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Is AIDS a TNF disease?

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Introduction

HIV, the etiologic agent of AIDS, belongs to the lentivirus subfamily of retroviruses. In striking contrast to oncoviruses, viruses of the lentiviral group, which also include the visna virus of sheep, caprine arthritis encephalitis virus, and equine encephalitis virus, do not transform cells but instead cause chronic progressive diseases [1]. Upon infection with viruses of this group, active viral replication commonly leads to the development of clinical symptoms *in vivo*. This feature is demonstrable *in vitro* by dramatic cytopathic effects upon virus/cell coculture. Since virus-producing cells are generally destroyed, this type of virus must be transmitted to other cells after replication in order to maintain the infection. However, it is well known that, after viral infection, such diseases require a long period of time before becoming full-blown. The virus appears to establish latent infection and stay dormant in many cells until activation signals have been received. In this regard, human AIDS is by no means exceptional.

AIDS is characterized by profound immunodeficiency. The hallmark of AIDS is a decreased number of CD4 + lymphocytes, which probably result from the high affinity of HIV envelope protein for the CD4 molecule and the subsequent selective killing of these cells. The number of cells infected with HIV is relatively small (≤ 1 in 100-1000), even in the peripheral blood mononuclear cells (PBMC) of AIDS patients [2-4]. Although several mechanisms for the selective depletion of CD4 + T-lymphocytes by HIV have been proposed [5], it is uncertain how any of them sufficiently explain the drastic depletion of CD4+ lymphocytes observed. The involvement of other cofactors is therefore being investigated.

While CD4⁺ T-lymphocytes are preferentially infected [6,7], these cells are not the exclusive targets of HIV infection. Recent evidence has shown that the spectrum of HIV target cells may be quite broad [8-10]: clear differences have been observed in the outcome of HIV infection in monocytes/macrophages versus T-lymphocytes. While the T-lymphocytes tend to be destroyed, monocytes/macrophages permit a persistent infection [11]. HIV can therefore be harbored as reservoirs by monocytes/macrophages as well as other cells in the body. The monocyte/macrophage type of response to HIV infection could be responsible for the establishment of latency in the host; this response may also cause pathogenic sequelae resulting from soluble factors produced by the infected cells.

In this review, we aim to highlight the influence of cytokines produced by the host immune system during HIV infection. We will focus on the effects of tumor necrosis factor (TNF) α and β , which have recently been studied extensively and which we believe are crucial factors in the development of AIDS [12]. To avoid complexity, we will use the name HIV for HIV-1 unless specifically indicated.

TNF- α and TNF- β enhance HIV replication

Human T-cell lines infected with HTLV-I are highly susceptible to HIV infection [13]. These cells, especially MT-2 and MT-4, demonstrate dramatic cytopathic effects in association with enhanced replication of HIV [14]. In addition, HIV-infected cells are susceptible to damage by the supernatant of MT-2 cells [15]. This cytotoxic effect has been observed in the fraction lacking HTLV-I viral particles and present after 56°C

heat-treatment. The result indicated that an MT-2-produced heat stable factor(s) was involved. The observed preferential cytotoxicity against HIV-infected cells suggested that this factor could become a potent, clinical anti-HIV agent. However, 2 days after treatment with this supernatant, the viral titer was almost the same as the control culture, even though 70% of treated cells were dead at this point. Assaying the viral titer 6h after treatment with supernatant revealed that the factor(s) produced by MT-2 enhanced the replication of HIV. The factor was finally identified as lymphotoxin (TNF- β), since these activities were neutralized by addition of monoclonal antibody against TNF- β to the supernatant [15]. This finding is consistent with reports that MT-2 cell produce TNF- β [16-18]. The same effect was also observed using TNF- α , a cytokine initially described as a tumoricidal agent of HIV-infected cells. TNF- α and TNF- β selectively killed HIV-infected cells and enhanced the replication of HIV [19,20]. These effects were seen on HIV-infected T-cell lines such as Molt-4, H9, and CEM.

Until 1987, it was widely believed that, in contrast to interleukin-1 (IL-1), TNF- α did not stimulate lymphocytes [21]; however, after treatment with TNF, a marked increase of HIV messenger (m) RNA was observed associated with an increase in HIV particles. Continuous, but not transient, treatment with TNF was necessary for optimal activation of virus production. Although viral expression is strikingly enhanced, accelerated viral replication appears to be the sole effect of the TNF, since the final titers of HIV assayed by 50% tissue culture infective dose (TCID₅₀) are the same irrespective of TNF treatment during the assay [22].

