CHAPTER II

LITERATURE REVIEW

History

Acquired immune deficiency syndrome (AIDS) was first recognized as a new and distinct clinical entity in 1981[46]. The first few cases were recognized because of unusual clusters of rare diseases such as Kaposi's sarcoma and *Pneumocystis carinii* pneumonia in a group of young, previously healthy, male homosexuals. Before then, this opportunistic pathogen had been associated with disease only in patients whose immune system had been seriously impaired as a result of drug therapy or congenital cellular immune deficiency. AIDS cases were soon reported in other populations, as well, including intravenous drug users (IVDUs) and hemophiliacs[47]. The observation that T-lymphocytes bearing the CD4 marker were depleted in these cases reinforced the conclusion that the disease arose from immune system failure. In 1982, the Center for Disease Control (CDC) of the United States of America named this kind of immune deficiency Acquired Immune Deficiency Syndrome or AIDS[48]. The first isolation of a retrovirus from an AIDS case was performed by Luc Montagnier and Barre-Sinoussi[1] at the Pasteur Institute in Paris in early 1983 and quickly confirmed by Robert Gallo[2].

Virion structure

HIV is a retrovirus and has two major subtypes, designated HIV-1 and HIV-2, which have similar structures and demonstrate the same cell tropism. The nucleotide sequence homology between HIV-1and HIV-2 is 42%.[49] The genome of HIV-1 is composed of three genes (*gag, pol, env*) encoding for the structural proteins of the mature virion whereas at least six genes (*tat, rev, vif, vpu,vpr,nef*) encode for proteins involved in the regulation of viral expression.[50]

The HIV virions have a spherical shape, about 90-130 nm in diameter, and consist of a lipid bilayer membrane or envelope that surrounds the cone-shaped

nucleocapsid. The nucleocapsid within each mature virion is composed of two molecules of the viral single-stranded RNA genome encapsulated by proteins proteolytically processed from the Gag precursor polypeptide. These *gag* gene products are the matrix protein, which is presumably located between the nucleocapsid and the virion envelope; the major capsid protein, which forms the capsid shell; and the nucleocapsid protein, which bind tightly to the viral RNA genome. Viral enzymes derived from the *pol* gene precursor polypeptide are also packaged into virions; these enzymes are protease, reverse transcriptase and integrase. In addition, Vpr are small proteins associated with nucleocapsids. The membrane of extracellular HIV particles contains knobs composed of a surface subunit and transmembrane subunit, designated gp120 and gp41, respectively.[51,52,53]

Receptors and cellular distribution

The target cells of HIV include CD4+ T lymphocytes, dendritic cells, Langerhans cells, monocytes, macrophages, and thymocytes. These cells all share in common the presence of the CD4 molecule on the cell surface that acts as the principle receptor for HIV.[54,55] However, surface CD4 alone appears insufficient to allow entry of HIV into cells. A surface protein called fusin or currently known as CXCR4 which appears to be a key co-receptor for T-tropic HIV strain.[17] In contrast, M-tropic HIV isolates appear to require another fusion co-receptor that is CCR-5 which is the receptor for the β-chemokines RANTES, MIP-1α and MIP-1β.[18,19,20,21] *In vitro*, HIV can also infect cells that do not express CD4 such as neuronal, endothelial, fibroblast, and NK cells.[56,57] The roles of CD4 independent infection *in vivo* is unknown.

Replication

The replication cycle of HIV-1 is divided into an early and a late phase. Each phase consists of sequential steps, and several of these steps involve specific interactions of viral proteins and nucleic acids with cellular factors. The early phase of replication begins with attachment of the envelope glycoprotein (gp120) of the HIV particle to a cell surface receptor (CD4) and then gp120 also binds to the co-receptor

(either β or α-chemokine receptor). After binding, the viral and cellular membranes fuse, and the nucleocapsid is released into the cytoplasm. The viral enzymes reverse transcriptase and integrase remain associated with this uncoated nucleocapsid, and the process of reverse transcription produces linear double-stranded viral DNA which is maintained in a nucleoprotein complex that is subsequently transported into the nucleus. In a reaction mediated by integrase, the ends of the linear double-stranded viral DNA are covalently joined to host cell DNA to produce the integrated provirus.

