Chapter II

Literature Reviews

Cytokines are polypeptide factors produced transiently or constitutively by a range of cell types, acting usually locally, to alter the physiology of target cells by binding to cell surface receptors and activating the expression of specific genes. It is characteristic of cytokines that they are often redundant in their effects (i.e., different cytokines can have the same effect). It is also characteristic that they are often pleiotropic in their actions (i.e., they have different effects on different cells). Presumably, since there is no evidence that different cell types have different receptors for individual cytokines, the multiplicity of actions is due to the activation of different subsets of genes in different cells, as a consequence of different signaling pathways downstream of the receptor. Different cytokines may interact, either synergistically or antagonistically, or merely in an additive manner (22).

Host cytokines play an important role in HIV type 1 (HIV-1) infection by promoting a long-lasting T helper type 1 (Th1) lymphocyte response to viral antigens. A vigorous Th1 response against HIV-1 results in the generation of HIV-1 targeted cytotoxic T lymphocytes (23,24). The Th1-type cytokines, interleukin (IL)-2, IL-12 and interferon (IFN) -$_{\gamma}$, are important for the development of cytotoxic T lymphocytes. IL-2 and IL-12 productions are reduced and simultaneously Th2 cytokine IL-4 gene expression does not increase in PBMC of HIV infected individuals regardless of the stage of disease (8,25,26). Interestingly, the important Th1 cytokine IFN-$_{\gamma}$ and Th2 cytokine IL-10 remain high throughout the course of infection in HIV infected individuals (27). Dysregulation of cytokine synthesis in HIV-associated disease extends to the proinflammatory cytokines such as TNF-$_{\alpha}$, IL-1$_{\beta}$ and IL-6 which had been elevated already during asymptomatic HIV infection (4,28,29). These proinflammatory cytokines, as well as IL-18 can up-regulate HIV production *in vitro* (6,13). However, IL-18 a recently cloned cytokine associated with Th1 and inflammatory processes, has not been studied in HIV infected subjects since it is involved in specific and nonspecific cell mediated immunity.

The chemokine superfamily, a new family of cytokines, is composed of small (8-10 kDa) structurally related polypeptides that play important roles in inflammatory and immunological responses by recruiting

specific subsets of leukocytes. Chemokines are produced by a variety of cell types, most notably by activated monocytes/macrophages and endothelial cells (30). Chemokines are also presumed to be involved in constitutive lymphocyte recirculation and homing (31). On the basis of the arrangement of the NH_2-terminal in two of the four conserved cystein residues, they are divided into two major groups, the CXC and CC subfamilies. One amino acid separates the two cysteines in the CXC chemokines, whereas the two cysteines are adjacent in the CC chemokines. Most CXC chemokines are potent chemoattractants for neutrophils, whereas most CC chemokines attract monocytes. The human genes for CXC chemokines are clustered on chromosome 4q12-q13, whereas those of CC chemokines are on chromosome 17q 11.2 (30). As indicated by the rapidly expanding literature, chemokines are increasingly studied because of their actions on leukocytes and their role in inflammation and immunity. Additional interest is arising from the recent discovery of a function of chemokines and chemokine receptors in HIV infection. Specific chemokine receptors play a pivotal role as co-receptors for HIV entry into target cells and specific chemokines can suppress infection by some strains of HIV-1. CCR-5, CCR-2b and CCR-3 act as coreceptors for monocytropic strains of HIV-1, while CXCR-4 acts as a coreceptor for T-tropic HIV strains (30).

The IL-18 discovery

In previous studies, Ushio *et al.* reported that mice injected with heat-killed *Propionibacterium acnes* secreted significantly higher levels of IFN- γ into the circulation upon challenge with T cell mitogens, whereas mice challenged with T cell mitogens alone did not. This IFN- γ production was also induced both *in vivo* and *in vitro*, by using lipopolysaccharide (LPS) as a mitogen instead of the T cell mitogens (12). Since it is unlikely that LPS stimulates the T cells directly, the possibility of some intermediate molecules to be involved was condisered.

After intravenous injection of LPS to induce toxic shock, an intermediate molecule was indeed identified from murine liver extract previously intraperitoneally injected with heat-killed *P. acnes* (9). The molecule in the mouse liver extracts was purified to homogeneity and was found to be an 18.3 kDa protein with a pI of 4.9, termed IFN- γ - inducing facter (IGIF). The amino acid sequence of the NH_2-

terminal portion of this molecule was determined and shown not to be similar to any protein in the EMBL, GeneBank, and PIR data bases, and subsequently called IL-18 (9).

