, R. Hofmann-Lehmann, J. Vlasak, W. Xu, S. Ayehunie, L. A. Cavacini, M. R. Posner, H. Katinger, G. Stiegler, B. J. Bernacky, T. A. Rizvi, R. Schmidt, L. R. Hill, M. E. Keeling, Y. Lu, J. E. Wright, T. C. Chou and R. M. Ruprecht (2000). "Human neutralizing monoclonal antibodies of the IgG1 subtype protect against mucosal simian-human immunodeficiency virus infection." *Nat Med* 6(2): 200-6.
- Baba, T. W., V. Liska, A. H. Khimani, N. B. Ray, P. J. Dailey, D. Penninck, R. Bronson, M. F. Greene, H. M. McClure, L. N. Martin and R. M. Ruprecht (1999). "Live attenuated, multiply deleted simian immunodeficiency virus causes AIDS in infant and adult macaques." *Nat Med* 5(2): 194-203.
- Badley, A. D., A. A. Pilon, A. Landay and D. H. Lynch (2000). "Mechanisms of HIV-associated lymphocyte apoptosis." *Blood* 96(9): 2951-64.
- Bangsberg, D. R., F. M. Hecht, E. D. Charlebois, A. R. Zolopa, M. Holodniy, L. Sheiner, J. D. Bamberger, M. A. Chesney and A. Moss (2000). "Adherence to protease inhibitors, HIV-

- 1 viral load, and development of drug resistance in an indigent population." *Aids* 14(4): 357-66.
- Barre-Sinoussi, F., J. C. Chermann, F. Rey, M. T. Nugeyre, S. Chamaret, J. Gruest, C. Dauguet, C. Axler-Blin, F. Vezinet-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(4599): 868-71.
- Benjouad, A., J. C. Gluckman, H. Rochat, L. Montagnier and E. Bahraoui (1992). "Influence of carbohydrate moieties on the immunogenicity of human immunodeficiency virus type 1 recombinant gp160." *J Virol* 66(4): 2473-83.
- Bentwich, Z., A. Kalinkovich and Z. Weisman (1995). "Immune activation is a dominant factor in the pathogenesis of African AIDS." *Immunol Today* 16(4): 187-91.
- Berberian, L., L. Goodlick, T. J. Kipps and J. Braun (1993). "Immunoglobulin VH3 gene products: natural ligands for HIV gp120." *Science* 261(5128): 1588-91.
- Berger, E. A. (1997). "HIV entry and tropism: the chemokine receptor connection." *Aids* 11 Suppl A: S3-16.
- Bernasconi, N. L., E. Traggiai and A. Lanzavecchia (2002). "Maintenance of serological memory by polyclonal activation of human memory B cells." *Science* 298(5601): 2199-202.
- Biron, C. A., K. B. Nguyen, G. C. Pien, L. P. Cousens and T. P. Salazar-Mather (1999). "Natural killer cells in antiviral defense: function and regulation by innate cytokines." *Annu Rev Immunol* 17: 189-220.
- Biswas, P., M. Ferrarini, B. Mantelli, C. Fortis, G. Poli, A. Lazzarin and A. A. Manfredi (2003). "Double-edged effect of Vgamma9/Vdelta2 T lymphocytes on viral expression in an in vitro model of HIV-1/mycobacteria co-infection." *Eur J Immunol* 33(1): 252-63.
- Biswas, P., B. Mantelli, A. Sica, M. Malnati, C. Panzeri, A. Saccani, H. Hasson, A. Vecchi, A. Saniabadi, P. Lusso, A. Lazzarin and A. Beretta (2003). "Expression of CD4 on human peripheral blood neutrophils." *Blood* 101(11): 4452-6.
- Blattner, W. A., K. Ann Oursler, F. Cleghorn, M. Charurat, A. Sill, C. Bartholomew, N. Jack, T. O'Brien, J. Edwards, G. Tomaras, K. Weinhold and M. Greenberg (2004). "Rapid clearance of virus after acute HIV-1 infection: correlates of risk of AIDS." *J Infect Dis* 189(10): 1793-801.
- Bohnhorst, J. O., M. B. Bjorgan, J. E. Thoen, R. Jonsson, J. B. Natvig and K. M. Thompson (2002). "Abnormal B cell differentiation in primary Sjogren's syndrome results in a depressed percentage of circulating memory B cells and elevated levels of soluble CD27 that correlate with Serum IgG concentration." *Clin Immunol* 103(1): 79-88.
- Borrow, P., H. Lewicki, X. Wei, M. S. Horwitz, N. Peffer, H. Meyers, J. A. Nelson, J. E. Gairin, B. H. Hahn, M. B. Oldstone and G. M. Shaw (1997). "Antiviral pressure exerted by HIV-1-specific cytotoxic T lymphocytes (CTLs) during primary infection demonstrated by rapid selection of CTL escape virus." *Nat Med* 3(2): 205-11.
- Botarelli, P., B. A. Houlden, N. L. Haigwood, C. Servis, D. Montagna and S. Abrignani (1991). "N-glycosylation of HIV-gp120 may constrain recognition by T lymphocytes." *J Immunol* 147(9): 3128-32.
- 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(6): 749-59.
- Broussard, S. R., S. I. Staprans, R. White, E. M. Whitehead, M. B. Feinberg and J. S. Allan (2001). "Simian immunodeficiency virus replicates to high levels in naturally infected African green monkeys without inducing immunologic or neurologic disease." *J Virol* 75(5): 2262-75.
- Cameron, P. U., P. S. Freudenthal, J. M. Barker, S. Gezelter, K. Inaba and R. M. Steinman (1992). "Dendritic cells exposed to human immunodeficiency virus type-1 transmit a vigorous cytopathic infection to CD4+ T cells." *Science* 257(5068): 383-7.
- Cao, Y., L. Qin, L. Zhang, J. Safrit and D. D. Ho (1995). "Virologic and immunologic characterization of long-term survivors of human immunodeficiency virus type 1 infection." *N Engl J Med* 332(4): 201-8.
- Chaisson, R. E., J. E. Gallant, J. C. Keruly and R. D. Moore (1998). "Impact of opportunistic disease on survival in patients with HIV infection." *Aids* 12(1): 29-33.
- Chase, A., Y. Zhou and R. F. Siliciano (2006). "HIV-1-induced depletion of CD4(+) T cells in the gut: mechanism and therapeutic implications." *Trends Pharmacol Sci* 27(1): 4-7.

- Chen, Z., P. Telfier, A. Gettie, P. Reed, L. Zhang, D. D. Ho and P. A. Marx (1996). "Genetic characterization of new West African simian immunodeficiency virus SIVsm: geographic clustering of household-derived SIV strains with human immunodeficiency virus type 2 subtypes and genetically diverse viruses from a single feral sooty mangabey troop." *J Virol* 70(6): 3617-27.
- Chiodi, F. (2006). "A link between immune hyperactivation of T cells during HIV-1 infection and the virus protease?" *Aids* 20(5): 769-71.
- Chun, T. W., L. Carruth, D. Finzi, X. Shen, J. A. DiGiuseppe, H. Taylor, M. Hermankova, K. Chadwick, J. Margolick, T. C. Quinn, Y. H. Kuo, R. Brookmeyer, M. A. Zeiger, P. Barditch-Crovo and R. F. Siliciano (1997). "Quantification of latent tissue reservoirs and total body viral load in HIV-1 infection." *Nature* 387(6629): 183-8.
- Clavel, F., M. Guyader, D. Guetard, M. Salle, L. Montagnier and M. Alizon (1986). "Molecular cloning and polymorphism of the human immune deficiency virus type 2." *Nature* 324(6098): 691-5.
- Coffin, J., A. Haase, J. A. Levy, L. Montagnier, S. Oroszlan, N. Teich, H. Temin, K. Toyoshima, H. Varmus, P. Vogt and et al. (1986). "Human immunodeficiency viruses." *Science* 232(4751): 697.
- Cohen, J. (2003). "Public health. AIDS vaccine trial produces disappointment and confusion." *Science* 299(5611): 1290-1.
