

# HIV-1 Infection In Children: A Clinical and Immunologic Overview

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**Abstract:** Globally, HIV-1 is most often transmitted heterosexually so that nearly half of all infected adults are women of child-bearing age. Infants may acquire infection from vertical transmission. Without treatment most HIV-1 infected children in Africa die before their third birthday; as a result child mortality has increased overall by 35-50%, and by greater than 100% in areas of high seroprevalence.

HIV-1 infection has a heterogeneous spectrum of clinical course. Compared to HIV-1-infected adults, survival times are considerably shorter for children who acquire the virus perinatally or during infancy. Factors contributing to accelerated disease progression in infants and children are poorly understood but may include relative immunological immaturity, thymic HIV-1-mediated destruction at a time of active thymopoiesis, and HLA class I sharing between mother and infant.

This review will initially discuss clinical and biological determinants of mother-to-child transmission and disease progression in HIV-infected infants and children. Our current knowledge of the mechanisms of T cell depletion is summarised and the host immune response to HIV-1 (innate and adaptive) described in the context of Pediatric HIV-1 infection.

**Keywords:** HIV, Children, Africa, HAART, Cellular Immunity, Progression.

## EPIDEMIOLOGY

### AIDS: A Global Perspective

Within a 20-year period AIDS has spread from a few high-risk groups to become a worldwide pandemic. According to statistics released from UNAIDS and the WHO over 40 million people have been infected with HIV-1 [117]. An estimated 14,000 new infections occur daily. 95% of these occur in developing nations where access to newer medical treatments are not readily available or affordable. AIDS is now the leading cause of death in sub-Saharan Africa and the fourth biggest killer worldwide. AIDS killed at least 2.3 million Africans in 2001 resulting in a significant drop in life expectancy. In the absence of an effective therapeutic vaccine and inadequate treatment and care, most HIV-1-infected individuals will die before the next decade.

### THE IMPACT OF AIDS ON THE WOMEN AND CHILDREN OF AFRICA

HIV-1 is predominantly spread through heterosexual transmission with the result that nearly half of all affected adults are young women of childbearing age. About one-third of those currently living with HIV/AIDS are individuals aged 15-24. Most are unaware of their status. HIV-1 prevalence studies in pregnant women have provided the most objective data for comparing epidemics in different countries. Seroprevalence rates in many antenatal settings have ranged from 15 to 30% and up to 44% in urban areas of Botswana [118].

### The Rise in the Numbers of HIV-1-Infected Children

The epidemic has had grave consequences on infants and children. A rapidly increasing number of children are infected by mother-to-child-transmission (MTCT) either perinatally or by breast-feeding. Vertical transmission rates of HIV-1 in African countries vary from 25-42% [81]. An estimated 600,000 new paediatric infections occur each year; of which some 1500/day (> 90%) take place in sub-Saharan Africa. 2.7 million children are currently HIV-1-infected [117]. The estimated impact of AIDS among infants and children under 5 years of age has resulted in an increase in mortality in that age group.

### MOTHER-TO-CHILD TRANSMISSION OF HIV-1

In the absence of antiretroviral prophylaxis, reported transmission rates of HIV-1 ranged from 16-20% among large-cohort studies in Europe & N. America [33, 93] but up to 42% in Africa. Differences in rates could be accounted for by variable prevalence of breast-feeding, prematurity & elective Caesarean section. Transmission occurs either at birth from infection *in-utero* and vertically during labour and delivery (66%), or during the postnatal period from exposure to infected breast-milk. In contrast, vertical transmission of HIV-1 infection is now a rare event in industrialised countries, through a combination of anti-retroviral therapy, obstetric management and the use of alternative infant feeding methods.

### *In Utero* Transmission of HIV-1

*In utero* transmission most likely occurs transplacentally or by maternofetal transfusion. Although HIV-1 *env* sequences have been detected within most placentas of HIV-1-seropositive women, the placenta usually acts as an effective barrier against transmission allowing for a relatively

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few intrauterine infections. Nevertheless, placental trophoblasts and macrophages (Hoffbauer cells) are susceptible to HIV-1 *in vitro* [5, 55], and HIV-1 can be amplified from chorionic villi from the majority of term placentas of HIV-1-infected women, even after exclusion of maternal contamination. In these studies, the presence of HIV-1 proviral DNA in the placenta correlated with the detection of virus in the cord blood of newborns [26]. *In-utero* transmission may be associated with accelerated disease progression in infected infants [80] and a relatively high rate of first trimester abortions among HIV-1-infected women [41].

### **Intrapartum transmission of HIV-1**

Intrapartum transmission can occur through maternofetal transfusion of blood during labour or contact of infant skin or mucous membranes with the infected blood or other maternal secretions during delivery. HIV-1 DNA has been detected in 30-40% of gastric and oropharyngeal aspirates from newborns of HIV-1-infected mothers. Although detection of virus in these samples correlated with transmission, most infants were uninfected [85, 2]. Risk factors specific to intrapartum transmission include prolonged rupture of membranes, invasive procedures such as amniocentesis, chorioamnionitis, high viral load during pregnancy or at delivery, and vaginal delivery [120]. In a large meta-analysis elective Caesarean section was shown to be protective [114].

### **Breast Milk Transmission of HIV-1**

HIV-1 has been detected in the breast milk and colostrum of HIV-1-infected women [83]. The rate of infants not infected at birth but infected through breast-feeding is estimated at 12-14% [10]. Approximately 29% of breast-fed infants of women who seroconvert following delivery contract HIV-1 infection [30]. The mechanism of transmission is most likely the frequent and prolonged exposure of infants' oral and gastrointestinal tracts to infected milk. A randomised trial from Nairobi indicated that most transmission occurred within the first 6 weeks of breast-feeding (66%), 75% within the first 6 months of life and 84% by 12 months of age [82]. In a separate study, transmission risk was significantly correlated with breast-milk HIV-1 viral load [101]. Other specific risk factors of breast-milk transmission include breast abscesses and cracked nipples [31]. Infants can be poorly susceptible to oral HIV-1 infection. The exact reasons for this relative 'resistance' are unclear.

### **Maternal Correlates associated with Adverse and Favourable Birth Outcomes**

#### **Maternal Well-Being**

There are a number of maternal correlates for which adverse birth outcomes are common. Behavioural factors associated with vertical transmission include illicit drug use during pregnancy [16] and the presence of drug withdrawal symptoms in the infant [106]. Maternal age, over 30 years [61] and low vitamin A levels [102] have been positively correlated with transmission. The presence of maternal CMV and EBV coinfections were similarly correlated. Other

coinfections included the presence of a number of sexually transmitted diseases including syphilis, herpes simplex, gonorrhoea and chlamydia [94].

#### **Obstetrical Factors**

Numerous obstetrical factors have been associated with vertical transmission, including history of previous adverse birth outcomes [116] and gestational time of less than 34 weeks [61]. This may be due to immaturity of infant mucosal barriers against infection. Although a number of studies have noted an increased rate of vertical transmission with preterm delivery, it remains unclear whether preterm delivery is a result or cause of the HIV-1 infection. Bleeding during pregnancy [116] and blood in the amniotic fluid primarily due to placental abruption or praevia [68] are all linked to increases in transmission.

#### **Biologic Determinants**

The relationship between high neutralization antibody titres against autologous or clinical HIV-1 isolates and vertical transmission status has not been established. Some studies have indicated a correlation between high neutralizing titres and a decrease in the incidence of vertical transmission [56, 100]. However, other studies have failed to confirm this association [52].

Clinical determinants of MTCT include severe immunosuppression with low CD4+ counts and high maternal viral load [27]. Mothers with HLA discordance from their infants were least likely to transmit infection [71].

## **PAEDIATRIC HIV-1 INFECTION: A CLINICAL OVERVIEW**

### **The Bimodal Distribution of Paediatric HIV-1 Infection**

Perinatally infected children progress more rapidly than adults [7]. Although 4% of the world's population HIV-1-infected subjects are children, 20% of all AIDS deaths have been in this group. Early studies before the era of highly active antiretroviral therapy (HAART) indicated that a subset of children (approximately 25%) progressed very rapidly to AIDS (within 1 year). The median time to AIDS for the remaining 75% was 7 years [32].