Molecular Characterization of IL-18

On the basis of partial amino acid sequences deduced from the purified proteins, the cDNA of mouse IL-18 (IGIF) was cloned. The gene encodes a 192-amino-acid biologically inactive precursor polypeptide (proIL-18) with a 35-amino-acid nonconventional signal sequence. The mature IL-18 contains 157 amino acids without N–glycosylation sites (10,32). A cDNA of human IL-18 cloned by cross-hybridization from human liver cDNA libraries was found to contain a single open reading frame encoding a 193-amino-acid proIL-18 (12). The human proIL-18 was found to have 65% homology with its mouse counterpart. It has not been clear whether IL-18 is secreted in its mature active form from any cells, since proIL-18 has no conventional signal peptide. Gu *et al.* showed that approximately 10% of the mature IL-18 was exported from COS cells transfected with an expression plasmid for proIL-18 along with a second one encoding wild-type IL-1β converting enzyme (ICE), whereas <1% of proIL-18 was exported (33). IL-18 and IL-1 show similarities as to their structure and processing enzyme. However, their biological functions, target specificities and receptor systems are completely different from one another (32).

IL-18 and IL-12 in synergy and differences

IL-18 is a factor that induces IFN-γ production from splenocytes, liver lymphocytes and type 1 T helper (Th1) cell clones. In addition, IL-18 enhances natural killer (NK) cell activity and proliferation of activated T cells (9,11,12). Thus, IL-18 apparently shares its biological functions with IL-12, which is known to have immunoregulatory activities, including the induction of IFN-γ production, the enhancement of NK cell cytoxicity, and the enhancement of activated T cell proliferation (34). Moreover, both IL 18 and IL 12 are secreted by activated macrophages as the liver of *P. acnes* treated mice after LPS challenge was shown to express IL-18 and IL-12 mRNA as opposed to untreated mice which do not express either cytokine (35). Unlike IL-12, IL-18 is expressed in cells other than those of the immune system. It is expressed by keratinocytes, osteoblasts cells, pituitary gland and adrenal cortical cells, and

intestinal epithelial cells (36). Therefore, the roles of IL-18 seem not to be restricted to induction of IFN-γ production. Although IL-18 and IL-12 have a synergistic effect on IFN-γ production by T cells and NK cells, their signal transduction cascades are independently activated through their receptors and transcription factors. IL-12 induces IFN-γ production by the target cells via activation of Stat3 and Stat4 of the signal transducer and activator of transcription (STAT) family proteins whereas IL-18 induces IFN-γ production via the well known transcription factor NF-κB (32,36).

IL-18, unlike IL-12, does not drive the development of Th1 cells by itself but it serves as a strong co-stimulatory factor potentially activating IL-12 driven Th1 development (37). Furthermore, IL-18 but not IL-12, significantly enhances IL-2 production and IL-2R α-chain expression on antigen or anti-CD3 stimulated murine Th1 clones and enriched human T cells (11,32,38).

Recently, analysis of the mechanism underlying the synergistic effect of IL-12 and IL-18 on IFN-γ induction has shown that T cells stimulated with IL-12 exhibit increased expression of the IL-18 receptor (IL-18R). The IL-18 receptor has recently been proven to be an IL-1 receptor related protein (IL-1Rrp) but not of the type 1 IL-1 receptor (IL-1RI) (39). Since expression of mRNA for the type 1 IL-1R is not upregulated in IL-12-stimulated Th1 cells despite high levels of IL-18 mRNA. This observation furthered our interpretation of the differential responsiveness of Th1 and Th2 cells to IL-18 and IL-1, both of which activate NF-κB. Thus, the presence of IL-18R may be important in distinguishing Th1 cells from Th2 cells which express IL-1RI but not IL-18R (37).

Just as T cells require co-stimulation with IL-12 to respond to IL-18 by significantly increasing IFN-γ production, B cells also require stimulation with IL-12 to become responsive to IL-18. To elucidate the mechanism by which IL-12 and IL-18 enhance IFN-γ production by B cells, B cells stimulated with IL-12 for 3 days were examined for their response to IL-18. These B cells responded to IL-18 with a dramatic increase in proliferation and production of IFN-γ suggesting that IL-12 also renders B cells responsive to IL-18 by induction of IL-18R (36). The present study has shown that a combination of IL-12 and IL-18 induces highly purified murine B cells activated with anti-CD40 antibody to produce IFN-γ which inhibits IL-4-dependent IgE and IgG1 production and enhances IgG2a production without inhibiting the B-cell proliferative response (40).