The late phase of replication begins with transcription and processing of viral RNA from the integrated proviral template and ends with release of progeny virions from the cell. In the provirus, the 5′ LTR encodes *cis*-acting sequences for cellular factors that control initiation of viral transcription by RNA polymerase II, and the 3′ LTR contains signals for processing the 3′ LTR of all viral transcripts. The viral transcriptional transactivator Tat acts through a signal at the 5′ end of newly initiated viral transcripts to augment initiation and/or elongation by the host cell RNA polymerase II complex. Unspliced, singly spliced, and multiply spliced viral transcripts are transported to the cytoplasm and translated into various viral proteins. The viral transactivator Rev recognizes a *cis*-acting element in the full-length viral transcript and controls the ratio of spliced and unspliced RNA. In addition, Rev may controls both transport of viral RNA from the nucleus and translation on cytoplasmic polysomes. A portion of full-length viral transcripts interacts with virion precursor polypeptides to produce immature nucleocapsids at the cell plasma membrane. These nucleocapsids acquire an envelope by extrusion through areas of the plasma membrane containing the envelope glycoprotein. In newly released extracellular particles, the Gag and Gag-Pol polyproteins are proteolytically processed by the viral protease to yield fully infectious virions.[58]

Mode of transmission

The character of the HIV pandemic in different regions has been largely influenced by the frequency of each of the three main modes of transmission- sexual, perinatal and parenteral. Sexual transmission is by far the most important, accounting for over 75% of all HIV infections world-wide. During the early phases of the

epidemic, homosexual transmission was the predominant mode in developed countries. However, during the past 5 years, there has been evidence of increasing trends for heterosexual transmission in industrialised countries. In the Caribbean, heterosexual transmission has now replaced homosexual transmission as the major mode of spread. In North America and Western Europe, the frequency of HIV infection is rising steadily among heterosexuals whereas it is plateauing and in some cases declining among homosexual/bisexual men and IVDUs. With increasing heterosexual transmission, the impact of the pandemic on women is rising sharply. As of late 1995, nine million women has been rose from 25% in 1990 to 45% by 1995. By the year 2000, the number of new infections among women will have highlighted the extent to which gender inequalities render women particularly susceptible to infection; they are often unable to make choices to protect themselves even when they have the information and the knowledge to do so. Enhancing the status of women is an important long-term strategy in the control of HIV, but meanwhile a female-controlled vaginal microbicide would enable women to protect themselves without dependence on their male partners.[57]

Mechanisms of CD4 T lymphocyte dysfunction

The immunosuppression in HIV infected patients, is due to quantitative as well as functional defects in CD4+ T lymphocytes caused by the following mechanisms[4, 60,61,62]

Single-cell killing

HIV may be directly involved in destruction of CD4 T+ cells: the budding of HIV from the cell surface disrupts cellular integrity, and the intracellular interactions between gp120 and CD4 may interfere the normal cellular metabolism.

Syncytia formation

The formation of syncytia involves fusion of the cell membrane of the infected CD4+ cells, which results in giant multinucleated cells. Large syncytia can form rendering the targeted cells non-functional and susceptible to lysis. Patients infected with SI strain have a more rapid progression to AIDS.

HIV-specific immune responses

Both humoral and cellular immune responses contribute to antiviral immunity. Antibodies directed against some regions of the envelope of HIV may yield an additional protective function related to their ability to mediate antibody-dependent cellular cytotoxicity (ADCC) after binding to NK cells, leading to the killing of HIV-infected cells. HIV-specific cytotoxic T lymphocytes (CTL) may play an important part in the immune response against HIV.

Autoimmune mechanism

Nonpolymorphism determinants of the major-histocompatibility-complex (MHC) class II molecule share some degree of structural homology with the gp120 and gp41 proteins of HIV, and antibodies to these HIV proteins could therefore cross-react with HLA class II molecules. These antibodies could prevent interaction between CD4 and class II molecules expressed on the antigen-presenting cells, thus impairing the cellular interaction required for efficient antigen presentation and inhibiting antigen-specific functions mediated by CD4 T helper cells.

Anergy

The CD4 cells become refractory to further *in vitro* stimulation through the activation of their CD3 molecules upon reaction of their component CD4 molecules with gp120 or gp120-anti-gp120 complexes.