### **The Clinical Course of HIV-1 Infection in Children**

HIV-1 infection in children causes a broad spectrum of disease. Manifestations include hepatosplenomegaly, failure to thrive, oral candidiasis, recurrent diarrhoea, parotitis, cardiomyopathy, hepatitis, nephropathy, developmental delay and encephalopathy, lymphoid interstitial pneumonitis, recurrent bacterial infections and specific malignancies. *Pneumocystis carinii* pneumonia is a very common serious opportunistic infection in children with HIV-1 infection and is associated with a high mortality. The pneumonia most often manifests between 3 to 6 months of age in infants with vertically-acquired infection. Primary and reactivated tuberculosis is a major cause of morbidity and mortality among HIV-1-infected children in Africa residing in communities where infection with the pathogen is endemic. Other co pathogens and opportunistic infections during paediatric HIV-1 infection include chronic or disseminated infections with herpesviruses (CMV, HSV and

VZV), and atypical mycobacteria. Causes of acute and chronic CNS infections include *Cryptococcus neoformans*, and *Toxoplasma gondii*. Leiomyosarcomas and lymphomas, including non-Hodgkin's B-cell Burkitts-type lymphomas, occur more frequently in children with HIV-1 infection than those who are immunocompetent. Kaposi's sarcoma has rarely been described among HIV-1-infected children residing in Western countries, but is not uncommonly seen among African children.

## ANTIRETROVIRAL THERAPY AND PREVENTION OF HIV-1 INFECTION IN CHILDREN

### Prevention of Mother-to-Child-Transmission (MTCT) of HIV

With the improved uptake of antenatal HIV testing and identification of infected women during pregnancy, MTCT of HIV can be dramatically reduced by providing antiretroviral therapy (ART) to mother antenatally, during delivery and to the baby postnatally; delivery by elective caesarean section (ELCS) and by avoidance of breast-feeding. These interventions reflect the recommendations of many studies including the landmark trial from the Pediatric AIDS Clinical Trials Group (PACTG 076), which demonstrated the efficacy of Zidovudine (AZT) in pregnancy & postnatally, reducing vertical transmission by 67% [22].

### The Goals of Antiretroviral Therapy During Pediatric HIV Infection

These are to maximally reduce plasma VL below the limit of detection (<50 copies/mL), prevent selection of drug resistant strains and maintain good immunological status (repopulation with CD4+ naïve T cells) to prevent clinical disease progression and opportunistic infections.

Four classes of antiretroviral drugs are available for HIV-1 therapy. Two classes target reverse transcriptase – the non-nucleoside reverse transcriptase inhibitors (NRTIs) and the non-NRTIs. A third class – the protease inhibitors target viral protease. The combination of these potent antiretroviral regimens has been pivotal in reducing the significant morbidity and mortality associated with HIV-1 infection in Western countries before 1996. However, a number of therapeutic challenges accompany the initiation and maintenance of HAART in all HIV-infected subjects.

### Difficulties with Implementation of HAART

#### The Emergence of Resistant Viral Variants

HIV-1 mutability is largely the result of errors introduced into the viral genome during replication. The HIV genome is approximately 10,000 nucleotides long and each new virion has an average of one mutation. This results in a large pool of quasispecies of viral variants that are incapable of productive infection, but some of which may provide an adaptive benefit to the virion such as the development of antiretroviral resistance. Aggressive, multidrug therapy as early in infection as possible has been advocated to fully suppress viral replication and to preclude the selection or emergence of resistant viral variants.

#### Adherence and Toxicities

There are difficulties with long-term adherence to therapy, particularly in infants and children where variable

drug administration, absorption & metabolism; maternal ART and vertical transmission of drug resistant virus; acceptability and palatability of formulations; refrigeration of syrup formulations in warm climates are all well documented.

Long-term follow-up of infected infants and children involves longitudinal determinations of prognostic markers including CD4 number and percentage, and viral load [38, 98]. These parameters provide a useful framework of when to initiate and change therapy, but involve frequent venipuncture in minors.

Long-term toxicities include lipodystrophy syndrome [121] and lipid abnormalities, cardiomyopathy, mitochondrial toxicity and lactic acidosis, renal tubular acidosis [19], hypersensitivity reactions, and CNS toxicity. In addition, the discovery that resting CD4+ T cells can provide a reservoir for HIV-1 have reinforced the need to develop new approaches to control and treat HIV infection.

## THE VIRAL KINETICS OF HIV-1 INFECTION

### Biological Determinants Contributing to Accelerated Disease Progression Among HIV-1-Infected Children

Perinatal infection occurs at a time of relative immunologic immaturity. If cellular immunity is critical in successful containment of HIV-1 then the infected infant can be severely immunosuppressed from birth onwards. The inability to control viraemia during acute infection could expose the thymus to HIV-1-mediated destruction at a time of active thymopoiesis [80]. This expanded thymocyte pool might also contribute to prolonged elevation of plasma HIV-1 RNA levels. Given that the virus is transmitted from the mother and that the degree of HLA class I sharing between mother and infant is high, the virus could evade the immune response of the newborn resulting in accelerated disease progression [40].

### Disease Progression and Viral Load, Comparing Adults & Children

In contrast to adults, HIV-1-related symptoms and/or CD4+ T cell depletion develop in most untreated vertically-infected children within the first few years of life [84]. In addition plasma HIV-1 RNA levels remained elevated over the first two years among infants [1] and do not decrease to <10<sup>5</sup> copies/mL through at least the third year of life. A continued reduction in plasma HIV-1 RNA levels (mean -0.2 to -0.3 log decline/year) has been observed in vertically infected children through 5 or 6 years of age [74]. It is unclear whether these differences in peripheral viral load affect disease progression [1, 104], although reductions in plasma HIV-1 RNA levels with HAART are associated with clinical benefit. The prolonged elevation of plasma HIV-1 RNA levels may be related to the kinetics of viral replication, the size of the pool of host cells that are permissive to viral replication, and deficient virus-specific immune responses.

### A Renewable Pool of Permissive Host Cells may Contribute to Persistently High Plasma HIV-1 RNA Levels in Infancy and Childhood

After the initiation of HAART >90% of virus in plasma was cleared in an initial rapid exponential decrease followed

by an exponential slower second phase decrease in adults experiencing primary infection [64] and with established disease [92]. Two sources for plasma virions were proposed:

1. Short-lived, productively infected cells (CD4+ T cells).
2. Long-lived cells with stably integrated HIV-1 provirus (tissue macrophages, dendritic cells (DCs) or latently infected CD4+ T cells undergoing activation) [92].

The biphasic decay may also represent an exponential decay of a single cellular source, with a decreasing exponent over time caused by a reduction in the number of virus-producing cells or the ability of the cells to produce virus (for example an increased number of cells moving from the activated to resting state). Estimates of half-lives for viral turnover were calculated to be similar in adults and perinatally-infected children [65].

A large and renewable pool of permissive host cells may contribute to persistently high plasma HIV-1 RNA levels in infancy and early childhood. CD4+ T lymphocytes and thymocytes might be particularly important substrates in infants. Relative lymphocytosis, thymopoiesis and an increased CD4+ T-cell pool size have been observed at these ages.

#### **Viral Set Point During Clinical Latency may Determine Disease Progression**

Studies in adult subjects have described the establishment of a viral set-point, which is maintained through clinical latency [63]. HIV-1 RNA levels will generally rebound to this same set point after the discontinuation of prolonged, effective HAART, and may be a strong predictor of the subsequent clinical course: the higher the set point, the more rapid the immunologic progression, and the shorter the period of clinical latency [46]. The factors that determine the viral set-point are unclear, but may be lowered by efficient host HIV-specific CD8+ T cell and T-helper responses [96] and in the presence of viral mutations that alter replicative competence, and include *Nef* deletions [25].

#### **T-CELL DEPLETION DURING HIV-1-INFECTION: A QUANTITATIVE AND QUALITATIVE DECLINE**

HIV-1 infection disrupts the homeostasis of balanced proliferation of progenitor cells and death of mature progeny by accelerated destruction of CD4+ T cells; these helper cells are pivotal in coordinating humoral and cellular immune responses. Quantitative estimates indicate the normal young adult possess approximately  $2 \times 10^{11}$  mature CD4+ T cells [43]. This number is halved by the time the CD4+ T cell count decreases to 200 cells/ $\mu$ L.

Qualitative defects during disease progression in the CD4+ T cell compartment include a decrease in the proportion of quiescent naïve CD45RA+CD62L+ T cells and an increase in the proportion of activated memory phenotype (CD45RO+). At the same time the T-cell receptor repertoire is restricted [39]. A number of hypotheses have been developed to account for CD4+ T cell depletion. Four plausible mechanisms will be discussed.

#### **Accelerated Destruction of Mature CD4+ T Cells**

Total body CD4+ T cells may be depleted in absolute number by destruction. *In-vitro* experiments with laboratory-adapted HIV-1 isolates in tissue culture revealed a cytopathic virus with tropism for CD4+ T cells. These cells were predicted to be equally susceptible *in-vivo*. The provision of HAART allowed investigators to estimate that continuous rounds of *de novo* infection sustained the viral load and that as many as  $2 \times 10^9$  infected CD4+ T cells were destroyed daily resulting in eventual exhaustion of the immune system [51].