- Conge, A. M., K. Tarte, J. Reynes, M. Segondy, J. Gerfaux, M. Zembala and J. P. Vendrell (1998). "Impairment of B-lymphocyte differentiation induced by dual triggering of the B-cell antigen receptor and CD40 in advanced HIV-1-disease." *Aids* 12(12): 1437-49.
- Craib, K. J., S. A. Strathdee, R. S. Hogg, B. Leung, J. S. Montaner, M. V. O'Shaughnessy and M. T. Schechter (1997). "Serum levels of human immunodeficiency virus type 1 (HIV-1) RNA after seroconversion: a predictor of long-term mortality in HIV infection." *J Infect Dis* 176(3): 798-800.
- Crotty, S. and R. Ahmed (2004). "Immunological memory in humans." *Semin Immunol* 16(3): 197-203.
- Cuss, A. K., D. T. Avery, J. L. Cannons, L. J. Yu, K. E. Nichols, P. J. Shaw and S. G. Tangye (2006). "Expansion of functionally immature transitional B cells is associated with human-immunodeficient states characterized by impaired humoral immunity." *J Immunol* 176(3): 1506-16.
- De Milito, A. (2004). "B lymphocyte dysfunctions in HIV infection." *Curr HIV Res* 2(1): 11-21.
- De Milito, A., C. Mørch, A. Sonnerborg and F. Chiodi (2001). "Loss of memory (CD27) B lymphocytes in HIV-1 infection." *Aids* 15(8): 957-64.
- de Moraes-Pinto, M. I., A. C. Almeida, G. Kenj, T. E. Filgueiras, W. Tobias, A. M. Santos, M. M. Carneiro-Sampaio, C. K. Farhat, P. J. Milligan, P. M. Johnson and C. A. Hart (1996). "Placental transfer and maternally acquired neonatal IgG immunity in human immunodeficiency virus infection." *J Infect Dis* 173(5): 1077-84.
- de Moraes-Pinto, M. I., C. K. Farhat, S. B. Carbonare, S. P. Curti, M. E. Otsubo, D. S. Lazarotti, R. C. Campagnoli and M. M. Carneiro-Sampaio (1993). "Maternally acquired immunity in newborns from women infected by the human immunodeficiency virus." *Acta Paediatr* 82(12): 1034-8.
- Deacon, N. J., A. Tsaykin, A. Solomon, K. Smith, M. Ludford-Menting, D. J. Hooker, D. A. McPhee, A. L. Greenway, A. Ellett, C. Chatfield, V. A. Lawson, S. Crowe, A. Maerz, S. Sonza, J. Learmont, J. S. Sullivan, A. Cunningham, D. Dwyer, D. Dowton and J. Mills (1995). "Genomic structure of an attenuated quasi species of HIV-1 from a blood transfusion donor and recipients." *Science* 270(5238): 988-91.
- Donaghy, H., A. Pozniak, B. Gazzard, N. Qazi, J. Gilmour, F. Gotch and S. Patterson (2001). "Loss of blood CD11c(+) myeloid and CD11c(-) plasmacytoid dendritic cells in patients with HIV-1 infection correlates with HIV-1 RNA virus load." *Blood* 98(8): 2574-6.
- Dorsey, B. D., R. B. Levin, S. L. McDaniel, J. P. Vacca, J. P. Guare, P. L. Darke, J. A. Zugay, E. A. Emini, W. A. Schleif, J. C. Quintero and et al. (1994). "L-735,524: the design of a potent and orally bioavailable HIV protease inhibitor." *J Med Chem* 37(21): 3443-51.
- Douek, D. C., L. J. Picker and R. A. Koup (2003). "T cell dynamics in HIV-1 infection." *Annu Rev Immunol* 21: 265-304.
- Dworkin, M. S., J. W. Ward, D. L. Hanson, J. L. Jones and J. E. Kaplan (2001). "Pneumococcal disease among human immunodeficiency virus-infected persons: incidence, risk factors, and impact of vaccination." *Clin Infect Dis* 32(5): 794-800.

- Eichberg, J. W., J. M. Zarling, H. J. Alter, J. A. Levy, P. W. Berman, T. Gregory, L. A. Lasky, J. McClure, K. E. Cobb, P. A. Moran and et al. (1987). "T-cell responses to human immunodeficiency virus (HIV) and its recombinant antigens in HIV-infected chimpanzees." *J Virol* 61(12): 3804-8.
- Ezzell, C. (1987). "AIDS drug gets green light." *Nature* 329(6142): 751.
- 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(11): 6433-8.
- Fiebig, E. W., D. J. Wright, B. D. Rawal, P. E. Garrett, R. T. Schumacher, L. Peddada, C. Heldebrandt, R. Smith, A. Conrad, S. H. Kleinman and M. P. Busch (2003). "Dynamics of HIV viremia and antibody seroconversion in plasma donors: implications for diagnosis and staging of primary HIV infection." *Aids* 17(13): 1871-9.
- Font, J., L. Pallares, J. Martorell, E. Martinez, A. Gaya, J. Vives and M. Ingelmo (1996). "Elevated soluble CD27 levels in serum of patients with systemic lupus erythematosus." *Clin Immunol Immunopathol* 81(3): 239-43.
- Franceschi, C., M. G. Franceschini, A. Boschini, T. Trenti, C. Nuzzo, G. Castellani, C. Smacchia, B. De Rienzo, R. Roncaglia, M. Portolani, P. Pietrosemoli, M. Meacci, M. Pecorari, A. Sabbatini, W. Malorni and A. Cossarizza (1997). "Phenotypic characteristics and tendency to apoptosis of peripheral blood mononuclear cells from HIV+ long term non progressors." *Cell Death Differ* 4(8): 815-23.
- Frank, I. and M. Pope (2002). "The enigma of dendritic cell-immunodeficiency virus interplay." *Curr Mol Med* 2(3): 229-48.
- Frauwirth, K. A. and C. B. Thompson (2002). "Activation and inhibition of lymphocytes by costimulation." *J Clin Invest* 109(3): 295-9.
- French, N., M. Moore, R. Haikala, H. Kayhty and C. F. Gilks (2004). "A case-control study to investigate serological correlates of clinical failure of 23-valent pneumococcal polysaccharide vaccine in HIV-1-infected Ugandan adults." *J Infect Dis* 190(4): 707-12.
- Gaines, H., M. von Sydow, A. Sonnerborg, J. Albert, J. Czajkowski, P. O. Pehrson, F. Chiodi, L. Moberg, E. M. Fenyo, B. Asjo and et al. (1987). "Antibody response in primary human immunodeficiency virus infection." *Lancet* 1(8544): 1249-53.
- Gaines, H., M. A. von Sydow, L. V. von Stedingk, G. Biberfeld, B. Bottiger, L. O. Hansson, P. Lundbergh, A. B. Sonnerborg, J. Wasserman and O. O. Strannegaard (1990). "Immunological changes in primary HIV-1 infection." *Aids* 4(10): 995-9.
- Gallo, R. C., P. S. Sarin, E. P. Gelmann, M. Robert-Guroff, E. Richardson, V. S. Kalyanaraman, D. Mann, G. D. Sidhu, R. E. Stahl, S. Zolla-Pazner, J. Leibowitch and M. Popovic (1983). "Isolation of human T-cell leukemia virus in acquired immune deficiency syndrome (AIDS)." *Science* 220(4599): 865-7.
- Gao, F., L. Yue, A. T. White, P. G. Pappas, J. Barchue, A. P. Hanson, B. M. Greene, P. M. Sharp, G. M. Shaw and B. H. Hahn (1992). "Human infection by genetically diverse SIVSM-related HIV-2 in west Africa." *Nature* 358(6386): 495-9.
- Garcia, F., M. Plana, G. M. Ortiz, S. Bonhoeffer, A. Soriano, C. Vidal, A. Cruceta, M. Arnedo, C. Gil, G. Pantaleo, T. Pumarola, T. Gallart, D. F. Nixon, J. M. Miro and J. M. Gatell (2001). "The virological and immunological consequences of structured treatment interruptions in chronic HIV-1 infection." *Aids* 15(9): F29-40.