Superantigens

The superantigen hypothesis regarding HIV infection stems from the observation that endogenous or exogenous retrovirally encoded superantigens stimulate murine CD4 T cells *in vivo*, leading to the anergy or deletion of a substantial percentage of CD4 T cells that have the specific variable β-regions. However, rather than causing deletions of specific subgroups of T cells, it is more likely that superantigens serve as potent activators of T cells, rendering them more susceptible to infection with the virus.

Apoptosis

There has been speculation that cross-linking of the CD4 molecules by HIV gp120 or gp120-anti-gp120 immune complexes prepares the cell for the programmed death, or apoptosis. Apoptosis, like the superantigens, would help each depleted cell be infected with HIV.

Immunopathogenesis of HIV infection

The rate of progression of HIV disease may be substantially different among HIV-infected individuals. Following infection of the host with any virus, the delicate balance between virus replication and immune response to the virus determines both the outcome of the infection, i.e. persistence versus elimination of the virus, and different rates of progression.[63,64,65]

Clinical course of HIV infection

The clinical course of HIV infection generally includes three phases or stages: (1). primary infection, (2). clinical latency, and (3). AIDS-defining illness. Such a course of infection is characteristic of the so-called typical progressors who represent the majority of HIV-infected individuals. The median time from initial infection to progression to AIDS in typical progressors is eight to ten years

1. Primary infection

Approximately three to six weeks after initial infection, 50-70% of HIV–infected individuals develop an acute mononucleosis-like syndrome. This period is associated with high levels of viremia, and within one week to three months there is an immune response to HIV. This immunity is apparently inadequate to suppress viral replication completely, since HIV expression persists in lymph nodes even when plasma viremia is difficult to detect. Detectable viremia declines markedly and stabilizes after the acute syndrome subsides. Although a substantial percentage of patients with HIV infection do not have a clinically recognizable acute syndrome after primary infection, the events described above probably occur even in the absence of symptoms.

2. Clinical latency

Most patients have a period of " clinical latency" that lasts for years after primary infection. During this period virtually all patients have a gradual deterioration of the immune system, manifested particularly by the progressive depletion of CD4+ T cells. Although this depletion may occur even without large increases in plasma concentrations of virus, viral replication in lymphoid organs, together with the spectrum of immunologic events that are directly or indirectly triggered by the virus, may contribute to it. Thus, HIV disease is clearly progressive during the so-called latent period.

3. AIDS-defining illness

AIDS-defining illness or clinically apparent disease is the inevitable outcome of the progressive deterioration of the immune system that occurs in most patients with HIV infection. Exceptions to the direct correlation between deteriorating immune function and clinically apparent disease are the progressive generalized lymphadenopathy; Kaposi's sarcoma, which can occur before the onset of severe immunosuppression; and neurologic disease that may reflect direct or indirect effects of the virus or its products on neurons. The profound immunosuppression that occurs during this phase of HIV infection is the end stage of the immunopathogenic events that began at the time of primary infection, and have continued for years through the clinically latent but microbiologically active stages of infection.

HIV disease progression

There are 4 major patterns of HIV disease in regard to the role of disease progression.

1. Typical progressors

The majority (70-80%) of HIV-infected individuals belong to the group of typical progressors. Following primary infection, as mentioned above, typical progressors experience a long period (up to six to eight years) of clinical latency. Despite the lack of symptoms, HIV disease is active as indicated by the persistent replication of virus and by the progressive loss of CD4+ T-cells. Individuals with CD4+ T-cell counts > 500 per μl generally remain free of symptoms, whereas the

appearance of constitutional symptoms is generally more frequent in individuals with CD4+ T-cell counts below 500 per μl. Exceptions to this paradigm are subjects with CD4+ T-cell counts higher than 500 per μl who develop progressive generalized lymphadenopathy, Kaposi's sarcoma, or neurologic diseases. Progression to clinically apparent disease or AIDS-defining illness generally occurs within eight to ten years in typical progressors. When CD4+ T-cell counts are below 200 per μl, the clinical picture may be characterized by severe and persistent constitutional signs and symptoms; at this level of CD4+ T cells, there is an increased susceptibility to opportunistic infections or neoplasms.