Quantitative image analysis and analysis of T-cell turnover revealed decreased numbers of CD4+ T cells and increased levels of cellular proliferation concentrated in the CD45RO+ population of memory CD4+ T cells and apoptosis in lymphoid tissue [43,55]. Given that cell division must be balanced by cell death, these experiments suggested that HIV-1 infection accelerated both destruction and production of CD4+ T cells. However, destruction alone could not account for the pronounced changes in CD4+ T cell function, or for redistribution of CD4+ T cells.

#### **Altered Movement and the Appearance of Loss**

Movement of cells from one location within the immune system is directed in part by homing receptors that mediate tissue-specific interactions [36]. Naïve CD45RA+CD62L+ T cells recirculate in an L-selectin (CD62L)-dependent manner from blood to lymph node and then back to blood. Memory (CD45RO+) T cells move through non-lymphoid tissue such as liver and lung, thereby increasing chances of encountering foreign antigen [17].

When antigen-presenting cells (APCs) initiate immune responses within lymphoid tissue, CD4+ T cells are stimulated and retained, while CD8+ T cells migrate into the circulation. This differential movement results in a transient decrease in total lymphocyte count and a CD8 lymphocytosis [67]. Changes of this type have been observed during acute and chronic HIV-1 infection and are augmented by the HIV-1-induced upregulation of CD62L on CD4+ T cells [12].

Effective HAART and a drop in HIV-1 RNA levels may decrease immune activation, allowing sequestered cells to recirculate back into the peripheral circulation [126]. Indeed reductions in viral load have been associated with decreased levels of adhesion molecules (VCAM-1, ICAM-1), which normally mediate lymphocyte sequestration into lymphoid tissue [78].

#### **Chronic Activation and T-Cell Death**

Uninfected T cells may die as a result infection elsewhere. HIV-1 infection results in a state of chronic activation driven in part by the antigenic stimulus of HIV-1 and in part by antigen-independent mechanisms or cytokines released by APCs and activated T cells [50]. This process has been termed 'activation-induced cell death' and has been observed in a number of other chronic infections including intestinal helminth infestations, tuberculosis and malaria [13].

### Impaired Renewal of T Cells

Early progenitors of CD4<sup>+</sup> T cells (multi-lineage and lineage-restricted haematopoietic cells) may also express CD4. Infection of cells with long half-lives may generate large numbers of differentiated progeny upon stimulation [77]. Such observations would account for the pancytopenia noted in end-stage patients with AIDS. Other authors have observed thymocyte depletion, loss of corticomedullary demarcation and the development of thymic medullary B-cell follicles. These changes are associated with presence of HIV-1 structural proteins in thymocytes and evidence of viral replication most commonly with CXCR4-utilizing isolates of HIV-1, which are tropic for CXCR4+CD3-CD4+CD8- intrathymic T progenitor cells. In contrast, CCR5-utilising isolates infect intrathymic myeloid and thymocyte subpopulations that are more mature, and result in thymocyte depletion after long periods of time [9].

Indirect evidence of thymic involvement includes decreased circulating CD4<sup>+</sup> and CD8<sup>+</sup> naïve T cells [97] and cells bearing T cell receptor rearrangement excision circles [TRECs] (markers of recent thymic emigrants) during disease progression. Signs of thymopoeisis appear to return after effective HAART [28], particularly if the subject is younger and does not have evidence of thymic involution [59].

Each of the above mechanisms of T cell depletion is presented by a number of investigators with strong supporting data. It would seem that in any given scenario, one mechanism might be dominant. Paediatric thymic dysfunction, for example, as a result of *in-utero* infection may account for the rapid disease progression observed in 20-30% of HIV-1-infected children. In such infants, a pattern of lymphocyte depletion develops that resembles a pattern observed in congenital thymic deficiency [59]. However, all four mechanisms may be important sequentially during chronic disease progression among young adults infected by sexual transmission.

### THE HOST IMMUNE RESPONSE TO HIV-1-INFECTION

Infection with HIV-1 generates an immune response that contains the virus but as a consequence of its cellular tropism and intrinsic characteristics is rarely, if ever, eliminated from the body. Broadly speaking, the host immune response can be divided into innate and adaptive immunity, and the latter into humoral (antibody mediated) and cellular components. In turn, the cellular arm of the adaptive immune response can be divided into T helper (CD4<sup>+</sup> mediated) and cytotoxic T lymphocyte (CD8<sup>+</sup> mediated) responses. This section will summarise host defence to HIV-1 infection with particular emphasis on cell-mediated immunity and provide overviews of innate and adaptive humoral immunity.

### THE INNATE IMMUNE SYSTEM DURING HIV-1 INFECTION

#### Soluble Mediators

Mannose binding lectins (MBLs) and complement bind to HIV-1 and either lyse the virus directly or help viral

phagocytosis by macrophages. Subjects with low levels of MBLs have an increased risk of HIV-1 infection and enhanced progression to disease [107]. Complement can also rapidly inactivate HIV-1 or serve as an opsonin for phagocytosis of the virus [37]. Cytokines, such as IL-12, IL-4 and IL-6 determine predominance of T helper 1(Th1) or Th2-type responses. TNF- $\alpha$ , and the interferons (IFNs) can influence the extent of HIV-1 replication [109]. Chemokines can recruit NK cells, T cells and macrophages to the site of HIV-1 Infection and increase the cytotoxic function of these cells [42].

### IFN-Producing Cells May Protect HIV-Infected Subjects by IFN Production

IFN-producing cells (IPCs) are immature DCs that mature into DCs (DC-2) and enhance Th2 responses [11]. IPCs secrete type I IFNs after exposure to viruses [99]. These cells express CD4 and the IL-3 receptor and are found primarily in lymphoid tissue [105]. They also make up 0.2-0.9% of all circulating PBMCs. Recently, loss of IPCs and IFN- $\gamma$  production were associated with high HIV-1 viral load. Moreover, normal IPC numbers and IFN- $\gamma$  production were noted in healthy HIV-infected subjects, naïve to HAART, who had remained infected for over 10 years with very low CD4<sup>+</sup>T-cell counts.

### CD8<sup>+</sup> T Cells Can Control HIV-1 Replication by an Innate Non-Antigen-Specific, Non-Species-Specific Nontoxic Antiviral Response

CD8<sup>+</sup> T cells can control HIV-1 replication in infected cells through both classic and an innate non-antigen-specific, non-species-specific nontoxic antiviral response (CNAR) [108]. These rapid responses are not restricted by HLA class I or II and do not appear to involve memory. In addition to an association with the long-term asymptomatic state, these responses have been described in HIV-exposed uninfected subjects [62].

### Neutrophils and DCs

In addition to their phagocytic activity, neutrophils release a number of inflammatory cytokines that control microbial infection [112]. DCs elicit protection from HIV-1 infection through production of cytokines and type I IFNs. They are important mediators of antigen recognition and activate both innate and adaptive immune responses through cytokine secretion [57].

### ADCC, NK Cells & $\gamma\delta$ T cells

ADCC has been reported to be inversely correlated with HIV-1 RNA levels in chronically infected subjects. Viral reduction resulted from lysis of infected cells and from direct inhibition of HIV-1 via secretion of the chemokines, RANTES and MIP-1- $\alpha$ . Moreover, several cytokines secreted by NK cells (IFN- $\gamma$ , TNF- $\alpha$ ,  $\beta$ -chemokines) have been shown to inhibit HIV-1 replication *in-vitro* [58].

$\gamma\delta$  T cells found at mucosal surfaces interact directly with nonpeptide antigens or with cellular stress proteins (heat shock proteins) and can lyse HIV-1-infected targets [35]. *In-vitro* studies suggest they may suppress HIV-1-

replication through secretion of chemokines and other soluble antiviral factors [124].

## HUMORAL IMMUNITY DURING HIV-1 INFECTION

HIV-1 is a persistent viral infection, with an ineffective humoral immune response that is partially protective in controlling infection. During acute and asymptomatic infection, a rigorous and sustained humoral response against a number of HIV-1 structural proteins fails to abrogate the infection [95]. The first antibodies detected recognise linear determinants to p24 and p17 in gag. Thereafter, antibodies to *env* and *pol* epitopes appear together with antibodies to regulatory and accessory proteins such as *rev*, *tat*, *vpr*, *vpu*, *vif* and *nef*.