- Garred, P., H. O. Madsen, U. Balslev, B. Hofmann, C. Pedersen, J. Gerstoft and A. Svejgaard (1997). "Susceptibility to HIV infection and progression of AIDS in relation to variant alleles of mannose-binding lectin." *Lancet* 349(9047): 236-40.
- Girard, M. P., S. K. Osmanov and M. P. Kiely (2006). "A review of vaccine research and development: The human immunodeficiency virus (HIV)." *Vaccine*.
- Goulder, P. J., R. E. Phillips, R. A. Colbert, S. McAdam, G. Ogg, M. A. Nowak, P. Giangrande, G. Luzzi, B. Morgan, A. Edwards, A. J. McMichael and S. Rowland-Jones (1997). "Late escape from an immunodominant cytotoxic T-lymphocyte response associated with progression to AIDS." *Nat Med* 3(2): 212-7.
- Graziosi, C., K. R. Gantt, M. Vaccarezza, J. F. Demarest, M. Daucher, M. S. Saag, G. M. Shaw, T. C. Quinn, O. J. Cohen, C. C. Welbon, G. Pantaleo and A. S. Fauci (1996). "Kinetics of cytokine expression during primary human immunodeficiency virus type 1 infection." *Proc Natl Acad Sci U S A* 93(9): 4386-91.
- Gross, J. A., J. Johnston, S. Mudri, R. Enselman, S. R. Dillon, K. Madden, W. Xu, J. Parrish-Novak, D. Foster, C. Lofton-Day, M. Moore, A. Littau, A. Grossman, H. Haugen, K. Foley, H. Blumberg, K. Harrison, W. Kindsvogel and C. H. Clegg (2000). "TACI and

- BCMA are receptors for a TNF homologue implicated in B-cell autoimmune disease."**  
**Nature 404(6781): 995-9.**
- Grossman, Z., M. Meier-Schellersheim, W. E. Paul and L. J. Picker (2006). "Pathogenesis of HIV infection: what the virus spares is as important as what it destroys."** Nat Med 12(3): 289-295.
- 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(4): 319-23.
- Haase, A. T. (1986). "Pathogenesis of lentivirus infections."** Nature 322(6075): 130-6.
- Haase, A. T. (1999). "Population biology of HIV-1 infection: viral and CD4+ T cell demographics and dynamics in lymphatic tissues."** Annu Rev Immunol 17: 625-56.
- Hagwood, N. L., A. Watson, W. F. Sutton, J. McClure, A. Lewis, J. Ranchalis, B. Travis, G. Voss, N. L. Letvin, S. L. Hu, V. M. Hirsch and P. R. Johnson (1996). "Passive immune globulin therapy in the SIV/macaque model: early intervention can alter disease profile."** Immunol Lett 51(1-2): 107-14.
- Hammer, S. M., K. E. Squires, M. D. Hughes, J. M. Grimes, L. M. Demeter, J. S. Currier, J. J. Eron, Jr., J. E. Feinberg, H. H. Balfour, Jr., L. R. Deyton, J. A. Chodakewitz and M. A. Fischl (1997). "A controlled trial of two nucleoside analogues plus indinavir in persons with human immunodeficiency virus infection and CD4 cell counts of 200 per cubic millimeter or less. AIDS Clinical Trials Group 320 Study Team."** N Engl J Med 337(11): 725-33.
- Hammonds, J., X. Chen, T. Fouts, A. DeVico, D. Montefiori and P. Spearman (2005). "Induction of neutralizing antibodies against human immunodeficiency virus type 1 primary isolates by Gag-Env pseudovirion immunization."** J Virol 79(23): 14804-14.
- Hanke, T., R. V. Samuel, T. J. Blanchard, V. C. Neumann, T. M. Allen, J. E. Boyson, S. A. Sharpe, N. Cook, G. L. Smith, D. I. Watkins, M. P. Cranage and A. J. McMichael (1999). "Effective induction of simian immunodeficiency virus-specific cytotoxic T lymphocytes in macaques by using a multiepitope gene and DNA prime-modified vaccinia virus Ankara boost vaccination regimen."** J Virol 73(9): 7524-32.
- Helfand, R. F., W. J. Moss, R. Harpaz, S. Scott and F. Cutts (2005). "Evaluating the impact of the HIV pandemic on measles control and elimination."** Bull World Health Organ 83(5): 329-37.
- Hintzen, R. Q., R. de Jong, C. E. Hack, M. Chamuleau, E. F. de Vries, I. J. ten Berge, J. Borst and R. A. van Lier (1991). "A soluble form of the human T cell differentiation antigen CD27 is released after triggering of the TCR/CD3 complex."** J Immunol 147(1): 29-35.
- Hirsch, M., R. Steigbigel, S. Staszewski, J. Mellors, E. Scerpella, B. Hirschel, J. Lange, K. Squires, S. Rawlins, A. Meibohm and R. Leavitt (1999). "A randomized, controlled trial of indinavir, zidovudine, and lamivudine in adults with advanced human immunodeficiency virus type 1 infection and prior antiretroviral therapy."** J Infect Dis 180(3): 659-65.
- Ho, D. D., A. U. Neumann, A. S. Perelson, W. Chen, J. M. Leonard and M. Markowitz (1995). "Rapid turnover of plasma virions and CD4 lymphocytes in HIV-1 infection."** Nature 373(6510): 123-6.
- Hofmann-Lehmann, R., J. Vlasak, A. L. Williams, A. L. Chenine, H. M. McClure, D. C. Anderson, S. O'Neil and R. M. Ruprecht (2003). "Live attenuated, nef-deleted SIV is pathogenic in most adult macaques after prolonged observation."** Aids 17(2): 157-66.
- Hunziker, L., M. Recher, A. J. Macpherson, A. Ciurea, S. Freigang, H. Hengartner and R. M. Zinkernagel (2003). "Hypergammaglobulinemia and autoantibody induction mechanisms in viral infections."** Nat Immunol 4(4): 343-9.
- Jacquot, S., T. Kobata, S. Iwata, C. Morimoto and S. F. Schlossman (1997). "CD154/CD40 and CD70/CD27 interactions have different and sequential functions in T cell-dependent B cell responses: enhancement of plasma cell differentiation by CD27 signaling."** J Immunol 159(6): 2652-7.
- Janoff, E. N., R. W. Scamurra, T. C. Sanneman, K. Eidman and J. R. Thurn (1999). "Human immunodeficiency virus type 1 and mucosal humoral defense."** J Infect Dis 179 Suppl 3: S475-9.
- 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, L. 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(6): 991-8.

- Johnson, B. K., G. A. Stone, M. S. Godec, D. M. Asher, D. C. Gajdusek and C. J. Gibbs, Jr. (1993). "Long-term observations of human immunodeficiency virus-infected chimpanzees." *AIDS Res Hum Retroviruses* 9(4): 375-8.
- Johnson, W. E. and R. C. Desrosiers (2002). "Viral persistance: HIV's strategies of immune system evasion." *Annu Rev Med* 53: 499-518.
- Jung, J., J. Choe, L. Li and Y. S. Choi (2000). "Regulation of CD27 expression in the course of germinal center B cell differentiation: the pivotal role of IL-10." *Eur J Immunol* 30(8): 2437-43.
- Kadowaki, N. and Y. J. Liu (2002). "Natural type I interferon-producing cells as a link between innate and adaptive immunity." *Hum Immunol* 63(12): 1126-32.
- Kaplan, L. J., R. S. Daum, M. Smaron and C. A. McCarthy (1992). "Severe measles in immunocompromised patients." *Jama* 267(9): 1237-41.
- Karray, S. and M. Zouali (1997). "Identification of the B cell superantigen-binding site of HIV-1 gp120." *Proc Natl Acad Sci U S A* 94(4): 1356-60.
- Kestler, H., T. Kodama, D. Ringler, M. Marthas, N. Pedersen, A. Lackner, D. Regier, P. Sehgal, M. Daniel, N. King and et al. (1990). "Induction of AIDS in rhesus monkeys by molecularly cloned simian immunodeficiency virus." *Science* 248(4959): 1109-12.