HIV-infected T-cell lines and freshly isolated PBMC from HIV-infected individuals responded to TNF resulting in elevated levels of HIV [23]. This suggests that the same enhancement of HIV expression by TNF is likely to occur *in vivo*. Other investigators have also shown that monokine-enriched supernatant from lipopolysaccharide (LPS)-stimulated monocytes increased the expression of HIV in ACH-2 cells, a subline of the CEM cell line chronically infected by HIV [24,25]. The enhancing activity of the factor was neutralized by antibody against TNF- α ; further experiments showed that TNF- α itself substantially stimulated expression of HIV in ACH-2 cells. Vyakarnam *et al.* [26] demonstrated an enhancement of HIV replication of up to 10⁴-fold in CD4⁺ cells and PBMC treated with TNF- α and TNF- β . HIV release was also enhanced by approximately 100- to 200-fold above the level in control cultures, compared with Molt-4 cells acutely infected with HIV soon after TNF- α treatment [22]. Although it has been reported that TNF- α does not affect HIV production in U1 cells [33], in subline of HIV-infected U937 cells, the enhancement of HIV replication by TNF- α was indeed seen in the U937 cell line, including U1 cells and primary macrophages [20,27].

The enhancement of HIV replication by TNF is linked to the induction of NF- κ B, a transcriptional factor expressed in immature and mature B-cells and in non-B-cells stimulated with phorbol ester (discussed below) [28]. The mechanism by which TNF selectively kill HIV-infected cells remains, however, to be clarified. One possible mechanism is that the increased replication of the virus perturbs the intracellular environment such that cell death follows. Alternatively, HIV infection may render cells susceptible to TNF by depleting an ill-defined cellular factor which has been proposed to be important in counteracting TNF-induced cytotoxicity [29]. Staurosporine, a

protein kinase-C(PK-C) inhibitor, augments TNF- α -induced cytotoxicity on HIV-infected cells but has no effect on the enhancement of HIV replication by TNF- α . This indicates that the former is PK-C-related but that the latter is independent of PK-C activity [30].

Other cytokines and HIV

The effects of several other cytokines on HIV expression have been studied in chronically HIV-infected cell lines of either the monocyte/macrophage or helper T-lymphocyte lineage. The effect of these cytokines on HIV-infected T-cells is significantly weaker than TNF; some cytokines only affect monocytes/macrophages in combination with TNF- α treatment (see Table 1).

Granulocyte/macrophage colony-stimulating factor

The granulocyte/macrophage colony-stimulating factor (GM-CSF) [31] was first reported by Hammer *et al.* [32] as having anti-HIV activity on the U937 promonocytic cell line. Using 30-300 μ /ml GM-CSF on U937 cells chronically infected with HIV, they showed a maximum reduction in reverse transcriptase activity of 37-55% between 10 and 14 days after treatment. However, Folks *et al.* [33] demonstrated that GM-CSF stimulated HIV production in U1 cells two fold after 48 h incubation; no additive or synergistic effect was found when GM-CSF was used in conjunction with TNF- α . This group has, however, recently reported that GM-CSF and TNF- α do indeed synergize in the induction of HIV expression in the U1 cells [34]. The discrepancy between the

results of Hammer *et al.* [32] and those of Folks *et al.* [33] may arise from the time period studied or variation between the cell lines used. As the latter group have mentioned, U1 cells may represent a clone atypical to infected monocytes in general [33]. More convincing evidence has been provided using primary macrophages and the JR-FL strain of HIV which is able to infect primary macrophages [35]. GM-CSF significantly stimulated the production of HIV p24 antigen in the culture medium of infected macrophages. Since GM-CSF is a factor that induces the growth of primary macrophages, the cytokine could appear to stimulate replication of HIV due to a host-cell growth effect. However, this interpretation is not probable since the enhancement factor for the virus replication far exceeded that of the growth of the cells. This cytokine appears to require a site in the HIV long terminal repeat (LTR) similar to, but distinct from, the NF- κ B binding site for the induction of enhanced replication in macrophages (Y. Koyanagi, personal communication, 1991). Furthermore, Poli *et al.* [36] recently demonstrated that HIV expression with GM-CSF was not associated with a significant transcriptional activation of the virus, in contrast to that with TNF- α .

Macrophage colony-stimulating factor

Koyanagi *et al.* [35] have reported that 500-1000 pM macrophage colony-stimulating factor (M-CSF) [37] enhances the production of HIV p24 antigen in the acute infection of primary macrophages. Since this cytokine induces the production of IL-1, it is possible that IL-1 is also involved in M-CSF induction of HIV (see below).