### Non-Neutralising Responses to *Env*

Most of the neutralising antibody response is directed against the gp120 subunit of the envelope glycoprotein [75]. Antigenic epitopes on gp120 and gp41 recognised by human antisera have been identified by epitope mapping of antisera and the analysis of monoclonal antibodies. HIV-1 cross-reactive antibodies from HIV-1 seropositive subjects recognise epitopes within the conserved regions of gp120 in a region overlapping the CD4 binding site (CD4 binding domain (CD4bd) antibodies). Epitopes within the first and fifth conserved domains of gp120, and those clustered in two regions on the gp41 ectodomain are also recognised. However, these antibodies do not neutralise virus [111].

### Neutralising Responses to *Env*

Only three monoclonal antibodies that neutralise primary isolates, each recognising a distinct epitope have been described. These are anti-gp120 antibodies IgG1 b12 and 2G12, and the anti-gp41 antibody 2F5 [89]. Unfortunately, for reasons, which remain unclear, the neutralising antibody responses detected in most HIV-1-infected at low levels appear inadequate in clearing HIV-1 and the virus rapidly escapes [29]. A possible explanation is that the antibody response is principally directed against viral debris rather than virions (i.e. non-native forms of the envelope released by lysis of infected cells or shedding from the viral surface) [76].

### The Poor Immunogenicity of *Env*

#### *Biochemical and Physical Properties*

Even in the presence of intact virions, the HIV-1 envelope is both of low antigenicity and immunogenicity. Low antigenicity is largely due to the oligomeric nature of the envelope proteins, together with very high levels of gp120 glycosylation, factors that combine to shield most potentially immunogenic epitopes of the mature virion from the immune system [91]. Furthermore, overlapping hypervariable loops (such as V1 and V2) may restrict accessibility of antibody to those parts of gp120 that are critical for viral entry [53].

#### *'Original Antigenic Sin'*

Since large amounts of gp160 produced by infected cells are recycled intracellularly, rather than being processed into gp120, a strong humoral response to gp160 may suppress

effective humoral responses against cross-reactive gp120 epitopes [90]. This mechanism has been termed 'original antigenic sin' [34].

### *Antigenic Variation*

Finally, because of its high mutation rate, HIV-1 may escape neutralising antibodies through antigenic variation. When a laboratory worker was infected with a neutralisation-sensitive strain of HIV-1, the virus rapidly developed a neutralisation-resistant phenotype *in-vivo*, indicative of rapid evolution of the virus to escape immune responses [8].

### The Poor Correlation of Neutralising Responses to *Env* with Non-Progression

Subjects with acute HIV-1 infection have significant decline of primary viraemia before neutralising antibodies are detectable. Although some with long-term control without HAART have strong neutralising responses, these responses are not present in all subjects who control virus [45]. Collectively, all these factors would explain why HIV-1-specific humoral immune responses have not been found to correlate with protection from disease progression in infected subjects.

## THE THYMUS DURING HIV-1 INFECTION

HIV-1 may directly kill thymocytes [110] and thymic DCs required for normal thymocyte development [119], and can damage thymic epithelial cells required for normal thymopoiesis [110] and thymocyte signalling. HIV-1 RNA-expressing cells are found both within the thymic perivascular and true thymic epithelial spaces during early and late HIV-1 infection. Early infection is associated with an increased lymphoid infiltrate of the thymic perivascular space, relative to normal thymus tissue, and with the presence of germinal centres in the thymic perivascular space. In late HIV-1 infection, thymic changes resemble, but are more exaggerated than, the atrophy associated with normal ageing [23, 47, 48, 119].

### TRECs During HIV-1 Infection

A decrease of TREC levels in the peripheral T cells of untreated HIV-1-infected patients has been described. Furthermore, HAART induced increases in peripheral CD4<sup>+</sup> T cell counts and TREC levels in younger HIV-1-infected children although some of the early rises in CD4<sup>+</sup> T cells were probably due to redistribution of CD4<sup>+</sup> T cells into the peripheral blood after treatment. These findings suggested a suppressive effect of HIV-1 viraemia on TREC<sup>+</sup> T cell production in the thymus [28].

### The T Cell Regeneration During HAART

The use of HAART in HIV-1-infected patients has led to suppression of plasma HIV-1 RNA, improvement in peripheral blood CD4<sup>+</sup> T cell counts, and in some cases thymic enlargement from increased thymopoiesis [47-49]. However increased thymic size could also result from infiltration of peripheral lymphocytes into the thymic perivascular space, particularly in untreated patients [48]. In humans, there are two distinct pathways for the generation of

TCR $\alpha\beta$  T cells: thymopoiesis and expansion of peripheral T cells [23,24]. Both mechanisms may contribute to the early rises of CD4<sup>+</sup> T cells in peripheral blood during HAART [66, 67, 69, 70, 87], although current opinion suggests thymopoiesis remains the primary mechanism [20, 24]. In children the source of the majority of restored CD4 T cells is the thymic production of new naive T cells. This may be important since in contrast to adults, not only is a quantitative recovery of CD4 cells occurring, but also the generation of new T-cell clones, which could recover the repertoire of specificities capable of responding to different pathogenic agents [24].

**THE CELLULAR IMMUNE RESPONSE TO HIV-1 INFECTION**

**HIV-1-Specific CD8<sup>+</sup> T Cell Activity During Acute Infection**

In nearly all subjects the cellular immune response to HIV-1 fails to eradicate or adequately control infection. This failure facilitates viral escape from immune control resulting in progressive immunodeficiency. During acute HIV-1 infection, a robust immune response by CD8<sup>+</sup> T cell peaks with the rise in viraemia to reduce viral load to establish an inverse relationship between CTL response and viral replication [Fig. 1 and 18]. Little is known about the function of these early HIV-1-specific T cells, but the response is restricted to a few clones [88].

**HIV-1-Specific CD8<sup>+</sup> T Cell Activity During Chronic Infection**

During chronic HIV-1 infection, expanded HIV-1-specific T cells persist at high frequencies; often 1–2% of all

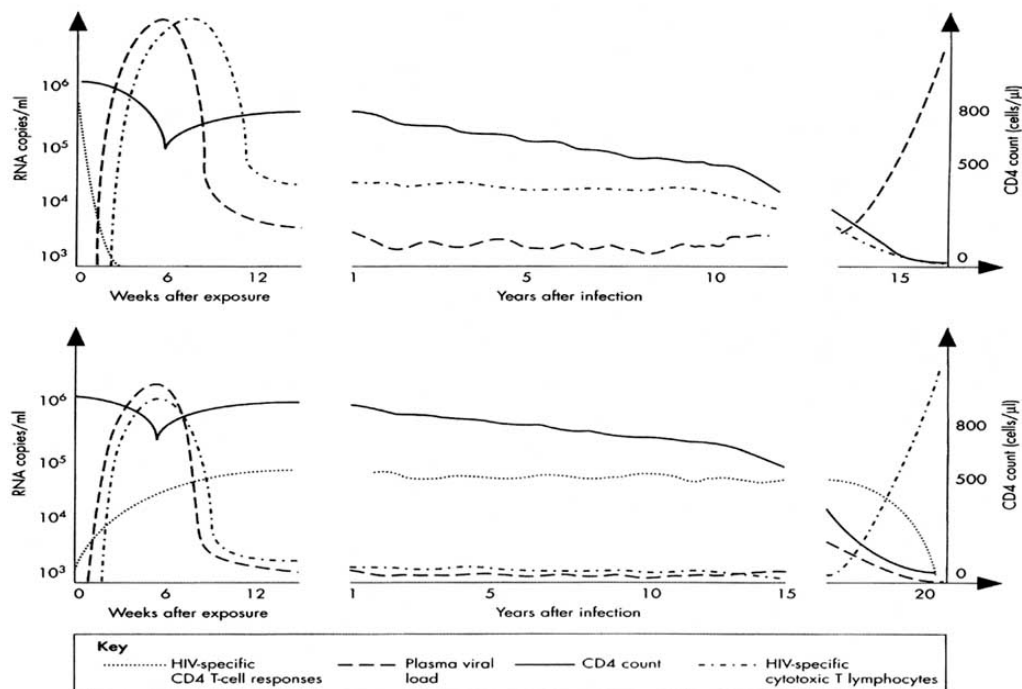
circulating CD8<sup>+</sup> T cells are specific for a dominant HIV-1 epitope [4]. There are similar numbers in lymph nodes [60]. These T cells probably turn over continuously; and like the acutely expanded T cells tend to die by apoptosis *ex vivo* [73]. The high number of responding T cells is probably dependent on continued antigen stimulation, since reduction of viraemia by HAART causes a steady decline in tetramer stained CD8<sup>+</sup> T cells [86]. Without treatment, the high number of HIV-1-specific CD8<sup>+</sup> T cells often persist into late infection. As in acute infection, these expanded T cells are often oligoclonal [127] and may be long-lived.