- Kilby, J. M., S. Hopkins, T. M. Venetta, B. DiMassimo, G. A. Cloud, J. Y. Lee, L. Alldredge, E. Hunter, D. Lambert, D. Bolognesi, T. Matthews, M. R. Johnson, M. A. Nowak, G. M. Shaw and M. S. Saag (1998). "Potent suppression of HIV-1 replication in humans by T-20, a peptide inhibitor of gp41-mediated virus entry." *Nat Med* 4(11): 1302-7.
- Kirchhoff, F., T. C. Greenough, D. B. Brettler, J. L. Sullivan and R. C. Desrosiers (1995). "Brief report: absence of intact nef sequences in a long-term survivor with nonprogressive HIV-1 infection." *N Engl J Med* 332(4): 228-32.
- Klein, M. (2003). "Prospects and challenges for prophylactic and therapeutic HIV vaccines." *Vaccine* 21(7-8): 616-9.
- Klein, U., R. Kuppers and K. Rajewsky (1997). "Evidence for a large compartment of IgM-expressing memory B cells in humans." *Blood* 89(4): 1288-98.
- Klein, U., K. Rajewsky and R. Kuppers (1998). "Human immunoglobulin (Ig)M+IgD+ peripheral blood B cells expressing the CD27 cell surface antigen carry somatically mutated variable region genes: CD27 as a general marker for somatically mutated (memory) B cells." *J Exp Med* 188(9): 1679-89.
- Kohler, H., S. Muller and P. L. Nara (1994). "Deceptive imprinting in the immune response against HIV-1." *Immunol Today* 15(10): 475-8.
- 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(7): 4650-5.
- Kroon, F. P., J. T. van Dissel, J. C. de Jong and R. van Furth (1994). "Antibody response to influenza, tetanus and pneumococcal vaccines in HIV-seropositive individuals in relation to the number of CD4+ lymphocytes." *Aids* 8(4): 469-76.
- Kroon, F. P., J. T. van Dissel, E. Ravensbergen, P. H. Nibbering and R. van Furth (1999). "Antibodies against pneumococcal polysaccharides after vaccination in HIV-infected individuals: 5-year follow-up of antibody concentrations." *Vaccine* 18(5-6): 524-30.
- Kruetzmann, S., M. M. Rosado, H. Weber, U. Germing, O. Tournilhac, H. H. Peter, R. Berner, A. Peters, T. Boehm, A. Plebani, I. Quinti and R. Carsetti (2003). "Human immunoglobulin M memory B cells controlling Streptococcus pneumoniae infections are generated in the spleen." *J Exp Med* 197(7): 939-45.
- Lalezari, J. P., K. Henry, M. O'Hearn, J. S. Montaner, P. J. Piliero, B. Trottier, S. Walmsley, C. Cohen, D. R. Kuritzkes, J. J. Eron, Jr., J. Chung, R. DeMasi, L. Donatacci, C. Drobnes, J. Delehanty and M. Salgo (2003). "Enfuvirtide, an HIV-1 fusion inhibitor, for drug-resistant HIV infection in North and South America." *N Engl J Med* 348(22): 2175-85.
- Lane, H. C., H. Masur, L. C. Edgar, G. Whalen, A. H. Rook and A. S. Fauci (1983). "Abnormalities of B-cell activation and immunoregulation in patients with the acquired immunodeficiency syndrome." *N Engl J Med* 309(8): 453-8.
- Leder, A. N., B. Flansbaum, G. Zandman-Goddard, R. Asherson and Y. Shoenfeld (2001). "Antiphospholipid syndrome induced by HIV." *Lupus* 10(5): 370-4.
- Ledru, E., H. Lecoeur, S. Garcia, T. Debord and M. L. Gougeon (1998). "Differential susceptibility to activation-induced apoptosis among peripheral Th1 subsets: correlation

- with Bcl-2 expression and consequences for AIDS pathogenesis." *J Immunol* 160(7): 3194-206.
- Lee, B., G. Leslie, E. Soilleux, U. O'Doherty, S. Baik, E. Levroney, K. Flummerfelt, W. Swiggard, N. Coleman, M. Malim and R. W. Doms (2001). "cis Expression of DC-SIGN allows for more efficient entry of human and simian immunodeficiency viruses via CD4 and a coreceptor." *J Virol* 75(24): 12028-38.
- Lefrere, J. J., L. Morand-Joubert, M. Mariotti, H. Bludau, B. Burghoffer, J. C. Petit and F. Roudot-Thoraval (1997). "Even individuals considered as long-term nonprogressors show biological signs of progression after 10 years of human immunodeficiency virus infection." *Blood* 90(3): 1133-40.
- Lefrere, J. J., F. Roudot-Thoraval, M. Mariotti, M. Thauvin, J. Lerable, J. Salpetrier and L. Morand-Joubert (1998). "The risk of disease progression is determined during the first year of human immunodeficiency virus type 1 infection." *J Infect Dis* 177(6): 1541-8.
- Lelievre, J. D., F. Mammano, D. Arnoult, F. Petit, A. Grodet, J. Estaquier and J. C. Ameisen (2004). "A novel mechanism for HIV1-mediated bystander CD4+ T-cell death: neighboring dying cells drive the capacity of HIV1 to kill noncytolytic primary CD4+ T cells." *Cell Death Differ* 11(9): 1017-27.
- Lemckert, A. A., S. M. Sumida, L. Holterman, R. Vogels, D. M. Truitt, D. M. Lynch, A. Nanda, B. A. Ewald, D. A. Gorgone, M. A. Lifton, J. Goudsmit, M. J. Havenga and D. H. Barouch (2005). "Immunogenicity of heterologous prime-boost regimens involving recombinant adenovirus serotype 11 (Ad11) and Ad35 vaccine vectors in the presence of anti-ad5 immunity." *J Virol* 79(15): 9694-701.
- Lenardo, M. J., S. B. Angleman, V. Bounkeua, J. Dimas, M. G. Duvall, M. B. Graubard, F. Hornung, M. C. Selkirk, C. K. Speirs, C. Trageser, J. O. Orenstein and D. L. Bolton (2002). "Cytopathic killing of peripheral blood CD4(+) T lymphocytes by human immunodeficiency virus type 1 appears necrotic rather than apoptotic and does not require env." *J Virol* 76(10): 5082-93.
- Lens, S. M., R. de Jong, B. Hooibrink, G. Koopman, S. T. Pals, M. H. van Oers and R. A. van Lier (1996). "Phenotype and function of human B cells expressing CD70 (CD27 ligand)." *Eur J Immunol* 26(12): 2964-71.
- Li, Q., L. Duan, J. D. Estes, Z. M. Ma, T. Rourke, Y. Wang, C. Reilly, J. Carllis, C. J. Miller and A. T. Haase (2005). "Peak SIV replication in resting memory CD4+ T cells depletes gut lamina propria CD4+ T cells." *Nature* 434(7037): 1148-52.
- Lim, S. G., A. Condez, C. A. Lee, M. A. Johnson, C. Elia and L. W. Poulter (1993). "Loss of mucosal CD4 lymphocytes is an early feature of HIV infection." *Clin Exp Immunol* 92(3): 448-54.
- Locher, C. P., R. M. Grant, E. A. Collisson, G. Reyes-Teran, T. Elbeik, J. O. Kahn and J. A. Levy (1999). "Antibody and cellular immune responses in breakthrough infection subjects after HIV type 1 glycoprotein 120 vaccination." *AIDS Res Hum Retroviruses* 15(18): 1685-9.
- Loenen, W. A., E. De Vries, L. A. Gravestein, R. Q. Hintzen, R. A. Van Lier and J. Borst (1992). "The CD27 membrane receptor, a lymphocyte-specific member of the nerve growth factor receptor family, gives rise to a soluble form by protein processing that does not involve receptor endocytosis." *Eur J Immunol* 22(2): 447-55.
- Mackay, F., P. Schneider, P. Rennert and J. Browning (2003). "BAFF AND APRIL: a tutorial on B cell survival." *Annu Rev Immunol* 21: 231-64.