Interleukin-1

Folks *et al.* [33] have previously reported that IL-1 [38] did not augment HIV replication in U1 cells. Osborn *et al.* [39] showed that, although IL-1 failed to augment HIV LTR-directed chloramphenicol acetyltransferase (CAT) activity in a human T-cell line (Jurkat), it did augment HIV LTR-directed CAT activity in a mouse T-cell line. IL-1 has also recently been shown to augment HIV LTR-directed CAT activity in a PK-C-independent manner in the human T-cell line Molt-4 infected with HIV [40]. However, this augmentation was significantly weaker than that observed by TNF stimulation. The activity of IL-1 is also dependent on the NF- κ B site in the HIV LTR so that, like TNF, the augmentory effect of IL-1 is blocked by mutations within this region of the LTR [39].

Interleukin-2

Interleukin-2 (IL-2) [41] does not appear to have any effect on U1, ACH-2, or Molt-4 cells infected by HIV [25,33,40]. However, this factor is indispensable for peripheral blood T-lymphocyte proliferation and consequent increase in the number of cells harboring the HIV provirus. It is not clear whether IL-2 directly stimulates the HIV LTR in primary T-lymphocytes. Kasid *et al.* [42] have reported that PBMC from cancer patients undergoing treatment with high dose IL-2 show enhanced proliferative activity and contain significant levels of mRNA for TNF- α , IL-6 and IL-2 receptor (R α). Even if IL-2 does not act directly on the HIV-LTR, it may act indirectly *in vivo* through the induction of TNF- α and IL-6.

Interleukin-3

Interleukin-3 (IL-3) [43] applied to ACH-2 and Molt-4 cells infected by HIV was shown to have no effect on HIV expression [25,40]; however, in primary macrophages, IL-3 substantially augments the production of HIV p24 [35].

Interleukin-4

Interleukin-4 (IL-4) [44] has no effect on ACH-2 and Molt-4 cells infected with HIV, or on Jurkat cells transiently transfected with an HIV LTR-driven reporter gene construct [25,39,40]. However, IL-4 significantly augments the replication of HIV in primary macrophages [45].

Interleukin-6

After demonstrating that TNF enhances the replication of HIV, we tried to ascertain whether IL-6 [46] was mediating this TNF effect, as TNF- α strongly induces IL-6 production. First, we exposed HIV-infected Molt-4 cells to the supernatant of TCL Na-I, an HTLV-I-transformed PBMC line which was used for the cloning of IL-6 (kindly provided by K. Sugamura, Tohoku University, Japan). However, this IL-6-rich supernatant did not appear to affect HIV infection, nor did recombinant IL-6 [40]. Although IL-6 does not affect HIV in T-cell lines, Poli *et al.* [27] reported that IL-6 induced HIV expression in U1 cells and primary macrophages infected by HIV_{AD-87}, a macrophagetropic strain of HIV. Synergy with TNF- α was also observed. The mechanism by which IL-6 induces HIV expression was presumed to be a post-

transcriptional event. IL-6 did not significantly increase the level of HIV mRNA over the constitutive level, yet viral protein expression (reverse transcriptase activity and HIV p24) was induced by IL-6 in U1 cells to levels comparable to TNF- α -induced levels. Although U1 cells are believed to possess only minimal constitutive expression of the HIV provirus [47], there still seems to be some amount of HIV-related transcript in these cells and their expression is critically regulated at post-transcriptional level.

Interferon- α , - β and - γ

Interferon (IFN)- α , - β and - γ can induce host-cell resistance to a wide range of viral infections, including many animal retroviruses [48]. These effects are considered to be partly mediated by the inhibition of viral assembly and release. Ho *et al.* [49] have discussed the anti-HIV activity of IFN- α *in vitro*: IFN- α had a dose-related suppressive effect on HIV replication when PBMC were used as a target for HIV infection. An anti-HIV effect of IFN was also reported in *in vitro* studies [50]. Wong *et al.* [51] have reported that, using the Hut-78 (T-cell line), RPMI-1788 (B-cell line), and normal CD4+ lymphocytes cloned from PBMC, the anti-HIV effect of IFN- γ was synergized by TNF- α [52]. This was an extension of the previous finding that IFN- γ combined with TNF had synergistic antiviral activity against various DNA and RNA viruses *in vitro* [51]. Hammer *et al.* [32] have demonstrated that IFN- γ , when added prior to infection, can reduce the viral production in chronically-infected U937 cells and nearly eliminate viral expression. Some groups have shown that human IFN- α can be more effective in reducing HIV replication *in vitro* than IFN- γ [53,54]. Hartshorn *et al.* [54] studied the anti-HIV activities of various interferons *in vitro*.