**Secretion of Cytokines and Chemokines by HIV-1-Specific CD8<sup>+</sup> T Cells**

CD8<sup>+</sup> T cells produce cytokines, which affect viral replication. These include IFN- $\alpha$ , which inhibits viral replication, and TNF- $\alpha$ , which can up regulate replication through activation of the HIV promoter in the virus 5' long terminal repeat [79]. HIV-1-specific CD8<sup>+</sup> T cells also produce the CC chemokines MIP-1 $\alpha$ , MIP-1  $\beta$  and RANTES, which suppress HIV-1 replication [122] by competition for, or downregulation of CCR5. Inhibition involves lytic mechanisms and release of CC chemokines and CD8<sup>+</sup> T-cell antiviral factor (CAF). CAF blocks LTR-mediated transcription in infected cells [122]. This factor may facilitate the development of latency.

**Target Cell Lysis and Apoptosis**

Cultured HIV-1-specific CD8<sup>+</sup> T cells lyse HIV-1-infected CD4<sup>+</sup> T cells *in vitro* despite the ability of *nef* to reduce expression of class I HLA molecules on their surface [21]. Target cell lysis is mediated by perforin and granzymes



**Fig. (1).** The relationship between viral replication, the HIV-1-specific CD8<sup>+</sup> T cell response and the selective pressure on epitope escape mutants. When viral load is high, as in acute infection, there is no selective force until the CD8<sup>+</sup> T cell responses appear. During chronic infection, HIV-1-specific CD8<sup>+</sup> T cell responses may be suboptimal giving rise to escape mutants.

[6, 103], or by FasL expression on antigen-activated CD8<sup>+</sup> T cells triggering apoptosis in cells that express Fas [44].

### Qualitative Defects in CTL Function During HIV-1 Infection

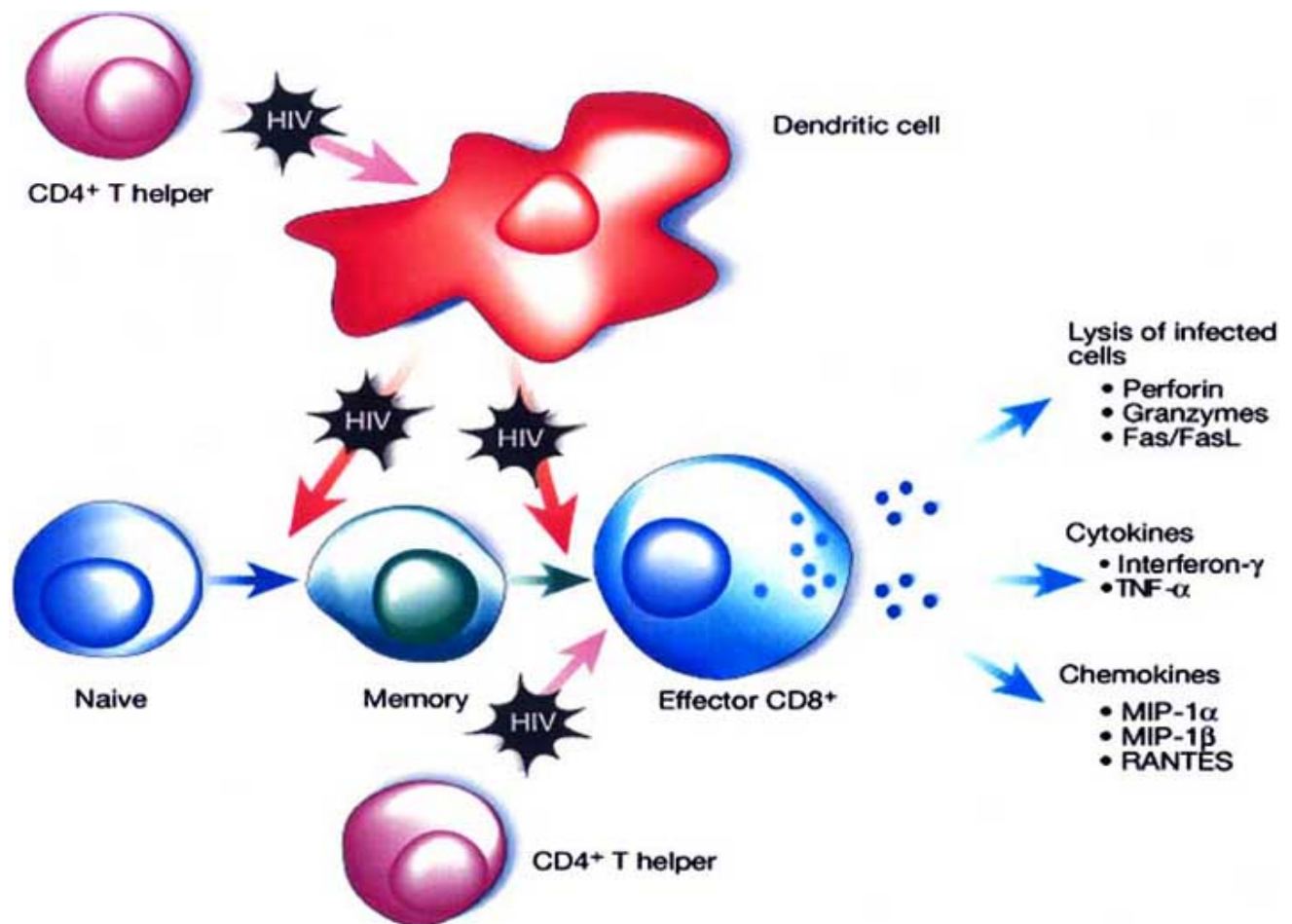
Recent studies have demonstrated that virus-specific CD8<sup>+</sup> T cells taken *ex vivo* can have functional defects that may undermine their control of virus. When HIV-1 epitope/HLA class I tetramer-staining was combined with intracellular staining for cytokines and chemokines, it was found that most HIV-1-specific cells in patients with chronic HIV-1 disease produced IFN- $\alpha$ , TNF- $\alpha$  and MIP-1 $\alpha$  on contact with their cognate antigen *ex vivo*. This pattern of cytokine secretion was similar to CMV-specific cells from HIV-1-uninfected donors. However, a striking difference was seen in the level of intracellular perforin. Less than 15% of HIV-1-specific cells contained perforin, which was reflected in poor *ex vivo* killing of appropriate target cells, compared with CMV-specific cells from the same donors, 50% of which expressed perforin and killed well [6].

It is uncertain why HIV-1-specific cells poorly express perforin: these cells lack expression of the glycoprotein

CD28 on their surface, but they retain CD27. In contrast, CMV-specific cells lose expression of both molecules. This loss is considered to be a marker of mature effector phenotype [6] and suggests HIV-1-specific CD8 T cells *in vivo* may be immature rather than end-stage effectors. The reason for immature phenotype of CD8<sup>+</sup> T cells may be a consequence of impaired T-cell help, since T-cell help is known to be important for priming the CD8<sup>+</sup> T-cell responses [115] for maintaining CD8<sup>+</sup> T-cell memory and for maturing CD8<sup>+</sup> T-cell function [72 and Fig. 2]. However, in subjects with maximal viral suppression on HAART and in LTNPs, the phenotype is no different, even if CD4 T cell help can be detected.

### Escape from CTL Viral Mutation

CD8<sup>+</sup> T cells can act against HIV-1 most effectively by killing infected cells before they generate new virus particles. Unless the killing process eliminates the virions rapidly, this process exerts a selective force, giving an advantage to cells infected with viruses that have mutated critical amino acids in the dominant epitopes. These infected cells escape lysis and propagate the mutant virus. The rate of mutation is



**Fig. (2).** CD4<sup>+</sup> T-cell dependence of CD8<sup>+</sup> T cells. CD4<sup>+</sup> T cells are important for priming DCs to initiate CD8<sup>+</sup> T-cell responses. They help maintain memory T cells and are important in maturation of CD8<sup>+</sup> T-cell function. All of these actions are impaired by HIV-1 infection. In addition, HIV-1 can directly infect and impair DC function.

influenced by the balance between the strength of killing and the level of viral replication, and may be also influenced by impairment of CD8+ T cell function [14].