As mentioned above, IL-12 and IL-18 synergize in inducing IFN-γ production by T cells via induction of IL-18R on T cells. In contrast, IL-18 alone augments NK activity and IFN-γ production in splenocytes of mice deficient in IL-12 (41). These results indicate that IL-18 acts on NK cells independently of IL-12. However, the cooperative action of IL-12 and IL-18 is indispensable for the *in vivo* activation of NK cells because IL-12$^{-/-}$IL-18$^{-/-}$mice display marked reduction in NK activity and severely impaired Th1 cell development (42).

Role of IL-18 and IL-12 in pathogenesis

Administration of anti-IL-18 antibodies prevents liver damage in mice inoculated with *P. acnes* and challenged with LPS, which induce toxic shock and liver injury (10). In addition, a severely impaired Th1 response was shown in *P. acnes*-injected IL-18$^{-/-}$mice, whereas a weak Th1 response was still observed in BCG-injected IL-18$^{-/-}$mice (42). These results reveal that IL-18 has an important role as to inflammatory processes and the Th1 profile. Moreover, the defensive effect against infectious microorganisms was examined. It has recently been shown that murine peritoneal exudate cells stimulated with a mixture of IL-12 and IL-18 acquire antifungal activity against *Cryptococcus neoformans* through production of IFN-γ by NK cells and the production of nitric oxide (NO) by macrophages (43). A single administration of IL-12 or IL-18 alone had no significant effect on a lethal infection with the sporozoite of *Plasmodium berghei*, the most virulent pathogen in mice (36). These results indicate that IL-12 and IL-18 are also synergistic *in vivo* as the combined administration of these cytokines, in a dose not exceeding the toxic dose, protects the mice from the infection or extends their survival time.

Other IL-18 related proinflammatory cytokines

Previous descriptions of the biological activities of IL-18 have relied on the presence of a co-stimulus to induce cytokines, and in particular, the induction of IFN-γ and other events promoting Th1 responses. In contrast, the IL-18 signal alone is sufficient for TNF-α synthesis because the property of IL-18 is consistent with its ability to activate the translocation of NF-κB in T cells. Mature IL-18 also induces IL-8, MIP-1α and IL-1β in human PBMC in the absence of any co-stimuli. Puren *et al.* concluded that

IL-18 possesses proinflammatory properties exerted by direct stimulation of gene expression and synthesis of TNF-α from CD3+/CD4+ and natural killer cells with subsequent production of IL-1β and IL-8 from the CD14+ population (44).

Monocyte/Macrophages - tissue factor/cytokines dysregulation in HIV infection

Mononuclear phagocytes are key effectors of the inflammatory component of the cellular immune response, and through cytokine production, interact with all phases of the immune system (45). Inflammatory activity in the cellular immune response includes monocyte activation of protease systems. The coagulation protease pathway, initiated by the expression of the transmembrane receptor, tissue factor (TF)(46), contributes to the delayed-type hypersensitivity (DTH) response through its generation of thrombin and fibrin (5). A depressed TF gene expression in LPS - stimulated HIV patient monocytes correlated with the decreasing TF and DTH responses is associated with disease progression (47). Its selective reduction may be a factor in the diminished resistance to secondary infections observed in AIDS.

In response to an inflammatory stimulus, such as an invading pathogen, macrophages(Mϕ) secrete proinflammatory cytokines, which include interleukin 1 (IL-1), interleukin 6 (IL-6) and tumor necrosis factor α (TNF-α). During a normal immune response, these cytokines play a part in coordinating the activities of T and B cells, as well as in directing cytotoxicity (45). Overexpression of these molecules can lead to fever, cachexia, and non-specific cellular activation (48). Lathey *et al.* have shown unstimulated PBMC to exhibit a significantly higher production of TNF-α and IL-1β transcripts in asymptomatic as compared with symptomatic patients (4,28). In contrast to IL-6, which can be detected at equal levels during all stages of disease (28). Since TNF has been implicated in the pathogenesis of AIDS cachexia, the activation of latent HIV infection and the manifestations of organ toxicity in HIV-infected patients, several groups have identified increased TNF production as a prognostic marker for disease severity (7,49,50). However, TNF-α produced by mononuclear phagocytes (MP), PBMC and T cells also has an inhibitory effect on HIV replication preceeding proviral DNA integration (51). Like TNF-α, other proinflammatory cytokines, including IL-1α, IL-1β and IL-6 have been shown to activate HIV replication or expression in the U1 cell line, monocyte-derived macrophages (MDM) and IL-2-stimulated PBMC (52). Furthermore, IFN-γ-inducing factors IL-12 and IL-18 have been demonstrated to increase HIV-1