2. Rapid progressors

A significant percentage (10-12%) of HIV- infected individuals experience an unusually rapid progression to AIDS within two to three years of primary infection. Rapid progressors may experience a prolonged acute viral syndrome and the period of true clinical latency may be absent or very brief. Downregulation of the initial burst of viremia may not be very efficient in rapid progressors; even after the initial decrease, the levels of viremia may rise rapidly. Inefficient control of the initial burst of viremia and rapid rise in viremia within the first or second year after primary infection reflect a poor control of HIV infection by the immune system. In this regard, a delay in the appearance of the primary immune response or a rapid disappearance of certain immune functions during the early stages of the chronic phase of infection may be detected in rapid progressors.

3. Long-term nonprogressors

A small percentage (less than 5 % on the basis of different cohorts) of HIV-infected individuals do not experience progression of disease for an extended period of time. Long-term nonprogressors by some definitions have CD4+ T-cell counts that are within the normal range and are stable over time; in addition, they generally have low levels of virologic parameters and preservation of lymphoid tissue architecture and immune function. From a clinical standpoint, long-term nonprogressors are asymptomatic; it seems that in these individuals, HIV infection has been arrested with regard to disease progression. It is unknown whether long-term nonprogressors have experienced a primary infection similar to that of other groups of

HIV- infected individuals, i.e. associated with an acute viral syndrome and burst of viremia.

4. Long-term survivors

In a small percentage of subjects who experience progression of HIV disease within a period of time similar to typical progressors, both clinical and laboratory parameters, although abnormal, remain stable for an extended period of time. The mechanisms, either virologic or immunologic, that are responsible for preventing further progression of HIV disease are unclear at present; the possibility of changes in virus genotype and/or phenotype, as well as that of preservation of certain HIV-specific immune are being investigated.

Host responses to HIV infection

Primary HIV-1 infection is characterized by high levels of infectious HIV-1 in plasma and peripheral blood mononuclear cells (PBMCs) during the first few weeks of infection; in one study levels of 1000-10000 tissue-culture-infective doses per millilitre of plasma and 100-10000 infective doses per million PBMC were found. The short and intense period of viral replication is followed in the ensuing weeks by a rapid decline in peripheral blood viral load (at least 100-fold) and concurrent resolution of the acute illness. Viral clearance might be due to the emergence of an effective host mechanism as instance, HIV-specific CTL and HIV-specific T-helper responses.[66,67]

Humoral response

The relation between humoral immune responses to HIV and disease progression remains uncertain. Neutralizing antibodies to gp41 and gp120 may contribute to viral clearance, although a direct correlation between decline in viral load and development of neutralizing antibody has not yet been demonstrated. These antibodies tend to develop after resolution of primary infection, suggesting that they are not the main mechanism of viral control. Neutralizing antibodies directed against the initially infecting quasi-species tend to persist for years, but do not evolve to subsequent variants, a possible factor in disease progression. Antibodies that inhibit

syncytium formation and antibodies that mediate antibody-dependent cellular cytotoxicity (ADCC) against virally infected cells also develop soon after infection. Their *in vivo* significance is unclear. Antibodies develop to all major HIV proteins, although with disease progression, antibodies to p24 tend to decrease in titer.[68]

Cellular response

An appreciable CD8+ lymphocytosis occurs during primary HIV-1 infection, generally beginning in the second week after onset of illness. Unlike the development of neutralizing antibodies, the increase in the number of CD8+cells during primary HIV-1 infection occurs concomitantly with the resolution of clinical symptoms and a decrease in the detectable levels of serum p24 antigen, suggesting that the CD8+ cell response to primary HIV-1 infection has a part in controlling viral replication *in vivo* as it has been shown to have *in vitro*. These CD8+ cells represent HLA-restricted, HIV-specific cytotoxic T cells, and the HIV epitopes are gradually being characterized.[69] Autologous CD8+ cells have been found to inhibit HIV replication *in vitro* by both cell-cell contact and by secretion of cytokines. However, some cytotoxic T lymphocytes (CTLs) are potentially detrimental to the host, as they might recognize and attack an uninfected cell presenting HIV antigens such as gp120 on its surface. Recently, it has been shown that restricted usage of T-cell receptor Vβ genes at the time of primary infection may correlate with a poor outcome as opposed to those subjects who generate a greater response using several Vβ genes. As HIV infection becomes chronic the CD8+ CTL response can become pauciclonal and directed towards a few immunodominant epitopes. Variants of the dominant HIV epitopes expressed by quasi-species within the infected host can specifically antagonize recognition of the parental epitope thwarting the ability of HIV-specific CTL to control the infection.[70,71,72]