Selection of mutants by CD8+ T cell is probably one of the main features of HIV-1 infection. Longitudinal studies of individual patients, matching dominant CD8+ T cell responses with changes in amino-acid sequence, have identified clear cases where a single change has abrogated presentation by the class I HLA molecules and these viruses have become dominant in the quasispecies [3, 14, 113, 124]. Furthermore, there is evidence to demonstrate that escape from one CD8+ T cell population is followed by a new CD8+ T cell response in a new epitope [3, 54]. However, this response may be less effective.

**Other Mechanisms of Escape** These include downregulation of classical HLA-A and HLA-B molecules and upregulation of Fas ligand secondary to *nef* activity in infected cells (discussed in earlier sections). Almost all HIV-1-specific T cells express Fas and so could be targets for killing by the FasL pathway.

Other means by which HIV can escape CD8+ T cell attack include sequestration of infected cells in the central nervous system with limited T cell access. Any latently-infected cells will be invisible to detection. Transition of virus from the R5 to the X4 type, when the co-receptor requirement is changed from CCR5 (natural receptor for the CC chemokines) to CXCR4 (natural receptor for stromal-derived factor) during disease progression, will mean that the virus becomes insensitive to inhibition by the CC chemokines released by antigen-activated T cells.

## CONCLUSIONS

There is a current paucity of knowledge on HIV-1 pathogenesis in exposed uninfected infants and HIV-1 infected children. Protective correlates and mechanisms of T cell depletion within a clinical Pediatric context have been discussed, however, a greater understanding of HIV-1-specific immunity in these groups is important to develop interventions to reduce transmission and to modify disease progression, which may supplement existing antiretroviral drug regimens.

## REFERENCES

[1] Abrams EJ, Weedon J, Steketee RW, Lambert G, Bamji M, Brown T, Kalish ML, Schoenbaum EE, Thomas PA, Thea DM. (1998). *Journal of Infectious Diseases*. 178:101-108.

[2] Ait-Khaled M, Lyall EG, Stainsby C, Taylor GP, Wright A, Weber JN, McClure MO, Tudor-Williams G. (1998). *Journal of Infectious Diseases*. 177:1097-100.

[3] Allen TM, O'Connor DH, Jing P, Dzuris JL, Mothe BR, Vogel TU, Dunphy E, Liebl ME, Emerson C, Wilson N, Kunstman KJ, Wang X, Allison DB, Hughes AL, Desrosiers RC, Altman JD, Wolinsky SM, Sette A, Watkins DI. (2000). *Nature*. 407:386-390.

[4] Altman JD, Moss PA, Goulder PJ, Barouch DH, McHeyzer-Williams MG, Bell JI, McMichael AJ, Davis MM (1996). *Science*. 274:94-96.

[5] Amirhessami-Aghili N, Spector SA. (1991) *Journal of Virology*. 65:2231-6.

[6] Appay V, Nixon DF, Donahoe SM, Gillespie GM, Dong T, King A, Ogg GS, Spiegel HM, Conlon C, Spina CA, Havlir DV, Richman DD, Waters A, Easterbrook P, McMichael AJ,

Rowland-Jones SL. (2000). *Journal of Experimental Medicine*. 192:63-75.

[7] Barnhart HX, Caldwell MB, Thomas P, Mascola L, Ortiz I, Hsu HW, Schulte J, Parrott R, Maldonado Y, Byers R. (1996). *Pediatrics* 9:710-16.

[8] Beaumont T, van Nuenen A, Broersen S, Blattner WA, Lukashov VV, Schuitemaker H. (2001). *Journal of Virology*. 75:2246-52.

[9] Berkowitz RD, Alexander S, Bare C, Linquist-Stepps V, Bogan M, Moreno ME, Gibson L, Wieder ED, Kosek J, Stoddart CA, McCune JM. (1998). *Journal of Virology*. 72, 10108-10117.

[10] Bertolli J, St Louis ME, Simonds RJ, Nieburg P, Kamenga M, Brown C, Tarande M, Quinn T, Ou CY. (1996). *Journal of Infectious Diseases*. 174:722.

[11] Biron C A, Nguyen KB, Pien GC, Couzens LP and Salazar-Mather TP. (1999). *Annual Review of Immunology*. 17:189-220.

[12] Bishop DK, Ferguson RM and Orosz CG. (1990). *Journal of Immunology*. 144:1153-1160.

[13] Borkow G, Leng Q, Weisman Z, Stein M, Galai N, Kalinkovich A, Bentwich Z. (2000). *Journal of Clinical Investigation*. 106:1053-1060.

[14] Brodie SJ, Lewinsohn DA, Patterson BK, Jiyamapa D, Krieger J, Corey L, Greenberg PD, Riddell SR. (1999). *Nature Medicine*. 5:34-41.

[15] Bucy RP, Hockett RD, Derdeyn CA, Saag MS, Squires K, Sillers M, Mitsuyasu RT, Kilby JM. (1999). *Journal of Clinical Investigation*. 103:1391-1398.

[16] Burns DN, Landesman S, Wright DJ, Waters D, Mitchell RM, Rubinstein A, Willoughby A, Goedert JJ. (1997). *Journal of Infectious Diseases*. 175:1206-1210.

[17] Butcher EC and Picker LJ. (1996) *Science*. 276:60-66.

[18] Chakraborty R and Rowland-Jones S. (1999) *Journal of HIV Therapy*. 4:1.

[19] Chakraborty R, Uy CS, Oleske JM, Coen PG, McSherry GD. (2003). *AIDS*. 17:673-7.

[20] Chavan S, Bennuri B, Kharbanda M, Chandrasekaran A, Bakshi S, Pahwa S. (2001). *Journal of Infectious Diseases*. 183:1445-54.

[21] Collins KL, Chen, BK, Kalams, SA, Walker BD and Baltimore D. (1998). *Nature*. 391:397-401.

[22] Connor EM, Sperling RS, Gelber R, Kiselev P, Scott G, O'Sullivan MJ, VanDyke R, Bey M, Shearer W, Jacobson RL, et al. (1994). *New England Journal of Medicine*. 331:1173-80.

[23] Correa R, Munoz-Fernandez MA. (2001). *AIDS*. 15:1959-63.

[24] Correa R, Munoz-Fernandez MA. (2002). *AIDS*. 16:1181-3.

[25] Deacon NJ, Tsykin A, Solomon A, Smith K, Ludford-Menting M, Hooker DJ, McPhee DA, Greenway AL, Ellett A, Chatfield C, et al. (1995). *Science*. 270:988-91.

[26] De Andreis C, Simoni G, Rossella F, Castagna C, Pesenti E, Porta G, Colucci G, Giuntelli S, Pardi G, Sempriani AE. (1996). *AIDS*. 10:711-5.

[27] Dickover RE, Garratty EM, Herman SA, Sim MS, Plaeger S, Boyer PJ, Keller M, Deveikis A, Stiehm ER, Bryson YJ. (1996). *Journal of the American Medical Association*. 275:599-605.

[28] Douek DC, McFarland RD, Keiser PH, Gage EA, Massey JM, Haynes BF, Polis MA, Haase AT, Feinberg MB, Sullivan JL, Jamieson BD, Zack JA, Picker LJ, Koup RA. (1999). *Nature*. 396:690-695.

[29] D'Souza MP, Livnat D, Bradac JA, Bridges SH. (1997). *Journal of Infectious Diseases*. 175:1056-1062.

[30] Dunn DT, Newell ML, Ades AE, Peckham CS. (1992). *Lancet*. 340:585.

[31] Ekpini ER, Wiktor SZ, Satten GA, Adjorlolo-Johnson GT, Sibailly TS, Ou CY, Karon JM, Brattegaard K, Whitaker JP, Gnaore E, De Cock KM, Greenberg AE. (1997). *Lancet*. 349:1054-1059.

[32] European Collaborative Study. (1994). *Pediatrics*; 94:815-9.

[33] European Collaborative Study Group. (1996). *AIDS*. 10:1675.

[34] Fazekas de St G, Webster RG. (1966). *Journal of Experimental Medicine*. 124:331-45.

[35] Fehniger TA, Herbein G, Yu H, Para MI, Bernstein ZP, O'Brien WA, Caligiuri MA. (1998). *Journal of Immunology*. 161:6433-6438.

[36] Fleury S, Rizzardi GP, Chapuis A, Tambussi G, Knabenhans C, Simeoni E, Meuwly JY, Corpataux JM, Lazzarin A, Miedema F, Pantaleo G. (2000). *Proceeding of the National Academy of Sciences USA*. 97:5393-5398.

[37] Garred P, Madsen HO, Balslev U, Hofmann B, Pedersen C, Gerstoft J, Svejgaard A. (1997). *Lancet*. 349:236-240.