production in PBMC and the U1 cell line, respectively (13,53). In addition to viral induction, *S. aureus* antigen-stimulated PBMC of HIV-1 infected individuals have impaired IL-12 p40 and p70 production (8). Neither IL-12 p35 mRNA nor IL-12 p40 mRNA are increased in lymph nodes with follicular hyperplasia (FHLN) of HIV infected individuals regardless of the stage of disease (25).

Th1 and Th2 cytokines in HIV infection

In 1993 Shearer and Clerici proposed that T cell dysfunction in HIV-1 infection was associated with a change in cytokines secreted by Th cells from a Th1 to a Th2 phenotype (54). In 1987 Maggi *et al.* reported a reduced number of T cell clones producing IL-2 and IFN-γ in AIDS patients (55). Recently, the same group generated a large panel of CD4+ T cell clones specific for PPD or *T. gondii* and observed a significant increase in the production of Th2 cytokines by clones generated from HIV-1 infected individuals, with an overall increased percentage of Th0 type clones (56). Consistent with this, T cell clones generated by random cloning procedures from CD4+ memory cells of asymptomatic HIV-1 infected individuals were more likely to be of the Th0 type, mainly due to increased IL-4 production, as were cells of an individual after HIV-1 infection compared to when uninfected (57). In general, the observed changes are relatively moderate (56,57). In addition to the increased percentage of Th0/Th2 CD4+ T cell clones, CD8+ T cell lines and clones have been generated from asymptomatic HIV-1 infected individuals that can provide helper activity for IgE synthesis (58) and show decreased cytolytic activity (59). These CD8+ T cells also have Th2-like cytokine secretion patterns (58,59).

In addition to analyses of T cell lines and T cell clones, studies on cytokine production after stimulation of PBMC *in vitro* have been published. Decreased IL-2 production by T cells of HIV-1 infected individuals *in vitro* has been extensively documented (26,27,60,61,62). However, with respect to other Th1 and Th2 cytokines, conflicting results have been reported. Clerici *et al.* showed decreased IL-2 production induced by recall antigen whereas increased mitogen-induced IL-4 production by PBMC of HIV-1 infected individuals in the asymptomatic phase depended on HIV progressive markers characterized by the *in vitro* antigen-stimulated T cell response of their PBMC (60). Meroni *et al.* observed that the early phase of HIV infection is characterized by reduced mitogen stimulated IL-2 and increased mitogen-stimulated IL-4 and IL-10 production (63). The production of both Th1 (IL-2 and

IFN-γ) and Th2 (IL-4) cytokines was found reduced in PHA-stimulated PBMC (26,27), whereas some reports showed unchanged IL-4 mRNA levels in unstimulated PBMC and lymph nodes (25,27). In contrast to some authors reporting reduction of IFN-γ production in HIV infected individuals, Fan *et al.* showed that IL-2 gene expression decreases and IFN-γ gene expression is elevated in unstimulated PBMC of HIV seropositive homosexual men compared with HIV seronegative homosexuals (61) and Klein *et al.* showed the same results, especially in the advanced stage of disease on CD4+T cells and CD8+ T cells, respectively, in PMA-ionomycin-stimulated PBMC of HIV infected individuals using cytoplasmic cytokine detection on a single cell level by FACS (62). Furthermore, the same group demonstrated that activated PBMC of HIV-1 positive, asymptomatic individuals showed an increased proportion of IL-4 and IL-10 producing cells among the CD4+ T cells in contrast to IL-2 and IFN-γ producing cells within the same cell population (62). These type 2 cytokines (IL-4 and IL-10) either had no effect whatsoever or enhanced *in vitro* T-cell programmed cell death (apoptosis) (64).