Recent research suggests that HIV undergoes rapid replication after the initial infection, and destroys many T cells but that it is met with a vigorous response by the immune system, and the viral load drops after the primary illness. However, over time the immune system often, but not always, fails to keep the virus under control, and HIV gains the upper hand in the struggle, the immune system fails, and full-blown AIDS develops. Norwak and McMichael[73] put forward the suggestion that the HIV

virus continually evolves and in doing so produces such a plethora of new epitopes that the immune system loses its way and fails to keep up with the new targets. However, if the initial immune response to conserved epitopes is strong, the immune defense will not be influenced by the mutation in other epitopes. And the body should control the virus indefinitely. If the response is directed more towards non-conserved epitopes, the HIV levels should rise as there is the emergence of mutants that escape from the immune recognition.

Cytokines and HIV disease

Cytokines have a highly complex network to regulate the immune system. This network is redundant and pleiotropic, and operates in an autocrine and paracrine manner to stimulate or suppress cellular proliferation and differentiation, and to modulate immune function.[74,75] Chronic immune activation induced by HIV infection results in dysregulation of the cytokine network. Alteration of cytokine production contributes to HIV pathogenesis by further stimulating viral replication, suppressing the ability of the immune system to mount a strong antiviral response, and inducing cytokine-mediated cytopathic effects.[76,77,78,79]

On the basis of the effect on virus expression and replication, cytokines may be categorized into three groups: (a). Inducers of virus expression[80,81,82] which include IL-1, IL-2, IL-3, IL-6, IL-12, granulocyte macrophage colony stimulating factor, M-CSF and TNF-α/β; (b). Suppressors of virus expression for instances: IFN-α, IFN-β, and IL-10 [78]; and (c). Bifunctional factors (IFN-γ, IL-4, and TGF-β), which may either induce or suppress of virus replication depend on the experimental system.[83] These cytokines may modulate virus replication in both T cells and macrophages. And *in vivo*, they may contribute to maintaining a constant level of virus expression and replication particularly in lymphoid tissue, during the entire course of HIV infection.

HIV infection is associated with increased expression of proinflammatory cytokines, especially during the later stages of disease. High levels of TNF-α, IL-1β, and IL-6 are secreted by peripheral blood mononuclear cells (PBMCs) and

macrophages from HIV-infected subjects.[9,10,84] These cytokines are also found at elevated levels in the serum, cerebrospinal fluid, and tissue, particularly in lymphoid tissue, a major site of HIV replication throughout the course of disease.[85,86] Proinflammatory cytokines, particularly TNF-α, are considered the most potent HIV-inducing cytokines. Both TNF-α and IL-1β activate the cellular transcription factor nuclear factor (NF) κB, a strong inducer of HIV long terminal repeat (LTR)-mediated transcription. IL-6 alone appears to increase HIV expression primarily by a post-transcription mechanism. High circulating levels of these cytokines may cause some of the clinical manifestations of HIV-1 infection (e.g. fever, chills, myalgia, headache, fatigue, leucopenia, and weight loss). Such early rises in cytokine levels occur before the development of HIV-specific antibodies and before the rise in CD8+ cells, suggesting that it is a first line of defense against HIV-1 infection; the precise source of each of these cytokines is unclear.