- [38] Gibb DM, Duong T, Tookey PA, Sharland M, Tudor-Williams G, Novelli V, Butler K, Riordan A, Farrelly L, Masters J, Peckham CS, Dunn DT; National Study of HIV in Pregnancy and Childhood Collaborative HIV Paediatric Study. (2003). *British Medical Journal*. 327:1019.
- [39] Gorochov G, Neumann AU, Kereveur A, Parizot C, Li T, Katlama C, Karmochkine M, Raguin G, Autran B, Debre P. (1998). *Nature Medicine*. 4:215-221.
- [40] Goulder PJ, Brander C, Tang Y, Tremblay C, Colbert RA, Addo MM, Rosenberg ES, Nguyen T, Allen R, Trocha A, Altfeld M, He S, Bunce M, Funkhouser R, Pelton SI, Burchett SK, McIntosh K, Korber BT, Walker BD. (2001). *Nature*. 412:334-8.
- [41] Gray RH, Wawer MJ, Serwadda D, Sewankambo N, Li C, Wabwire-Mangen F, Paxton L, Kiwanuka N, Kigozi G, Konde-Lule J, Quinn TC, Gaydos CA, McNairn D. (1998). *Lancet*. 351:98.
- [42] Graziosi C, Gantt KR, Vaccarezza M, Demarest JF, Daucher M, Saag MS, Shaw GM, Quinn TC, Cohen OJ, Welbon CC, Pantaleo G, Fauci AS. (1996). *Proceeding of the National Academy of Sciences USA*. 93:4386-4391.
- [43] Haase AT. (1999). *Annual Review of Immunology*. 17:625-656.
- [44] Hadida F, Vieillard V, Mollet L, Clark-Lewis I, Baggioolini M, Debre P. (1999). *Journal of Immunology*. 163:1105-1109.
- [45] Harrer T, Harrer E, Kalams SA, Elbeik T, Staprans SI, Feinberg MB, Cao Y, Ho DD, Yilma T, Caliendo AM, Johnson RP, Buchbinder SP, Walker BD. (1996). *AIDS Research in Human Retroviruses*. 12:585-92.
- [46] Hatano H, Vogel S, Yoder C, Metcalf JA, Dewar R, Davey RT, Polis MA. (2000). *AIDS*. 14:1357-63.
- [47] Haynes BF, Hale LP. (1998). *Immunology Research*. 18:175-92.
- [48] Haynes BF, Hale LP, Weinhold KJ, Patel DD, Liao HX, Bressler PB, Jones DM, Demarest JF, Gebhard-Mitchell K, Haase AT, Bartlett JA. (1999). *Journal of Clinical Investigation*. 103:453-60.
- [49] Haynes BF, Markert ML, Sempowski GD, Patel DD, Hale LP. (2000). *Annual Review of Immunology*. 18:529-60.
- [50] Hazenberg MD, Hamann D, Schuitemaker H, Miedema F. (2000). *Nature Immunology*. 1:285-289.
- [51] Ho DD, Neumann AU, Perelson AS, Chen W, Leonard JM, Markowitz M. (1995). *Nature*. 373:123-126.
- [52] Husson RN, Lan Y, Kojima E, Venzon D, Mitsuya H, McIntosh K. (1995). *Journal of Pediatrics*. 126:865-871.
- [53] Johnson WE, Desrosiers RC. (2002). *Annual Review Medicine*. 53:499-518.
- [54] Kanazawa S, Okamoto T, Peterlin BM. (2000). *Immunity*. 12:61-70.
- [55] Kesson AM, Fear WR, Kazazi F, Mathijs JM, Chang J, King NJ, Cunningham AL. (1993). *Journal of Infectious Diseases*. 168:571-9.
- [56] Kliks SC, Wara DW, Landers DV, Levy JA: Features of HIV-1 that could influence maternal-child transmission. (1994). *Journal of the American Medical Association*. 272:467-474.
- [57] Klebanoff SJ, Coombs RW. (1992). *Journal of Clinical Investigation*. 89:2014-2017.
- [58] Knight SC. Bone-marrow-derived dendritic cells and the pathogenesis of AIDS. (1996). *AIDS*. 10:807-817.
- [59] Kourtis AP, Ibegbu C, Nahmias AJ, Lee FK, Clark WS, Sawyer MK, Nesheim S. (1996). *New England Journal of Medicine*. 335:1431-6.
- [60] Kuroda MJ, Schmitz JE, Charini WA, Nickerson CE, Lord CI, Forman MA, Letvin NL. (1999). *Journal of Virology*. 73:1573-1579.
- [61] Landesman SH, Kalish LA, Burns DN, Minkoff H, Fox HE, Zorrilla C, Garcia P, Fowler MG, Mofenson L, Tuomala R. (1996). *New England Journal of Medicine*. 334:1617-1623.
- [62] Levy JA, Mackewicz CE, Barker E. (1996). *Immunology Today*. 17:217-224.
- [63] Lifson JD, Nowak MA, Goldstein S, Rossio JL, Kinter A, Vasquez G, Willtrout TA, Brown C, Schneider D, Wahl L, Lloyd AL, Williams J, Elkins WR, Fauci AS, Hirsch VM. (1997). *Journal of Virology*. 71:9508-14.
- [64] Little SJ, McLean AR, Spina CA, Richman DD, Havlir DV. (1999). *Journal of Experimental Medicine*. 190:841-50.
- [65] Luzuriaga K, Wu H, McManus M, Britto P, Borkowsky W, Burchett S, Smith B, Mofenson L, Sullivan JL. (1999). *Journal of Virology*. 73:362-67.
- [66] Mackall CL, Hakim FT, Gress RE. (1997). *Seminars in Immunology*. 9:339-46.
- [67] Mackay CR, Marston WL, Dudler L. (1990). *Journal of Experimental Medicine*. 171, 801-817.
- [68] Mandelbrot L, Le Chenadec J, Berrebi A, Bongain A, Benifla JL, Delfraissy JF, Blanche S, Mayaux MJ. (1998). *Journal of the American Medical Association*. 280:55-60.
- [69] Markert ML, Alvarez-McLeod AP, Sempowski GD, Hale LP, Horvatinovich JM, Weinhold KJ, Bartlett JA, D'Amico TA, Haynes BF. (2001). *AIDS Research in Human Retroviruses*. 17:1635-43.
- [70] McCune JM, Loftus R, Schmidt DK, Carroll P, Webster D, Swor-Yim LB, Francis IR, Gross BH, Grant RM. (1998). *Journal of Clinical Investigation*. 101:2301-8.
- [71] McDonald KS, Embree J, Njenga S, Nagelkerke NJ, Ngatia I, Mohammed Z, Barber BH, Ndinya-Achola J, Bwayo J, Plummer FA. (1998). *Journal of Infectious Diseases*. 177:551.
- [72] McMichael AJ, Rowland-Jones SL. (2001). *Nature*. 410:980 - 987.
- [73] Meyaard L, Otto SA, Jonker RR, Mijster MJ, Keet RP, Miedema F. (1992). *Science*; 257:217-219.
- [74] Mofenson LM, Korelitz J, Meyer WA 3rd, Bethel J, Rich K, Pahwa S, Moye J Jr, Nugent R, Read J. (1997). *Journal of Infectious Diseases*. 175:1029-1038.
- [75] Moore JP, Cao Y, Ho DD, Koup RA. (1994). *Journal of Virology*. 68:5142-5155.
- [76] Moore JP, Cao Y, Leu J, Qin L, Korber B, Ho DD. (1996). *Journal of Virology*. 70:427-444.
- [77] Moses A, Nelson J, Bagby, G. (1998). *Blood*. 91, 1479-1495.
- [78] Mosier DE. 1995. *Nature*. 375, 193-194.
- [79] Mosmann TR, Li L, Sad S. (1997). *Seminars in Immunology*. 9:87-92.
- [80] Nahmias AJ, Clark WS, Kourtis AP, Lee FK, Cotsonis G, Ibegbu C, Thea D, Palumbo P, Vink P, Simonds RJ, Nesheim SR. (1998). *Journal of Infectious Diseases*. 178:680.
- [81] Nduati R. (2000). *Advances in Experimental and Medical Biology*. 478:201-10.
- [82] Nduati R, John G, Mbori-Ngacha D, Richardson B, Overbaugh J, Mwachia A, Ndinya-Achola J, Bwayo J, Onyango FE, Hughes J, Kreiss J. (2000). *Journal of the American Medical Association*. 283:1167.
- [83] Nduati RW, John GC, Richardson BA, Overbaugh J, Welch M, Ndinya-Achola J, Moses S, Holmes K, Onyango F, Kreiss JK. (1995). *Journal of Infectious Diseases*. 172:1461.
- [84] Newell ML, Peckham C, Dunn D. The European collaborative study. (1994). *Pediatrics*. 94:815-819.
- [85] Nielsen K, Boyer P, Dillon M, Wafer D, Wei LS, Garratty E, Dickover RE, Bryson YJ. (1996). *Journal of Infectious Diseases*. 173:1001-4.
- [86] Ogg GS, Jin X, Bonhoeffer S, Moss P, Nowak MA, Monard S, Segal JP, Cao Y, Rowland-Jones SL, Hurley A, Markowitz M, Ho DD, McMichael AJ, Nixon DF. (1999). *Journal of Virology*. 73, 797-800.
- [87] Pakker NG, Notermans DW, de Boer RJ, Roos MT, de Wolf F, Hill A, Leonard JM, Danner SA, Miedema F, Schellekens PT. (1998). *Nature Medicine*. 4:208-14.
- [88] Pantaleo G, Demarest JF, Soudeyns H, Graziosi C, Denis F, Adelsberger JW, Borrow P, Saag MS, Shaw GM, Sekaly RP, Fauci AS. (1994). *Nature* 370:463-467.
- [89] Parren PW, Gauduin MC, Koup RA, Poignard P, Sattentau QJ, Fiscaro P, Burton DR. (1997). *Immunology Letters*. 58:125-132.
- [90] Parren PW, Moore JP, Burton DR, Sattentau QJ. (1999). *AIDS*. 13:S137-162.
- [91] Parren PW, Sattentau QJ, Burton DR. (1997). *Nature Medicine*. 3:366-367.
- [92] Perelson AS, Essunger P, Cao Y, Vesanan M, Hurley A, Saksela K, Markowitz M, Ho DD. (1997). *Nature*. 378:188-191.
- [93] Pitt J, Brambilla D, Reichelderfer P, Landay A, McIntosh K, Burns D, Hillyer GV, Mendez H, Fowler MG. (1997). *Journal of Infectious Diseases*. 175:567.
- [94] Pitt J, Schluchter M, Jensen H, Kovacs A, LaRussa P, McIntosh K, Boyer P, Cooper E, Goldfarb J, Hammill H, Hodes D, Peavy H, Sperling R, Tuomala R, Shearer W. (1998). *Journal of AIDS*. 19:462-470.
- [95] Poccia F, Battistini L, Cipriani B, Mancino G, Martini F, Gougeon ML, Colizzi V. (1999). *Journal of Infectious Diseases*. 180, 858-861.