They used PBMC and tumor cell lines (H9 and U937) as targets for HIV, demonstrated that IFN- α and IFN- β had similar dose-dependent antiviral activity on PBMC and tumor cell lines, while IFN- γ had minimal antiviral activity on PBMC, but definite antiviral activity on tumor cell lines. Michaelis and Levy [55] studied the effect of IFN- β on HIV *in vitro*, demonstrating that IFN- β reduces the replication of HIV in acutely infected PBMC. Although the effectiveness of IFN- α and IFN- β on HIV infection does not appear to be significantly different, IFN- β is less toxic in humans and could prove more useful in the clinical treatment of HIV infection. However, a continuous high dose of IFN- β , and, presumably IFN- α , is required for optimal anti-HIV activity, since removal and/or low concentrations of the agent generally led to the return of virus production by the infected cells after long-term culture. Moreover, with these IFN, resistance of treated cells to the antiviral effect may occur over time [55]. With regard to the effects of IFN- γ on primary macrophages, Koyanagi *et al.* [35] have shown that HIV replication is enhanced by pre-treatment of the cells with IFN- γ for 3 days before infection but that treatment with IFN- γ after infection for 14 days resulted in a decrease of HIV production. Vyakarnam *et al.* [26] have suggested that IFN- γ enhances syncytium formation fivefold in CD4+ lymphocytes and 15-fold in PBMC.

Among the cytokine activities reported, IFN are the only cytokines that are widely believed to inhibit HIV replication. However, some believe that IFN- γ has no effect on HIV and it has shown equivocal results on HIV replication (Table 1).

Platelet-derived growth factor

Platelet-derived growth factor (PDGF) [56] is reported to have no activity on ACH-2 cells in terms of HIV replication [25].

Transforming growth factor- β

Transforming growth factor (TGF)- β [57] significantly inhibits HIV reverse transcriptase production and synthesis of viral proteins in U1 cells stimulated with phorbol ester (TPA) or IL-6 [58]. Suppression of virus expression by TGF- β was demonstrated through a 7-day culture period. Since TGF- β reduced the production and accumulation of HIV transcripts induced by TPA, Poli *et al.* [58] proposed that TGF- β interfered with the different steps of the viral production in U1 cells, rather than the post-translational block of HIV production proposed for IFN- α . A similar inhibitory effect on HIV production was seen in primary macrophages acutely infected by HIV, both in the absence and the presence of IL-6 [58]. In contrast, no significant effects of TGF- β were observed either in ACH-2 or in primary T-cells infected *in vitro*.

Induction of cytokine production by HIV

We have described how various cytokines can affect HIV production. Recent data show that HIV infection directly induces the production of cytokines; for example, HIV increased IL-6 mRNA and IL-6 secretion soon after exposure of mononuclear cells from healthy donors to either bioactive or inactivated HIV [59]. Further purification of the target cells showed that HIV-induced IL-6 production was derived from the

monocyte/macrophage fraction, and not from T-cells. By employing purified mononuclear phagocytes from normal peripheral blood, both IL-1 and TNF- α induction was observed within a few hours after exposure to HIV virions; this cytokine induction was also observed using heat-inactivated HIV [60]. Since soluble CD4 was able to block this action, it was concluded that the effect was mediated through the CD4 molecule. For example, the addition of purified gp120 from HIV to human monocytes resulted in the production of other cytokines, IL-1 and prostaglandin E2 [61]. Hence, monocytes/macrophages can produce various HIV-enhancing cytokines after viral binding, and the CD4 molecule appears to be somehow involved in signal transduction to stimulate this cytokine production.

There are, however, several contradictory reports concerning the role of HIV in IL-1, IL-6, or TNF- α induction from fresh PBMC. Munis *et al.* [62] showed that productive and cytopathic infection with a macrophagetropic strain of HIV does not alter the regulation of TNF- α expression in primary macrophages. These authors used both a TNF- α -mediated cytotoxic assay and a polymerase chain reaction (PCR) assay for TNF- α mRNA, which they suggested could detect between one and 10 cells with fully activated TNF- α expression [62]. Ammann *et al.* [63] essentially described the same negative result for HIV cytokine induction in PBMC from AIDS patients. In addition, Molina *et al.* [64] recently reported that, under endotoxin-free conditions, no increase in IL-1, IL-6, or TNF- α mRNA or protein was detectable in fresh PBMC exposed to HIV or recombinant gp120 compared with cytokine levels in mock-exposed cultures. Given these findings, it is clear that HIV does not always induce the production of cytokines.