- Malaspina, A., S. Moir, J. Ho, W. Wang, M. L. Howell, A. O'Shea M, G. A. Roby, C. A. Rehm, J. M. Mican, T. W. Chun and A. S. Fauci (2006). "Appearance of immature/transitional B cells in HIV-infected individuals with advanced disease: Correlation with increased IL-7." *Proc Natl Acad Sci U S A* 103(7): 2262-7.
- Malaspina, A., S. Moir, S. M. Orsega, J. Vasquez, N. J. Miller, E. T. Donoghue, S. Kottilil, M. Gezmu, D. Follmann, G. M. Vodeiko, R. A. Levandowski, J. M. Mican and A. S. Fauci (2005). "Compromised B cell responses to influenza vaccination in HIV-infected individuals." *J Infect Dis* 191(9): 1442-50.
- Manz, R. A., A. Thiel and A. Radbruch (1997). "Lifetime of plasma cells in the bone marrow." *Nature* 388(6638): 133-4.
- Martinez-Maza, O., E. Crabb, R. T. Mitsuyasu, J. L. Fahey and J. V. Giorgi (1987). "Infection with the human immunodeficiency virus (HIV) is associated with an in vivo increase in B lymphocyte activation and immaturity." *J Immunol* 138(11): 3720-4.

- Mascola, J. R., G. Stiegler, T. C. VanCott, H. Katinger, C. B. Carpenter, C. E. Hanson, H. Beary, D. Hayes, S. S. Frankel, D. L. Birx and M. G. Lewis (2000). "Protection of macaques against vaginal transmission of a pathogenic HIV-1/SIV chimeric virus by passive infusion of neutralizing antibodies." *Nat Med* 6(2): 207-10.
- Masur, H., J. E. Kaplan and K. K. Holmes (2002). "Guidelines for preventing opportunistic infections among HIV-infected persons--2002. Recommendations of the U.S. Public Health Service and the Infectious Diseases Society of America." *Ann Intern Med* 137(5 Pt 2): 435-78.
- McCune, J. M. (2001). "The dynamics of CD4+ T-cell depletion in HIV disease." *Nature* 410(6831): 974-9.
- McNeil, A. C., W. L. Shupert, C. A. Iyasere, C. W. Hallahan, J. A. Mican, R. T. Davey, Jr. and M. Connors (2001). "High-level HIV-1 viremia suppresses viral antigen-specific CD4(+) T cell proliferation." *Proc Natl Acad Sci U S A* 98(24): 13878-83.
- Mellors, J. W., A. Munoz, J. V. Giorgi, J. B. Margolick, C. J. Tassoni, P. Gupta, L. A. Kingsley, J. A. Todd, A. J. Saah, R. Detels, J. P. Phair and C. R. Rinaldo, Jr. (1997). "Plasma viral load and CD4+ lymphocytes as prognostic markers of HIV-1 infection." *Ann Intern Med* 126(12): 946-54.
- Mellors, J. W., C. R. Rinaldo, Jr., P. Gupta, R. M. White, J. A. Todd and L. A. Kingsley (1996). "Prognosis in HIV-1 infection predicted by the quantity of virus in plasma." *Science* 272(5265): 1167-70.
- Melvin, A. J. and K. M. Mohan (2003). "Response to immunization with measles, tetanus, and Haemophilus influenzae type b vaccines in children who have human immunodeficiency virus type 1 infection and are treated with highly active antiretroviral therapy." *Pediatrics* 111(6 Pt 1): e641-4.
- Milicic, A., D. A. Price, P. Zimbwa, B. L. Booth, H. L. Brown, P. J. Easterbrook, K. Olsen, N. Robinson, U. Gileadi, A. K. Sewell, V. Cerundolo and R. E. Phillips (2005). "CD8+ T cell epitope-flanking mutations disrupt proteasomal processing of HIV-1 Nef." *J Immunol* 175(7): 4618-26.
- Miura, Y. and Y. Koyanagi (2005). "Death ligand-mediated apoptosis in HIV infection." *Rev Med Virol* 15(3): 169-78.
- Miyawaki, T., T. Uehara, R. Nibu, T. Tsuji, A. Yachie, S. Yonehara and N. Taniguchi (1992). "Differential expression of apoptosis-related Fas antigen on lymphocyte subpopulations in human peripheral blood." *J Immunol* 149(11): 3753-8.
- Mizuma, H., S. Zolla-Pazner, S. Litwin, W. el-Sadr, S. Sharpe, B. Zehr, S. Weiss, W. C. Saxinger and M. Marmor (1987). "Serum IgD elevation is an early marker of B cell activation during infection with the human immunodeficiency viruses." *Clin Exp Immunol* 68(1): 5-14.
- Moir, S., A. Malaspina, K. M. Ogwaro, E. T. Donoghue, C. W. Hallahan, L. A. Ehler, S. Liu, J. Adelsberger, R. Lapointe, P. Hwu, M. Baseler, J. M. Orenstein, T. W. Chun, J. A. Mican and A. S. Fauci (2001). "HIV-1 induces phenotypic and functional perturbations of B cells in chronically infected individuals." *Proc Natl Acad Sci U S A* 98(18): 10362-7.
- Moir, S., A. Malaspina, O. K. Pickeral, E. T. Donoghue, J. Vasquez, N. J. Miller, S. R. Krishnan, M. A. Planta, J. F. Turney, J. S. Justement, S. Kotttilil, M. Dybul, J. M. Mican, C. Kovacs, T. W. Chun, C. E. Birse and A. S. Fauci (2004). "Decreased survival of B cells of HIV-viremic patients mediated by altered expression of receptors of the TNF superfamily." *J Exp Med* 200(7): 587-99.
- Moir, S., K. M. Ogwaro, A. Malaspina, J. Vasquez, E. T. Donoghue, C. W. Hallahan, S. Liu, L. A. Ehler, M. A. Planta, S. Kotttilil, T. W. Chun and A. S. Fauci (2003). "Perturbations in B cell responsiveness to CD4+ T cell help in HIV-infected individuals." *Proc Natl Acad Sci U S A* 100(10): 6057-62.
- Moog, C., H. J. Fleury, I. Pellegrin, A. Kirn and A. M. Aubertin (1997). "Autologous and heterologous neutralizing antibody responses following initial seroconversion in human immunodeficiency virus type 1-infected individuals." *J Virol* 71(5): 3734-41.
- Moore, J. and A. Trkola (1997). "HIV type 1 coreceptors, neutralization serotypes, and vaccine development." *AIDS Res Hum Retroviruses* 13(9): 733-6.
- Moore, J. P., Y. Cao, L. Qing, Q. J. Sattentau, J. Pyati, R. Koduri, J. Robinson, C. F. Barbas, 3rd, D. R. Burton and D. D. Ho (1995). "Primary isolates of human immunodeficiency virus type 1 are relatively resistant to neutralization by monoclonal antibodies to gp120, and their neutralization is not predicted by studies with monomeric gp120." *J Virol* 69(1): 101-9.

- Morris, L., J. M. Binley, B. A. Clas, S. Bonhoeffer, T. P. Astill, R. Kost, A. Hurley, Y. Cao, M. Markowitz, D. D. Ho and J. P. Moore (1998). "HIV-1 antigen-specific and -nonspecific B cell responses are sensitive to combination antiretroviral therapy." *J Exp Med* 188(2): 233-45.
- Moss, W. J., F. Cutts and D. E. Griffin (1999). "Implications of the human immunodeficiency virus epidemic for control and eradication of measles." *Clin Infect Dis* 29(1): 106-12.
- Nagase, H., K. Agematsu, K. Kitano, M. Takamoto, Y. Okubo, A. Komiyama and K. Sugane (2001). "Mechanism of hypergammaglobulinemia by HIV infection: circulating memory B-cell reduction with plasmacytosis." *Clin Immunol* 100(2): 250-9.