Another major disruption in the cytokine pattern observed in HIV disease is a progressive loss in the ability to produce immunoregulatory cytokines, such as IL-2 and IL-12.[12,13,87,88] Both cytokines are critical for effective cell-mediated immune responses, as they stimulate proliferation and lytic activity of cytotoxic T lymphocytes (CTL) and natural killer (NK) cells. These cell-mediated immune effectors represent the primary mechanism whereby most viral infections are cleared. In addition, IL-12 is essential for stimulating the production of T helper (Th) 1-type cytokines, including IL-2 and IFN-γ, which favour the development of cell-mediated immune responses. While it is clear that the Th1 limb of cellular immune responses is impaired during the course of HIV infection,[89,90,91] controversy surrounds the proposed dominance of Th2-like responses (i.e. secretion of IL-4, IL-5, and IL-10) during progression of HIV disease. Clerici et al.[92,93] showed that stimulated PBMC of HIV- infected patients exhibit a preferential Th2 pattern of cytokine secretion with disease progression;[94] however, other investigators have found a skewing of the cytokine secretion pattern of T cells of HIV-infected patients towards a Th0 state rather than towards a Th2 state.[95,96,97] In either case, the finding that HIV replication is more efficient in Th0 compared to Th1 clones highlights the importance of Th1 response in the pathogenesis of HIV disease.

Chemokines and HIV disease

Chemokines belong to a superfamily of mediators with immunoregulatory functions involved in inflammatory process. Virtually every tissue in the body can produce chemokines. The chemokine superfamily is distinguished by shared structural similarities, including the four conserved cysteine residues used to define the C, C-C, C-X-C and C-X_3-C chemokine subgroups. The chemokine and chemokine receptor families have steadily been growing to almost 40 chemokines and at least 15 chemokine receptors have been characterised with many more candidates currently under investigation. C-X-C chemokines or α-chemokines act predominantly on neutrophils, while C-C chemokines or β-chemokines modulate macrophage and T-cell responses. There are at least nine β-chemokine receptors: CCR-1 to CCR-8, CCR10 and four α-chemokine receptors, CXCR-1 to CXCR-4 have been reported.[15,16]

Recent findings suggest that certain chemokines and chemokine receptors may play a role in HIV infection. First, the members of the β-chemokine family, regulated upon activation, normal T-cell expressed and secreted (RANTES), macrophage inflammatory protein-1 alpha and beta (MIP-1α and MIP-1β) were demonstrated to be potent inhibitors of macrophage-tropic (M-tropic) HIV replication *in vitro*.[22,23,24,25,98] Second, the specific chemokine receptors were shown to be efficient coreceptors for HIV entry into target cells. While M-tropic HIV strains utilize primarily the chemokine receptor CCR5 (and, to a lesser extent, CCR3), T-cell line-tropic (T-tropic) isolates that tend to evolve some years after infection acquire the ability to use CXCR-4 (fusin) for viral entry.[17,18,19,99] After the virus binds to CD4 on the target cell, the chemokine receptors interact with the viral envelope protein (*env*) and trigger conformational changes in *env* thought to be necessary for fusion between the viral and cellular membranes. The viral determinants regulating this cellular tropism appear to be associated with the V3 region of the HIV envelope protein.[100,101] Finally, CCR5 expression has been suggested to have clinical relevance. A mutant allele of the CCR gene that contains an internal 32 base pairs deletion resulting in a truncated protein has a major impact on susceptibility to HIV infection and on the rate of disease progression in HIV-infected individuals.[102,103] Homozygosity for the

CCR5 mutation results in near-total protection from HIV-1 infection.[104] Heterozygosity for the CCR5 mutation results in decreased expression of CCR5 on the cell surface and reduced infectability of CD4+ cells with M-tropic isolates of HIV-1 compared with CD4+ cells of CCR5 wild-type individuals.

Numerous cell types produce a variety of chemokines, and modulation of the production of these factors may influence HIV replication in a strain-specific manner.[105] Chemokine production, induced during inflammation, is enhanced by several cytokines, including TNF-α, IL-1β and IL-2. Thus, in HIV-infected subjects during the early stages of disease, the ability of TNF-α to stimulate β-chemokine production and thereby suppress M-tropic entry may override its HIV-inducing effects. However, in individuals harbouring predominantly T-tropic quasi-species during the later stages of HIV disease, only the HIV-inducing activity of TNF-α would be influential. In fact, TNF-α -mediated induction of β-chemokine secretion may actually enhance entry and replication of T-tropic strains of HIV.