The chemokine receptors and HIV entries

The principle cell types targeted by HIV-1 *in vivo* are helper T lymphocytes and cells of the monocyte-macrophage lineage via the CD4 receptor pathway, the primary high-affinity receptor for HIV-1. Although the expression of CD4 is a major factor defining the tropism of HIV for these target cells, it had long been recognized that human CD4 alone is not sufficient to render most non-human cell lines (and even some human cell types) permissive for HIV-1 fusion and entry (65). This suggested that an accessory factor(s) present in most human cells was needed for fusion. The most recent discovery revealed that expression of the β-chemokine receptor CCR-5 in CD4+, non-permissive human and non-human cells rendered them susceptible to infection by non-syncytium-inducing HIV strains (NSI) or macrophage - tropic strains (M-tropic), and allowed *env*-mediated membrane fusion, thereby identifying CCR-5 as a second receptor for NSI primary virus (14,15). The LESTR (leukocyte-expressed seven-transmembrane-domain receptor) or α-chemokine receptor CXCR-4 (fusin) is another coreceptor for syncytium- inducing HIV strains (SI) or T-cell line adapted (T-tropic) HIV strains (66).

CCR-5 and CXCR-4 are seven-transmembrane G-protein-coupled receptors, serving as the receptors for the CC chemokines (β-chemokines) RANTES, MIP-1α and MIP-1β and for CXC

chemokine (α-chemokine) stromal cell - derived factor -1 (SDF-1), respectively (14,15,66). Apart from their lymphocyte trafficking functions, these chemokines can suppress HIV infection into the primary CD4 T cells at the viral entry level depending on the HIV phenotypic strains (14,15,66).

Chemokines and HIV infection

In order to study how the β-chemokines MIP-1α, MIP-1β and RANTES inhibit HIV-1 replication, some researchers have used a virus entry assay based on single-cycle infection by an *env*-deficient virus, NL4/3 *Δ env*, which also carries the luciferase reporter gene, complemented by envelope glycoproteins expressed *in trans* (HIV-luciferase pseudotyped with macrophage-tropic Env) (14,15,67). Some investigators showed the β-chemokines inhibiting HIV-1 replication by using a cocktail of anti-β-chemokine antibodies (17, 21). The capacity of the CXC chemokine SDF-1 to prevent infection by T-cell line adapted HIV-1 was tested first on HeLa cells transfected with CD4 and carrying an integrated HIV-1 long terminal repeat (LTR) - driven reporter gene, *lac Z* of *E. coli* inducible by the HIV-1 encoded transactivating protein Tat of HIV-1 pseudotyped with T-tropic Env or T-cell line adapted HIV-1 after infection (66). From all of these results, it was concluded that HIV-1 replication is associated with the chemokines and the chemokine receptors at the viral entry level. Experiments using individual recombinant β-chemokines also indicated that variations in sensitivity can be observed with CD4+ cells naturally infected with NSI viruses. In all cases, RANTES was the most potent inhibitor of HIV replication, followed by MIP-1β and MIP-1α (21). In contrast, these chemokines were shown inefficient in blocking infection of primary macrophage cultures by CCR-5-tropic HIV-1 (68).

The roles of RANTES, MIP-1α and MIP-1β in HIV disease progression have just been elucidated (20,21,69,70). At all stages of disease, PHA - stimulated PBMC of HIV-1 infected individuals showed more incresed RANTES and MIP-1α production than those of HIV seronegative individuals. In contrast, no significant difference was observed between HIV progressive patients and asymptomatic individuals regarding maximum production of these β-chemokines by activated peripheral blood cells, (21) but PHA-driven production of both RANTES and MIP-1β was significantly higher in long-term nonprogressors (LTNP) than in rapid and late progressors (20). The elevated β-chemokine production in AIDS patients would suggest that they are not preventing disease progression either by their ability to

competitively inhibit HIV infection via its coreceptor on CD4+ cells, or by a protective mechanism based on their chemotactic activities. The latter activities presumably regulate immune cell trafficking and inflammation. However, the low levels of endogenously produced RANTES, MIP-1α and MIP-1β (<10 ng/ml, each) can autoregulate endogenous NSI HIV production during the early phase of naturally infected CD4+ T cells cultured from an asymptomatic HIV infected individual but these chemokines did not effect HIV production during more advanced phase of culture (21). In summary, several studies have provided evidence that production of the CC chemokines RANTES, MIP-1α and MIP-1β in HIV disease is not significantly associated with disease progression in contrast to virus load and CD4 counts (21,69). However, CD4+ T-cell clones of nonprogressing individuals secrete high levels of these chemokines whereas CD4+ T-cell clones of AIDS patients produce no RANTES and little or no MIP-1α and MIP-1β (70). These findings indicate that endogenous production of β-chemokines by CD4+ T cell clones correlates with the clinical state of HIV-1 infected individuals (70). Furthermore, the increased β-chemokines RANTES, MIP-1α and MIP-1β expression also correlate with IFN-γ gene expression in the same lymphoid tissue (FHLN) of HIV positive patients regardless of the stage of disease (25). In addition, these results were similar to the correlation between the response to these β-chemokines and Th1 cytokines *in vitro,* especially, the direct correlation between MIP-1α and IFN-γ (71).