- Ng, L. G., A. P. Sutherland, R. Newton, F. Qian, T. G. Cachero, M. L. Scott, J. S. Thompson, J. Wheway, T. Chtanova, J. Groom, I. J. Sutton, C. Xin, S. G. Tangye, S. L. Kalled, F. Mackay and C. R. Mackay (2004). "B cell-activating factor belonging to the TNF family (BAFF)-R is the principal BAFF receptor facilitating BAFF costimulation of circulating T and B cells." *J Immunol* 173(2): 807-17.
- Nilsson, A., A. de Milito, F. Mowafi, G. Winberg, O. Bjork, E. Z. Wolpert and F. Chiodi (2005). "Expression of CD27-CD70 on early B cell progenitors in the bone marrow: implication for diagnosis and therapy of childhood ALL." *Exp Hematol* 33(12): 1500-7.
- Novembre, F. J., M. Saucier, D. C. Anderson, S. A. Klumpp, S. P. O'Neil, C. R. Brown, 2nd, C. E. Hart, P. C. Guenthner, R. B. Swenson and H. M. McClure (1997). "Development of AIDS in a chimpanzee infected with human immunodeficiency virus type 1." *J Virol* 71(5): 4086-91.
- Ochsenbein, A. F., D. D. Pinschewer, S. Sierro, E. Horvath, H. Hengartner and R. M. Zinkernagel (2000). "Protective long-term antibody memory by antigen-driven and T help-dependent differentiation of long-lived memory B cells to short-lived plasma cells independent of secondary lymphoid organs." *Proc Natl Acad Sci U S A* 97(24): 13263-8.
- Onlamoon, N., K. Pattanapanyasat and A. A. Ansari (2005). "Human and nonhuman primate lentiviral infection and autoimmunity." *Ann N Y Acad Sci* 1050: 397-409.
- Opravil, M., W. Fierz, L. Matter, J. Blaser and R. Luthy (1991). "Poor antibody response after tetanus and pneumococcal vaccination in immunocompromised, HIV-infected patients." *Clin Exp Immunol* 84(2): 185-9.
- Ortiz, G. M., D. F. Nixon, A. Trkola, J. Binley, X. Jin, S. Bonhoeffer, P. J. Kuebler, S. M. Donahoe, M. A. Demoitie, W. M. Kakimoto, T. Ketas, B. Clas, J. J. Heymann, L. Zhang, Y. Cao, A. Hurley, J. P. Moore, D. D. Ho and M. Markowitz (1999). "HIV-1-specific immune responses in subjects who temporarily contain virus replication after discontinuation of highly active antiretroviral therapy." *J Clin Invest* 104(6): R13-8.
- Osterhaus, A. D., C. A. van Baalen, R. A. Gruters, M. Schutten, C. H. Siebelink, E. G. Hulskotte, E. J. Tijhaar, R. E. Randall, G. van Amerongen, A. Fleuchaus, V. Erfle and G. Sutter (1999). "Vaccination with Rev and Tat against AIDS." *Vaccine* 17(20-21): 2713-4.
- Otten, U., P. Ehrhard and R. Peck (1989). "Nerve growth factor induces growth and differentiation of human B lymphocytes." *Proc Natl Acad Sci U S A* 86(24): 10059-63.
- Palacios, R., J. Santos, P. Valdivielso and M. Marquez (2002). "Human immunodeficiency virus infection and systemic lupus erythematosus. An unusual case and a review of the literature." *Lupus* 11(1): 60-3.
- Pantaleo, G., J. F. Demarest, H. Soudeyns, C. Graziosi, F. Denis, J. W. Adelsberger, P. Borrow, M. S. Saag, G. M. Shaw, R. P. Sekaly and et al. (1994). "Major expansion of CD8+ T cells with a predominant V beta usage during the primary immune response to HIV." *Nature* 370(6489): 463-7.
- Pantaleo, G., C. Graziosi, L. Butini, P. A. Pizzo, S. M. Schnittman, D. P. Kotler and A. S. Fauci (1991). "Lymphoid organs function as major reservoirs for human immunodeficiency virus." *Proc Natl Acad Sci U S A* 88(21): 9838-42.
- Pantaleo, G., C. Graziosi, J. F. Demarest, L. Butini, M. Montroni, C. H. Fox, J. M. Orenstein, D. P. Kotler and A. S. Fauci (1993). "HIV infection is active and progressive in lymphoid tissue during the clinically latent stage of disease." *Nature* 362(6418): 355-8.
- Pantaleo, G., C. Graziosi and A. S. Fauci (1993). "New concepts in the immunopathogenesis of human immunodeficiency virus infection." *N Engl J Med* 328(5): 327-35.
- Pantaleo, G., S. Menzo, M. Vaccarezza, C. Graziosi, O. J. Cohen, J. F. Demarest, D. Montefiori, J. M. Orenstein, C. Fox, L. K. Schrager and et al. (1995). "Studies in subjects with long-term nonprogressive human immunodeficiency virus infection." *N Engl J Med* 332(4): 209-16.

- Perelson, A. S., D. E. Kirschner and R. De Boer (1993). "Dynamics of HIV infection of CD4+ T cells." *Math Biosci* 114(1): 81-125.
- Perelson, A. S., A. U. Neumann, M. Markowitz, J. M. Leonard and D. D. Ho (1996). "HIV-1 dynamics in vivo: virion clearance rate, infected cell life-span, and viral generation time." *Science* 271(5255): 1582-6.
- Phillips, R. E., S. Rowland-Jones, D. F. Nixon, F. M. Gotch, J. P. Edwards, A. O. Ogunlesi, J. G. Elvin, J. A. Rothbard, C. R. Bangham, C. R. Rizza and et al. (1991). "Human immunodeficiency virus genetic variation that can escape cytotoxic T cell recognition." *Nature* 354(6353): 453-9.
- Pialoux, G., H. Gahery-Segard, S. Sermet, H. Poncelet, S. Fournier, L. Gerard, A. Tartar, H. Gras-Masse, J. P. Levy and J. G. Guillet (2001). "Lipopeptides induce cell-mediated anti-HIV immune responses in seronegative volunteers." *Aids* 15(10): 1239-49.
- Pilgrim, A. K., G. Pantaleo, O. J. Cohen, L. M. Fink, J. Y. Zhou, J. T. Zhou, D. P. Bolognesi, A. S. Fauci and D. C. Montefiori (1997). "Neutralizing antibody responses to human immunodeficiency virus type 1 in primary infection and long-term-nonprogressive infection." *J Infect Dis* 176(4): 924-32.
- Pitcher, C. J., C. Quittner, D. M. Peterson, M. Connors, R. A. Koup, V. C. Maino and L. J. Picker (1999). "HIV-1-specific CD4+ T cells are detectable in most individuals with active HIV-1 infection, but decline with prolonged viral suppression." *Nat Med* 5(5): 518-25.
- Poignard, P., R. Sabbe, G. R. Picchio, M. Wang, R. J. Gulizia, H. Katinger, P. W. Parren, D. E. Mosier and D. R. Burton (1999). "Neutralizing antibodies have limited effects on the control of established HIV-1 infection in vivo." *Immunity* 10(4): 431-8.
- Pomerantz, R. J. and D. L. Horn (2003). "Twenty years of therapy for HIV-1 infection." *Nat Med* 9(7): 867-73.
- Poon, B., J. F. Hsu, V. Gudeman, I. S. Chen and K. Grovit-Ferbas (2005). "Formaldehyde-treated, heat-inactivated virions with increased human immunodeficiency virus type 1 env can be used to induce high-titer neutralizing antibody responses." *J Virol* 79(16): 10210-7.
- Price, D. A., P. J. Goulder, P. Klenerman, A. K. Sewell, P. J. Easterbrook, M. Troop, C. R. Bangham and R. E. Phillips (1997). "Positive selection of HIV-1 cytotoxic T lymphocyte escape variants during primary infection." *Proc Natl Acad Sci U S A* 94(5): 1890-5.
- Reitter, J. N., R. E. Means and R. C. Desrosiers (1998). "A role for carbohydrates in immune evasion in AIDS." *Nat Med* 4(6): 679-84.
- Richman, D. D. (2001). "HIV chemotherapy." *Nature* 410(6831): 995-1001.