NF- κ B and HIV

Phorbol ester and TNF- α and - β : potent inducers of NF- κ B

Phorbol ester activates the replication of HIV in chronically-infected Molt-4 cells; it also selectively kills HIV-infected Molt-4 cells [65]. At the molecular level, HIV LTR-directed gene expression has been shown to increase on treatment with mitogens, including TPA. This effect has been correlated with the induction of a DNA binding protein, NF- κ B, with binding sites in the viral enhancer [66] (Fig.1). This enhancer consists of a tandemly repeated 11 base pair (bp) sequence, κ B, also found in the enhancer regions of SV40, cytomegalovirus, and the κ B immunoglobulin (Ig) gene. In this experiment, Jurkat cells were used as the target, with both phytohemagglutinin (PHA) and TPA as coinducers, since HIV induction in T-cells was thought to require two signals to activate the cells to secrete lymphokines; however, TPA alone activates the HIV LTR to the same extent as a combination of PHA and TPA in Jurkat cells, and the stimuli required for lymphokine production are not needed for HIV LTR activation [67].

Since TNF- α and TNF- β enhance the replication of HIV and selectively kill HIV-infected cells, we speculated that TNF also activate HIV through the induction of NF- κ B. When an LTR deletion mutant lacking the NF- κ B site was used as a reporter plasmid, any enhancement of HIV LTR activation by TNF, as observed with wild-type LTR, was abolished in Molt-4, Jurkat and COS cells [68]. By comparing the activities of a wild-type HIV LTR and a mutant containing 6 bp changes in the NF- κ B site, the effect of TNF- α on HIV LTR expression was also shown to be NF- κ B-site-dependent in Jurkat cells, 70Z murine pre-B-cells, and U937 cells [39].

Activation of the HIV LTR by TPA or TNF- α must therefore occur via distinct pathways: (1) TPA and TNF- α synergistically induces NF- κ B and, (2) TNF- α , but not TPA, induces NF- κ B in HT-2 murine helper T-cells [39]. IL-2 production was not seen during the induction of the HIV LTR by TNF- α , again suggesting that HIV LTR gene expression can be induced without activating lymphokine secretion. The HIV LTR is also activated during monocyte differentiation by TNF- α [69]. Poli *et al.* [70] showed that anti-TNF- α antibodies substantially suppressed the expression of HIV p24 protein in TPA-stimulated ACH-2 and U1 cells, suggesting that TPA acts via the induction of TNF- α secretion [70]. However, as reported above, the effect of TPA on the HIV LTR could be blocked by staurosporine, a PK-C inhibitor, whereas the effect of TNF- α could not be suppressed in this way [30]. This finding clearly indicates that TPA and TNF- α employ different pathways in the activation of NF- κ B; induction by TPA is PK-C-dependent whereas that by TNF- α is PK-C-independent. A similar result was reported in gel retardation studies using nuclear extract from Jurkat cells pre-exposed to PK-C inhibitors such as staurosporin and H7 [71].

A mechanism by which TPA acts to induce NF- κ B has recently been proposed [72,73]. In uninduced cells, NF- κ B is present in the cytosol, inactivated by forming a complex with an inhibitory protein, I- κ B. Once cells are stimulated by TPA, PK-C phosphorylates I- κ B, which frees NF- κ B from inhibition and allows it to translocate to the nucleus where it activates target gene expression through binding to NF- κ B sites. NF- κ B consists of two subunits, p55 and p65, both of which have recently been identified as belonging to the *rel* oncogene family [74-77]. I- κ B is presumed to bind to, and hence mask, the DNA binding domain of p65. Ghosh *et al.* [78] showed that

PK-C-dependent phosphorylation of I- κ B prevented it from associating with NF- κ B by observing the activation of NF- κ B *in vitro* after adding PK-C to partially purified NF- κ B/I- κ B complex from uninduced cells. However, it is still not clear whether phosphorylation of I- κ B in an NF- κ B/I- κ B complex can free NF- κ B. Furthermore, how do TNF act to induce NF- κ B without invoking PK-C activity? It is possible that TNF function via a protein kinase-A (PK-A)-dependent pathway.