The ability of the β-chemokines to inhibit HIV replication likely reflects the competitive inhibition of HIV attachment to the chemokine receptors on target cells. The role of the β-chemokines in HIV-1 disease progression *in vivo* remains unclear. Conflicting data have been obtained regarding a relationship between the level of these chemokines and progression of HIV disease.[106,107,108,109,110,111] Although one study has shown that bulk CD4, but not CD8 T cells from HIV-1-infected asymptomatic subjects produce moderately elevated levels of β-chemokines[112] that might affect HIV-1 replication, other study indicate no correlation between β-chemokine levels and nonprogression.[29,30] However, the levels of β-chemokines in AIDS patients are comparable to and can exceed those levels in nonprogressing individuals, indicating that the overall β-chemokine production may have little effect on HIV-1 disease.

IL-18 (IFN-γ inducing factor, IGIF)

IL-18, originally termed IFN-γ inducing factor (IGIF), with a molecular mass of 18.3 kDa and a pI of 4.9, in 1995 was found to be a novel cytokine of mice

preconditioned with heat-killed *Propionibacterium acnes* (*P. acnes*). A cDNA of the murine and human IGIF was cloned and the recombinant IGIF exhibited pleiotropic immunological activities in addition to the induction of IFN-γ, and thus the conventional name of IL-18 was proposed.[33] IL-18 contains a single open reading frame encoding a 193 amino acid proIL-18. It has been proposed that IL-18 contains 12 strands of β-sheet forming the β-trefoil fold, similar to the IL-1 family of proteins, and the biologically inactive proIL-18 is cleaved by interleukin-1β (IL-1β)-converting enzyme.[113,114]

It has been uncertain whether IL-18 is secreted as a mature active form from any cells, since proIL-18 has no conventional signal peptide. It has been shown that IL-18 was secreted from murine Kupffer cells, macrophages, epidermal cells, dermal keratinocytes, adrenal gland and cytoplasm of intestinal epithelial cells.[115] IL-18 may play a role in the development of Th1-type immune responses[116], especially in the contact sensitivity reaction induced by skin cells, and may also play an important role as a neuroimmunomodulator in the immune system following a stressful experience. In addition to those functions, IL-18 may play an important role in the induction of mucosal immunity from the neonatal period to adulthood.

in vitro IL-18 possesses diverse biological functions, including induction of IFN-γ production by T cells and NK cells, increase in IL-2 production and induction of IL-2Rα-chain expression, reduction of IL-10 production, induction of proliferation of murine T cells, Th1 clones, and human enriched T cells and enhancement of the Fas-Fas ligand-mediated cytotoxic activity of murine NK clones.[117,118] IL-18 has positive effects against HSV-1 and Meth A sarcoma in mice and is elevated in synovial fluid and serum of patients with rheumatoid arthritis.[119] Recently, IL-18 has been found to stimulate HIV production in the chronically infected U1 monocytic cell line.[37]

Treatment of HIV infection

Antiretroviral therapy

Approaches to drug therapy for HIV infection focus on different stages of the viral life cycle and different aspects of the biology of the virus.[120] The ideal

antiretroviral therapy would be active against cell-free virus, against both latent and productive stages of HIV, against all strains of HIV in all body sites including the brain, would be non-toxic, cheap, orally available and have indefinite action. No agent currently available posseses these qualities and thus therapy is not commenced immediately after diagnosis.[121,122]

The currently licensed antiretroviral nucleoside analogues are zidovudine (ZDV or AZT), didanosine (ddI), zalcitabine (ddC), stavudine (d4T), lamivudine (3TC), and abacavir (ABC). Zidovidine monotherapy invariably fails, usually after 6-18 months. Failure is probably a result of mutations in the reverse transcriptase gene resulting in phenotypic resistance, and perhaps also altered drug metabolism and patient tolerance for an active dose. Zidovidine in combination with lamivudine, didanosine or zalcitabine is more effective than monotherapy or alternating monotherapies, leading to more pronounced reduction in circulating viral load and greater and more sustained clinical responses.

Non-nucleoside reverse transcriptase inhibitors (NNRTIs) are agents which bind to the non-active site of reverse transcriptase and are more active *in vitro* than the nucleoside analogues. Currently approved NNRTIs are nevirapine, delaverdine and efavirenz. A combination of a non-nucleoside reverse transcriptase inhibtor with nucleoside analogues such as AZT+3TC or d4T+ddI appears to prevent the early development of resistance.