- Richman, D. D., T. Wrin, S. J. Little and C. J. Petropoulos (2003). "Rapid evolution of the neutralizing antibody response to HIV type 1 infection." *Proc Natl Acad Sci U S A* 100(7): 4144-9.
- Rodes, B., C. Toro, E. Paxinos, E. Poveda, M. Martinez-Padial, J. M. Benito, V. Jimenez, T. Wrin, S. Bassani and V. Soriano (2004). "Differences in disease progression in a cohort of long-term non-progressors after more than 16 years of HIV-1 infection." *Aids* 18(8): 1109-16.
- 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(5342): 1447-50.
- Royce, R. A., A. Sena, W. Cates, Jr. and M. S. Cohen (1997). "Sexual transmission of HIV." *N Engl J Med* 336(15): 1072-8.
- Sampalo, A., M. Lopez-Gomez, J. Jimenez-Alonso, F. Ortiz, F. Samaniego and F. Garrido (1993). "CD5+ B lymphocytes in HIV infection: relationship to immunological progression of disease." *Clin Immunol Immunopathol* 66(3): 260-8.
- Samson, M., F. Libert, B. J. Doranz, J. Rucker, C. Liesnard, C. M. Farber, S. Saragosti, C. Lapoumeroulie, J. Cognaux, C. Forceille, G. Muyldermans, C. Verhofstede, G. Burtonboy, M. Georges, T. Imai, S. Rana, Y. Yi, R. J. Smyth, R. G. Collman, R. W. Doms, G. Vassart and M. Parmentier (1996). "Resistance to HIV-1 infection in caucasian individuals bearing mutant alleles of the CCR-5 chemokine receptor gene." *Nature* 382(6593): 722-5.
- Samuelsson, A., C. Brostrom, N. van Dijk, A. Sonnerborg and F. Chiodi (1997). "Apoptosis of CD4+ and CD19+ cells during human immunodeficiency virus type 1 infection--correlation with clinical progression, viral load, and loss of humoral immunity." *Virology* 238(2): 180-8.

- Samuelsson, A., A. Sonnerborg, N. Heuts, J. Coster and F. Chiodi (1997). "Progressive B cell apoptosis and expression of Fas ligand during human immunodeficiency virus type 1 infection." *AIDS Res Hum Retroviruses* 13(12): 1031-8.
- Santra, S., M. S. Seaman, L. Xu, D. H. Barouch, C. I. Lord, M. A. Lifton, D. A. Gorgone, K. R. Beaudry, K. Svehla, B. Welcher, B. K. Chakrabarti, Y. Huang, Z. Y. Yang, J. R. Mascola, G. J. Nabel and N. L. Letvin (2005). "Replication-defective adenovirus serotype 5 vectors elicit durable cellular and humoral immune responses in nonhuman primates." *J Virol* 79(10): 6516-22.
- 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. Scallan, 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(5403): 857-60.
- Sha, B. E., A. A. Harris, C. A. Benson, W. L. Atkinson, P. A. Urbanski, J. A. Stewart, W. W. Williams, R. L. Murphy, J. P. Phair, S. A. Levin and et al. (1991). "Prevalence of measles antibodies in asymptomatic human immunodeficiency virus-infected adults." *J Infect Dis* 164(5): 973-5.
- Shea, A., D. A. Sarr, N. Jones, L. Penning, G. Eisen, A. Gueye-Ndiaye, S. Mboup, P. Kanki and H. Cao (2004). "CCR5 receptor expression is down-regulated in HIV type 2 infection: implication for viral control and protection." *AIDS Res Hum Retroviruses* 20(6): 630-5.
- Shirai, A., M. Cosentino, S. F. Leitman-Klinman and D. M. Klinman (1992). "Human immunodeficiency virus infection induces both polyclonal and virus-specific B cell activation." *J Clin Invest* 89(2): 561-6.
- Shiver, J. W., T. M. Fu, L. Chen, D. R. Casimiro, M. E. Davies, R. K. Evans, Z. Q. Zhang, A. J. Simon, W. L. Trigona, S. A. Dubey, L. Huang, V. A. Harris, R. S. Long, X. Liang, L. Handt, W. A. Schleif, L. Zhu, D. C. Freed, N. V. Persaud, L. Guan, K. S. Punt, A. Tang, M. Chen, K. A. Wilson, K. B. Collins, G. J. Heidecker, V. R. Fernandez, H. C. Perry, J. G. Joyce, K. M. Grimm, J. C. Cook, P. M. Keller, D. S. Kresock, H. Mach, R. D. Troutman, L. A. Isopi, D. M. Williams, Z. Xu, K. E. Bohannon, D. B. Volkin, D. C. Montefiori, A. Miura, G. R. Krivulka, M. A. Lifton, M. J. Kuroda, J. E. Schmitz, N. L. Letvin, M. J. Caulfield, A. J. Bett, R. Youil, D. C. Kaslow and E. A. Emini (2002). "Replication-incompetent adenoviral vaccine vector elicits effective anti-immunodeficiency-virus immunity." *Nature* 415(6869): 331-5.
- Silvestri, G., D. L. Sodora, R. A. Koup, M. Paiardini, S. P. O'Neil, H. M. McClure, S. I. Staprans and M. B. Feinberg (2003). "Nonpathogenic SIV infection of sooty mangabeys is characterized by limited bystander immunopathology despite chronic high-level viremia." *Immunity* 18(3): 441-52.
- Slifka, M. K., R. Antia, J. K. Whitmire and R. Ahmed (1998). "Humoral immunity due to long-lived plasma cells." *Immunity* 8(3): 363-72.
- Smith, B. A., S. Gartner, Y. Liu, A. S. Perelson, N. I. Stilianakis, B. F. Keele, T. M. Kerkering, A. Ferreira-Gonzalez, A. K. Szakal, J. G. Tew and G. F. Burton (2001). "Persistence of infectious HIV on follicular dendritic cells." *J Immunol* 166(1): 690-6.
- Sprauer, M. A., L. E. Markowitz, J. K. Nicholson, R. C. Holman, A. Deforest, L. G. Dales and N. K. Khoury (1993). "Response of human immunodeficiency virus-infected adults to measles-rubella vaccination." *J Acquir Immune Defic Syndr* 6(9): 1013-6.
- Staszewski, S., J. Morales-Ramirez, K. T. Tashima, A. Rachlis, D. Skiest, J. Stanford, R. Stryker, P. Johnson, D. F. Labriola, D. Farina, D. J. Manion and N. M. Ruiz (1999). "Efavirenz plus zidovudine and lamivudine, efavirenz plus indinavir, and indinavir plus zidovudine and lamivudine in the treatment of HIV-1 infection in adults. Study 006 Team." *N Engl J Med* 341(25): 1865-73.
- Sullivan, B. L., E. J. Knopoff, M. Saifuddin, D. M. Takefman, M. N. Saarloos, B. E. Sha and G. T. Spear (1996). "Susceptibility of HIV-1 plasma virus to complement-mediated lysis. Evidence for a role in clearance of virus in vivo." *J Immunol* 157(4): 1791-8.
- Szelc, C. M., C. Mitcheltree, R. L. Roberts and E. R. Stiehm (1992). "Deficient polymorphonuclear cell and mononuclear cell antibody-dependent cellular cytotoxicity in pediatric and adult human immunodeficiency virus infection." *J Infect Dis* 166(3): 486-93.
- Tangye, S. G., Y. J. Liu, G. Aversa, J. H. Phillips and J. E. de Vries (1998). "Identification of functional human splenic memory B cells by expression of CD148 and CD27." *J Exp Med* 188(9): 1691-703.

- Tasker, S. A., M. R. Wallace, J. B. Rubins, W. B. Paxton, J. O'Brien and E. N. Janoff (2002). "Reimmunization with 23-valent pneumococcal vaccine for patients infected with human immunodeficiency virus type 1: clinical, immunologic, and virologic responses." *Clin Infect Dis* 34(6): 813-21.