Monocyte/Macrophages and HIV pathogenesis and cellular tropism

The asymptomatic phase of HIV-1 infection is characterized by low frequencies of infected cells in peripheral blood(72), and the predominant HIV-1 variants replicate both in primary T cells and macrophages and are non-syncytium-inducing (NSI) (73). HIV-1-infected macrophages in tissue compartments are believed to be the viral reservoir because in the asymptomatic phase of infection, peripheral blood T cells harbor macrophage-tropic viruses compatible with recent infection of these T cells by HIV-1 derived from macrophages (73,74). In the course of an infection, a gradual shift in the composition of virus populations towards T-cell tropic HIV-1 variants is observed (73). In 50-60% of infected individuals, this shift is accompanied by the emergence of SI variants (75). These SI variants in general are absent during the asymptomatic phase of infection. Macrophages are the main initial target cells for the virus (76), and during the first phase of infection only NSI variants are present. Newborn babies exposed to mixed populations of SI and NSI HIV-1 may be more likely to be infected initially with

NSI viruses, suggesting that NSI variants have a selective advantage in primary infection (77). The period of time required to generate specific mutations is correlated with the SI phenotype (78).

Recent observations have provided evidence that the PBMC of some highly exposed, uninfected subjects are resistant to infection with prototypical M-tropic viruses, but are readily infectable with T-tropic viruses because they show a mutation comprising a homozygous 32-bp deletion within the gene encoding the M- but not the T-tropic receptor CCR-5. This allele was found to be common in Caucasian populations at a frequency of approximately 1%, but was not found in people of African or Asian ancestry (79). This serves as compelling evidence that a mutation in CCR-5 renders individuals apparently resistant to infection with HIV-1 and hence implies that entry of M-tropic strains into susceptible target cells is critically important for establishing the infection. These results also correlate with earlier findings that only M-tropic strains could be cultured from individuals shortly after acquisition of infection (80). The evidence also correlates with the recovery of HIV from macrophages documented during early stages of infection in which virus isolation from T cells is unsuccessful and detectable levels of antibodies against HIV are absent (3).

The progression to acquired immunodeficiency syndrome (AIDS) is associated with a shift from an M-tropic to a T-tropic viral phenotype (73,78). Although the selective pressure driving this phenotypic transition is not well defined, CD3/CD28-stimulated CD4+ cells may exert selective pressure in favor of T-tropic isolate production through the combination of high levels of β-chemokine production and lack of CCR-5 expression (81). IL-4 could promote the shift towards T-tropic HIV variants *in vitro* by increasing CXCR-4 and decreasing CCR-5 expression in primary CD4+T cells (82). Moreover, several CC chemokines, including those inhibiting entry and replication of M-tropic HIV strains, increase the replication of T-tropic HIV strains in CD4+ T cells by increasing cell surface colocalization of CD4 and the T-tropic HIV coreceptor CXCR-4 (83). This selective HIV phenotypic transition is associated with increased IL-4 production during the asymptomatic phase of infection (60,63), and also with the increased gene expression of MIP-1α and MIP-1β in brain tissue from patients with AIDS dementia (18). The cells expressing chemokines in HIV-1-infected brains were identified morphologically as microglia and astrocytes(18). These results also correlate with *in vitro* studies concluding that MIP-1α and MIP-1β are induced by HIV infection of monocyte-derived macrophages(MDM) (18,19). In addition to the cytokine/chemokines and the CD4 T cell activation favoring the suppression of HIV phenotypic shift to

T-tropic or SI viruses, common opportunistic pathogens in HIV disease such as *Mycobacterium avium* complex and *Pneumocystis carinii*, can activate HIV viral replication in tissue macrophages (84,85). This investigation concludes that macrophages are a repository of HIV during opportunistic infections. Finally, primary T - tropic HIV isolates can use both CXCR-4 and CCR-5 CD4 positive target cells for infection (86), and some investigators have shown that primary human macrophages can support T-cell line adapted virus replication under monocyte isolation and culture conditions (87). These findings support the potential of most SI isolates to contain a mixed population of SI and NSI quasi-species (86), and of human macrophages to serve as a second target cells for SI viruses depending on their activation state.