- [96] Pontesilli O, Klein MR, Kerkhof-Garde SR, Pakker NG, de Wolf F, Schuitemaker H, Miedema F. (1997). *Immunology Letters*. 57:125-30.
- [97] Rabin RL, Roederer M, Maldonado Y, Petru A, Herzenberg LA, Herzenberg LA. (1995). *Journal of Clinical Investigation*. 95, 2054-2060.
- [98] Resino S, Bellon JM, Resino R, Navarro ML, Tomas Ramos J, de Jose MI, Mellado MJ, Munoz-Fernandez MA. 2004. *Clinical Infectious Diseases*. 38:1605-12.
- [99] Rissoan MC, Soumelis V, Kadowaki N, Grouard G, Briere F, de Waal Malefyt R, Liu YJ. (1999). *Science*. 283:1183-1186.
- [100] Scarlatti G, Albert J, Rossi P, Hodara V, Biraghi P, Muggiasca L, Fenyo EM. (1993). *Journal of Infectious Diseases*. 168:207-210.
- [101] Semba RD, Kumwenda N, Hoover DR, Taha TE, Quinn TC, Mtimavalye L, Biggar RJ, Broadhead R, Miotti PG, Sokoll LJ, van der Hoeven L, Chipangwi JD. (1999). *Journal of Infectious Diseases*. 180:93.
- [102] Semba RD, Miotti PG, Chipangwi JD, Saah AJ, Canner JK, Dallabetta GA, Hoover DR. (1994). *Lancet*. 343:1593.
- [103] Shankar P, Xu Z, Lieberman J. (1999). *Blood*. 94:3084-3093.
- [104] Shearer WT, Quinn TC, LaRussa P, Lew JF, Mofenson L, Almy S, Rich K, Handelsman E, Diaz C, Pagano M, Smeriglio V, Kalish LA. (1997). *New England Journal of Medicine*. 336:1137-1349.
- [105] Siegal FP, Kadowaki N, Shodell M, Fitzgerald-Bocarsly PA, Shah K, Ho S, Antonenko S, Liu YJ. (1999). *Science*. 284:1835-1837.
- [106] Simonds RJ, Steketee R, Nesheim S, Matheson P, Palumbo P, Alger L, Abrams EJ, Orloff S, Lindsay M, Bardeguez AD, Vink P, Byers R, Rogers M, Studies TPACT. (1998). *AIDS*. 12:301-308.
- [107] Smith KY, Valdez H, Landay A, Spritzler J, Kessler HA, Connick E, Kuritzkes D, Gross B, Francis I, McCune JM, Lederman MM. (2000). *Journal of Infectious Diseases*. 181:141-147.
- [108] Soumelis V, Scott I, Gheyas F, Bouhour D, Cozon G, Cotte L, Huang L, Levy JA, Liu YJ. (2001). *Blood*. 98:906.
- [109] Spear GT, Sullivan BL, Landay AL, Lint TF. (1990). *Journal of Virology*. 64:5869-5873.
- [110] Stanley SK, McCune JM, Kaneshima H, Justement JS, Sullivan M, Boone E, Baseler M, Adelsberger J, Bonyhadi M, Orenstein J, Fox CH, Fauci AS. (1993). *Journal of Experimental Medicine*. 178:1151-63.
- [111] Steimer KS, Scandella CJ, Skiles PV, Haigwood NL. (1991). *Science*; 254:105-108.
- [112] Stranford SA, Skurnick J, Louria D, Osmond D, Chang SY, Sninsky J, Ferrari G, Weinhold K, Lindquist C, Levy JA. (1999). *Proceeding of the National Academy of Sciences USA*. 96:1030-1035.
- [113] Tan R, Xu X, Ogg GS, Hansasuta P, Dong T, Rostron T, Luzzi G, Conlon CP, Screaton GR, McMichael AJ, Rowland-Jones S. (1999). *Blood*. 93:1506-1510.
- [114] The International Perinatal HIV Group: (1999). *New England Journal of Medicine*. 340:977.
- [115] Trimble LA, Lieberman J. (1998). *Blood*. 91:585-594.
- [116] Tuntland T, Odinecs A, Pereira CM, Nosbisch C, Unadkat JD. (1999). *American Journal of Obstetrics and Gynecology*. 180:198-206.
- [117] UNAIDS. December 2001: new HIV estimates and additional data. 2001: [http://www.unaids.org/epidemic\\_update/report/index.html](http://www.unaids.org/epidemic_update/report/index.html).
- [118] US Bureau of the Census. Recent HIV Seroprevalence Levels by Country; February 1999. Research note 26. Washington DC.
- [119] Valentin H, Nugeyre MT, Vuillier F, Bousnell L, Schmid M, Barre-Sinoussi F, Pereira RA. (1994). *Journal of Virology*. 68:3041-50.
- [120] Van Dyke RB, Korber BT, Popek E, Macken C, Widmayer SM, Bardeguez A, Hanson IC, Wiznia A, Luzuriaga K, Viscarello RR, Wolinsky S. (1999). *Journal of Infectious Diseases*. 179:319.
- [121] Vigano A, Mora S, Testolin C, Beccio S, Schneider L, Bricalli D, Vanzulli A, Manzoni P, Brambilla P. (2003). *Journal of AIDS*. 32:482-9.
- [122] Wagner L, Yang OO, Garcia-Zepeda EA, Ge Y, Kalams SA, Walker BD, Pasternack MS, Luster AD. (1998). *Nature*. 391:908-11.
- [123] Wallace M, Bartz SR, Chang WL, Mackenzie DA, Pauza CD, Malkovsky M. (1996). *Clinical and Experimental Immunology*. 103:177-184.
- [124] Walter EA, Greenberg PD, Gilbert MJ, Finch RJ, Watanabe KS, Thomas ED, Riddell SR. (1995). *New England Journal of Medicine*. 333:1038-1044.
- [125] Wang L, Chen J J, Gelman BB, Konig R, Cloyd MW. (1999). *Journal of Immunology*. 162:268-276. Wilson JD, Ogg GS, Allen RL, Goulder PJ, Kelleher A, Sewell AK, O'Callaghan CA, Rowland-Jones SL, Callan MF, McMichael AJ. (1998). *Journal of Experimental Medicine*. 188:785-790.