- Thompson, J. S., S. A. Bixler, F. Qian, K. Vora, M. L. Scott, T. G. Cachero, C. Hession, P. Schneider, I. D. Sizing, C. Mullen, K. Strauch, M. Zafari, C. D. Benjamin, J. Tschopp, J. L. Browning and C. Ambrose (2001). "BAFF-R, a newly identified TNF receptor that specifically interacts with BAFF." *Science* 293(5537): 2108-11.
- Thompson, J. S., P. Schneider, S. L. Kalled, L. Wang, E. A. Lefevre, T. G. Cachero, F. MacKay, S. A. Bixler, M. Zafari, Z. Y. Liu, S. A. Woodcock, F. Qian, M. Batten, C. Madry, Y. Richard, C. D. Benjamin, J. L. Browning, A. Tsapis, J. Tschopp and C. Ambrose (2000). "BAFF binds to the tumor necrosis factor receptor-like molecule B cell maturation antigen and is important for maintaining the peripheral B cell population." *J Exp Med* 192(1): 129-35.
- Titanji, K., A. Nilsson, C. Mørch, A. Samuelsson, A. Sonnerborg, S. Grutzmeier, M. Zazzi and A. De Milito (2003). "Low frequency of plasma nerve-growth factor detection is associated with death of memory B lymphocytes in HIV-1 infection." *Clin Exp Immunol* 132(2): 297-303.
- Torcia, M., L. Bracci-Laudiero, M. Lucibello, L. Nencioni, D. Labardi, A. Rubartelli, F. Cozzolino, L. Aloe and E. Garaci (1996). "Nerve growth factor is an autocrine survival factor for memory B lymphocytes." *Cell* 85(3): 345-56.
- Tsubota, H., C. I. Lord, D. I. Watkins, C. Morimoto and N. L. Letvin (1989). "A cytotoxic T lymphocyte inhibits acquired immunodeficiency syndrome virus replication in peripheral blood lymphocytes." *J Exp Med* 169(4): 1421-34.
- Vacca, J. P., B. D. Dorsey, W. A. Schleif, R. B. Levin, S. L. McDaniel, P. L. Darke, J. Zugay, J. C. Quintero, O. M. Blahy, E. Roth and et al. (1994). "L-735,524: an orally bioavailable human immunodeficiency virus type 1 protease inhibitor." *Proc Natl Acad Sci U S A* 91(9): 4096-100.
- Walker, C. M., D. J. Moody, D. P. Stites and J. A. Levy (1986). "CD8+ lymphocytes can control HIV infection in vitro by suppressing virus replication." *Science* 234(4783): 1563-6.
- Walker, L. S., A. Gulbranson-Judge, S. Flynn, T. Brocker and P. J. Lane (2000). "Co-stimulation and selection for T-cell help for germinal centres: the role of CD28 and OX40." *Immunol Today* 21(7): 333-7.
- Wallace, M., S. R. Bartz, W. L. Chang, D. A. Mackenzie, C. D. Pauza and M. Malkovsky (1996). "Gamma delta T lymphocyte responses to HIV." *Clin Exp Immunol* 103(2): 177-84.
- Wallace, M. R., D. G. Hooper, S. J. Graves and J. L. Malone (1994). "Measles seroprevalence and vaccine response in HIV-infected adults." *Vaccine* 12(13): 1222-4.
- van der Vuurst de Vries, A. R., H. Clevers, T. Logtenberg and L. Meygaard (1999). "Leukocyte-associated immunoglobulin-like receptor-1 (LAIR-1) is differentially expressed during human B cell differentiation and inhibits B cell receptor-mediated signaling." *Eur J Immunol* 29(10): 3160-7.
- van Oers, M. H., S. T. Pals, L. M. Evers, C. E. van der Schoot, G. Koopman, J. M. Bonfrer, R. Q. Hintzen, A. E. von dem Borne and R. A. van Lier (1993). "Expression and release of CD27 in human B-cell malignancies." *Blood* 82(11): 3430-6.
- Wang, Z., H. W. Horowitz, T. Orlikowsky, B. I. Hahn, V. Trejo, A. S. Bapat, R. S. Mittler, R. J. Rayanade, S. Y. Yang and M. K. Hoffmann (1999). "Polyspecific self-reactive antibodies in individuals infected with human immunodeficiency virus facilitate T cell deletion and inhibit costimulatory accessory cell function." *J Infect Dis* 180(4): 1072-9.
- Watanabe, M. E. (2003). "Skeptical scientists skewer VaxGen statistics." *Nat Med* 9(4): 376.
- Wei, X., J. M. Decker, S. Wang, H. Hui, J. C. Kappes, X. Wu, J. F. Salazar-Gonzalez, M. G. Salazar, J. M. Kilby, M. S. Saag, N. L. Komarova, M. A. Nowak, B. H. Hahn, P. D. Kwong and G. M. Shaw (2003). "Antibody neutralization and escape by HIV-1." *Nature* 422(6929): 307-12.
- Wei, X., S. K. Ghosh, M. E. Taylor, V. A. Johnson, E. A. Emini, P. Deutsch, J. D. Lifson, S. Bonhoeffer, M. A. Nowak, B. H. Hahn and et al. (1995). "Viral dynamics in human immunodeficiency virus type 1 infection." *Nature* 373(6510): 117-22.
- Weinberg, J. B., T. J. Matthews, B. R. Cullen and M. H. Malim (1991). "Productive human immunodeficiency virus type 1 (HIV-1) infection of nonproliferating human monocytes." *J Exp Med* 174(6): 1477-82.

- Veljkovic, V., S. Muller and H. Kohler (2003). "Does VaxGen hide the breakthrough infections?"**  
**Lancet 361(9370): 1743-4.**
- Weller, S., M. C. Braun, B. K. Tan, A. Rosenwald, C. Cordier, M. E. Conley, A. Plebani, D. S. Kumararatne, D. Bonnet, O. Tournilhac, G. Tchernia, B. Steiniger, L. M. Staudt, J. L. Casanova, C. A. Reynaud and J. C. Weill (2004). "Human blood IgM "memory" B cells are circulating splenic marginal zone B cells harboring a prediversified immunoglobulin repertoire."** Blood 104(12): 3647-54.
- Wong, J. K., M. Hezareh, H. F. Gunthard, D. V. Havlir, C. C. Ignacio, C. A. Spina and D. D. Richman (1997). "Recovery of replication-competent HIV despite prolonged suppression of plasma viremia."** Science 278(5341): 1291-5.
- Voss, G., K. Manson, D. Montefiori, D. I. Watkins, J. Heeney, M. Wyand, J. Cohen and C. Bruck (2003). "Prevention of disease induced by a partially heterologous AIDS virus in rhesus monkeys by using an adjuvanted multicomponent protein vaccine."** J Virol 77(2): 1049-58.
- Wyatt, R. and J. Sodroski (1998). "The HIV-1 envelope glycoproteins: fusogens, antigens, and immunogens."** Science 280(5371): 1884-8.
- Yarchoan, R., R. W. Klecker, K. J. Weinhold, P. D. Markham, H. K. Lyerly, D. T. Durack, E. Gelmann, S. N. Lehrman, R. M. Blum, D. W. Barry and et al. (1986). "Administration of 3'-azido-3'-deoxythymidine, an inhibitor of HTLV-III/LAV replication, to patients with AIDS or AIDS-related complex."** Lancet 1(8481): 575-80.
- Zanetti, A. R., A. Amendola, S. Besana, A. Boschini and E. Tanzi (2002). "Safety and immunogenicity of influenza vaccination in individuals infected with HIV."** Vaccine 20 Suppl 5: B29-32.
- Zhang, L., W. Yu, T. He, J. Yu, R. E. Caffrey, E. A. Dalmasso, S. Fu, T. Pham, J. Mei, J. J. Ho, W. Zhang, P. Lopez and D. D. Ho (2002). "Contribution of human alpha-defensin 1, 2, and 3 to the anti-HIV-1 activity of CD8 antiviral factor."** Science 298(5595): 995-1000.
- Zinkernagel, R. M. (2003). "On natural and artificial vaccinations."** Annu Rev Immunol 21: 515-46.