NF- κ B and associated regulatory proteins are thought to serve as substrates for more than one protein kinase such as PK-C and PK-A; the induction of NF- κ B by IL-1 can be mimicked by cyclic adenosine monophosphate (cAMP) analogs and cAMP-elevating drugs in the murine pre-B-cell line 70Z/3 [79]. Release of NF- κ B activity from NF- κ B/I- κ B complex by PK-A *in vitro* has also been shown using nuclear extracts from 70Z/3 cells [78]. However, the effect of TNF on HIV-infected T-cells, cAMP analogs and cAMP-elevating drugs have failed to provide consistent results identifying cAMP as a mediator of TNF- α -induced HIV expression (personal observations). In addition, PK-A was not found to be involved in NF- κ B activation by TNF- α in studies using a cAMP analog and a PK-A inhibitor on Jurkat cells [71].

Other immunologic inducers of NF- κ B

In addition to TPA and TNF, antigens have also been thought to stimulate HIV production; however, the mechanism of antigen-induced HIV stimulation is not precisely understood. The effect of anti-CD3 antibodies on HIV LTR-stimulation in Jurkat cells was studied, given that anti-CD3 antibody stimulation is thought to mimic

the interaction between the T-cell receptor (TCR)/CD3 complex and the antigenic peptide major histocompatibility complex (MHC) [67]. Immobilized anti-CD3 antibody stimulated the HIV LTR; costimulation with anti-CD28 antibodies augmented HIV LTR expression to a level comparable with that observed with TPA. However, anti-CD28 antibodies alone could not stimulate the HIV LTR. CD28 is known to be an additional accessory molecule involved in T-cell activation and can enhance the mitogenic signal derived from TCR/CD3 interactions [80]. Experiments using 5'-deletion mutants of the HIV LTR showed that the NF- κ B site and sequences in the upstream U3 region are required for this CD3/CD28-mediated response, which is also PK-C-dependent. In contrast to these results, another group has suggested that anti-CD28 antibodies alone can stimulate the HIV LTR through NF- κ B induction [81]. This antigen-induced HIV stimulation may not work efficiently *in vivo*, because CD4 molecules on T-cells infected by HIV are often occupied by HIV envelope protein, gp120, which will interfere with the interaction between CD4+ T-cells and antigen-presenting cells.

Physiological roles of TNF- α/β in T-cells

What is the physiological role of TNF- α/β -mediated induction of NF- κ B in T-cells? Greene *et al.* [82] have proposed that TNF- α and IL-1 play an important role in the induction of IL-2R α in the early stages of T-cell development [82]. IL-2R α is known to be an inducible receptor and, with IL-2R β , forms a high-affinity IL-2 receptor complex. IL-2R α is also known to be induced through the activation of NF- κ B. In mature T-cells, IL-2R α expression normally requires stimulation through the TCR/CD3

complex; however, in immature, CD3-negative/low T-cells, this mode of IL-2R α upregulation appears unlikely, in spite of its apparent requirement given the expeditious proliferation of immature T-cells. As suggested by others [82], IL-2R α expression may be induced by TNF- α and IL-1, both of which are secreted by macrophages and endothelial cells present in the thymus.

Another important *cis*-element of the HIV LTR is the triad of Sp1 binding sites (Fig. 1). However, under TNF- α stimulation, the NF- κ B sites can functionally substitute for the Sp1 sites in directing HIV gene expression [83]. A summary of the immunologic inducers of NF- κ B is presented in Table 2.

Cytokines and AIDS: a hypothesis

Almost all cytokines can be produced from multiple cell types. In addition, the action of most cytokines is pleiotropic in nature and may be modified by other mediators. A single factor, such as LPS or a virus, can induce many different cytokines from the same type of cell. The cytokine itself can also either induce or down-regulate the production of other cytokines [84]. In our opinion, AIDS represents a cytokine or TNF disease (Fig. 2). In the cytokine network of AIDS, TNF- α and TNF- β appear to be crucial molecules, enhancing the replication of HIV as well as inducing their own expression and that of other cytokines [73,85]. TNF- α has been demonstrated to stimulate the release of other cytokines, such as IL-1, IL-6, and GM-CSF in various cell types, and is therefore considered to be a key member of the cytokine cascade in a first-defense mechanism [86]. Moreover, although controversial, the binding of HIV

may directly activate monocytes/macrophages to release various cytokines. If this *in vitro* model is operative *in vivo*, then TNF- α , IL-1 and IL-6 could be induced from monocytes/macrophages upon infection with HIV. TNF- α , TNF- β , and IL-1 activate HIV directly through the induction of NF- κ B, and IL-6 shows a synergistic effect with TNF- α . IL-2 induced by IL-1 activates T-cells in an autocrine manner. In addition, activated T-cells produce cytokines such as TNF- α , TNF- β , IL-3, IL-4, GM-CSF, and IL-6, all of which, in turn, accelerate HIV replication in T-lymphocytes and macrophages in an autocrine and paracrine manner (see Fig. 2).