Four protease inhibitors, saquinavir, ritonavir, indinavir and nelfinavir have been licensed for treatment of HIV infection. Protease inhibitors are an attractive target for therapeutic intervention because they act at a postintegration step of HIV replication. Although they are potent HIV inhibitor resistance does develop *in vivo* particularly when used as monotherapy.

To date, the therapy for HIV infection centers on highly active antiretroviral therapy or HAART, particularly the use of nucleoside analogues and protease inhibitors.[38] However, there are problems with drug treatment of HIV, including resistance, adherence to treatment, and systemic or recurrent local side effects. A new

avenue of research includes the potential combination of highly effective antiviral drugs with immune response based approaches that enhance CD8-derived HIV suppression and cytolysis of CD4 viral factories.

Immunotherapy

Several generalized specific immunomodulators are being evaluated at various stages of HIV disease. In addition to IFN-α, other therapies such as immunoglobulin, HIV-specific immunoglobulin, HIV-specific therapeutic vaccines, recombinant soluble CD4, cytokines such as IL-2, TNF inhibitor such as pentoxyfiline and thalidomide, glutathione, and cellular therapies are all currently undergoing evaluation.

One approach is the administration of an immunomodulator (IL-2) agent aimed at enhancing, or restoring the patient's immune function. IL-2 therapy has been clinically investigated for many years.[123] IL-2 is a 133 amino-acid protein with a molecular weight of 15.5 kDa. It is a potent immunoregulatory cytokine with pleiotropic activity that plays a pivotal role in T lymphocyte activation and proliferation, stimulates the production of lymphokines like TNF-α and IFN-γ, stimulates proliferation and cytotoxicity of NK cells, increases proliferation and secretion of immunoglobulin by B lymphocytes and increases cytomegalovirus-specific cytotoxicity of lymphocytes of patients with AIDS.[40] IL-2 is produced mainly by CD4+ T-helper lymphocytes stimulated by other cytokines such as IL-1 and IL-6, or by immunogenic peptides presented in concert with MHC molecules. The clinical applications of IL-2 have centered on the treatment of cancers because IL-2 can activate lymphocytes to become lymphokine-activated killer (LAK) cells that have a broad range of tumor cell targets.[124] IL-2 has been studied in combination with cellular therapy using tumor-infiltrating lymphocytes (TIL) to eradicate the tumor.[125] Finally, IL-2 has been administered in patients with AIDS related Kaposi's sacroma. IL-2 could restore the ability of the immune system to produce CD4+ T lymphocytes from T-lymphocyte precursors, stimulate an expansion of peripheral CD4+ T lymphocytes, or prolong the half-life of these cells.

IL-2 therapies have been shown to exhibit a significant dichotomy between raising the number of CD4 cells and improving the immune function.[126,127] Previous trials of intraveneous IL-2 plus antiretroviral therapy have generated promising data. These studies have shown that treatment with 6-12 MIU/day of intravenous IL-2 for 5 consecutive days every 8 weeks led to elevated and sustained CD4 cell counts.[40,128] Major limitations of IL-2 therapy administered by continuous intravenous infusion are its inconvenience, the necessary hospitalisation and the frequency of dose-limiting toxicity such as the capillary leak syndrome with fluid retention and significant hypotension, fever, rash and a plethora of subjective complaints including fatigue, myalgias, and gastrointestinal disturbances. In recent dose-finding trials, subcutaneous IL-2 injections were associated with fewer and less severe side effects, when given every 8 weeks. Hengge UR et al.[129] showed similar data to previous trials but with increased delayed-type hypersentivity reactivity with therapy, suggesting an overall improvement in immune function. A combination of IL-2 and antiretroviral therapy resulted in a larger and more persistent increase in CD4 count than antiretroviral therapy alone. De Paoli and colleagues[45] found that IL-2 can induce the reconstitution of CD4/CD45RA (naive) lymphocyte subset and recover the ability of these cells to produce *in vitro* IL-2, IL-4 and IFN-γ *in vitro*. No data on the effects of IL-2 administration on the cytokine and chemokine mRNA production *in vivo* is available. In particular, it is not known if or how the IL-2, IL-18, RANTES and MIP-1α mRNA productions are modified during IL-2 therapy.