Given the diverse biological functions of these cytokines, we propose that many of the symptoms associated with AIDS can be explained by their release. Enhanced production of IL-1 and TNF- α , both well-known endogenous pyrogens, could explain the fever seen in AIDS patients. TNF- α (also termed cachectin) may be involved in AIDS-associated cachexia, although its biological role in cachexia *in vivo* has not yet been established. A similar assumption was made by Haase in 1986 [87]; however, in our model, we consider TNF- α and TNF- β as central mediators of AIDS pathogenesis (Fig. 2). Even in individuals newly exposed to HIV, TNF-mediated activities may hasten the establishment of infection and progression to full-blown AIDS. The various potential modes of TNF in HIV pathogenesis are discussed below.

CD4+ T-cell depletion

TNF- α and TNF- β are known to work as immunomodulators and effector molecules in monocyte-mediated cytotoxicity [88,89] and as membrane-bound effector molecules

in cell-mediated cytotoxicity [90]. TNF fulfill their responsibility by the activation of an immune response and can directly kill HIV-infected cells. However, their activity could also betray the immune system by activating lately infected cells and, as a result, disseminating HIV viral progeny to infect another set of CD4 + cells. Indeed, not only the secreted form of TNF- α , but also membrane-bound TNF- α enhances the replication of HIV through HIV LTR activation (O. Prakash, personal communication, 1991). With host cells actively secreting TNF and the immune system sending out TNF-bound cytotoxic effector cells against HIV-infected cells, this cycle will continue in a positive feed-back manner until the host can no longer supply CD4+ cells (Fig. 2). Immunologic mechanisms have been proposed to explain CD4+ T-cell depletion in AIDS, and supporting evidence has been published [91-95]. It is possible that TNF- α and IL-1 potentiate this mechanism, because these cytokines are reported to enhance antibody-dependent cell-mediated cytotoxicity [96]. This is how we envision the central role of TNF in the depletion of CD4+ T-cells *in vivo*. Opportunistic infections caused by CD4+ depletion will result in persistently higher levels of TNF- α/β secretion, which will also contribute to this positive feed-back circuit. A recently proposed alternative mechanism of CD4+ T-cell depletion is that TGF- β , as one of the most potent immunosuppressive factors, plays the central role. TGF- β titers are increased in the supernatants of PBMC and sera from HIV-infected donors as compared to uninfected controls, and TGF- β preferentially inhibits proliferation of CD4+ T-lymphocytes *in vitro*[97,98].

AIDS-related Kaposi's sarcoma

Miles *et al.* [99] reported that AIDS Kaposi's sarcoma (KS)-derived cells produce and respond to IL-6 and suggested that IL-6 is an autocrine growth factor for AIDS KS cells. An immunopathological study showed marked increased epidermal staining for IL-6 and TNF- α in AIDS-related KS elements [100]. TNF- α can be produced from keratinocytes by physiological stimuli such as ultraviolet light [86], which may contribute to the induction of IL-6 in the skin and development of KS in AIDS.

Ensoli *et al.* [101] have suggested that the HIV-1 Tat protein plays a role in the maintenance and progression of KS in AIDS because it specifically promotes the growth of spindle cells derived from AIDS KS [101]. Normal mesenchymal cells, thought to be the precursors of KS spindle cells in the lesion, do not proliferate after addition of Tat. However, the combination of IL-1 and TNF- α induces normal mesenchymal cells to become responsive to the mitogenic effect of Tat (B Ensoli, personal communication, 1991). This indicates that IL-1 and TNF- α induce not only HIV gene expression but may also be involved in the pathogenic mechanisms of KS.

Central nervous system and gastrointestinal disorders

Marked dysfunction of the central nervous system (CNS) is observed in AIDS patients despite only subtle or minimal lesions [102-104]; it has been suggested that cytokines released from HIV-infected macrophages might be involved in this aspect of AIDS pathology. Post-mortem analyses of AIDS patients with CNS involvement have shown that cerebral white matter was the most frequently involved structure [103]. From this

observation, together with the high frequency of peripheral nerve demyelination, de la Monte *et al.* [103] suggested that myelin-producing cells such as oligodendrocytes might be especially vulnerable in this disease. TNF- α can damage myelin and oligodendrocytes *in vitro* [105], and some glioma-derived cell lines have been shown to be susceptible to the anti-proliferative effect of TNF- α [106]. Although TNF- α is reported to destroy CNS oligodendrocytes, IL-1 stimulates the growth of astrocytes [107] and, indeed, astrogliosis is one of the most frequent pathological features in AIDS encephalopathy.

Besides TNF- α and IL-1, other cytokines or neuropeptides are reported to be involved in CNS dysfunction in AIDS. An HIV-infected macrophage cell line, but not an HIV-infected lymphocyte cell line, was shown to release toxic agents that damage neurons *in vitro* [108]. Physiological and biological studies revealed that this neurotoxic agent was a novel factor proposed to produce neurologic disease in AIDS. Furthermore, the envelope protein of HIV seems to interfere with the activity of neurotropic factors indispensable for neuronal growth. HIV envelope protein, gp120, has been shown to share homology with neuroleukin, a neuron growth factor, such that gp120 was able to competitively inhibit its neurotropic effect *in vitro* [109]. In addition, neuronal cell killing by gp120 was blocked by vasoactive intestinal peptide (VIP), a neuron growth factor which was also shown to have homology with gp120 [110]. Another neurotoxic agent produced by HIV-infected macrophages, but not by HIV-infected T-cells, was recently reported by Pulliam *et al.* [111]. Apparently, it was different from TNF- α because LPS-activated macrophages did not produce this factor and effects such as morphological and functional abnormalities in a normal human

brain culture system could not be reproduced by $\text{TNF-}\alpha$.

It has recently been suggested that $\text{TGF-}\beta$ may be a mediator of CNS dysfunction in AIDS [112]. $\text{TGF-}\beta$ was found to be aberrantly expressed in the brains of AIDS patients where HIV transcripts were detected. However, the expression of $\text{TGF-}\beta$ was not limited to HIV-positive cells; primary macrophages infected by HIV *in vitro* were also shown to increase their expression of $\text{TGF-}\beta$, which triggered the release of $\text{TGF-}\beta$ from cultured astrocytes that were not infected with HIV. $\text{TGF-}\beta$ may recruit HIV-infected cells into the CNS, enabling viral spread and augmenting cytokine production from astrocytes, such as $\text{TNF-}\alpha$, which will cause neuronal damage.

Chronic diarrhea and malabsorption syndrome are often observed in AIDS patients in the absence of any known pathogens except HIV [113]. Levy [114] has suggested that HIV infection of enterochromaffin cells in the intestinal mucosa might deregulate motility and digestive functions of the intestine. In addition, the possible involvement of macrophage-produced cytokines in these disorders in the lamina propria should be considered.

Laboratory findings

Several reports have shown that serum levels of $\text{TNF-}\alpha$ and IL-1 are substantially elevated with the development of AIDS [115-118]. In particular, Lahdevirta *et al.* [115] demonstrated that $\text{TNF-}\alpha$ levels fell within the range of healthy control values in 11 out of 13 asymptomatic carriers and AIDS-related complex (ARC) patients, but were

elevated in all of nine AIDS patients and in five out of nine ARC patients suffering from secondary infection and/or weight loss. In this regard, it is noteworthy that an increase of HIV p24 antigen was observed in AIDS and ARC patients after therapeutic application of TNF- α [119].

Soluble IL-2R α (sIL-2R α) levels are increased in the sera of HIV-infected individuals [120]. As above, if the physiological role of TNF- α is to induce IL-2R α in immature T-cells, it is likely that an elevated serum level of TNF affects IL-2R α expression, which sometimes becomes sIL-2R α . Urinary and serum neopterin, products of stimulated macrophages, were shown to be elevated among ARC and AIDS patients [121]. Phase I trials of TNF- α in advanced cancer patients have shown that TNF- α significantly enhances serum IL-2R α and neopterin levels [122]. Since IFN- γ is a strong inducer of neopterin [121], IFN- γ induced by exogenous TNF- α , though indirect, might contribute to the elevation of neopterin levels *in vivo*. Hypergammaglobulinemia is also frequently seen in AIDS patients [123]. This finding could be explained by increased IL-6 levels in response to TNF- α , resulting in the over-stimulation of B lymphocytes to produce IgG. In this regard, Amadori *et al.* [124] showed that *in vitro* activation of B lymphocytes from HIV patients was inhibited by anti-IL-6 antibodies.

Perspective

In this review, we have discussed the effects of various cytokines on HIV infection. Other than TNF- α and TNF- β , the effects of cytokines on HIV expression are weak

and their mechanisms of action are still not clearly understood. Some cytokines show synergic effects with TNF. There can be no doubt that TNF- α and TNF- β are the cytokines which have the most potent activity on HIV-infected cells; they are strong inducers of NF- κ B and they can selectively kill HIV-infected T-cells. Under normal physiological conditions, one possible role of TNF is to induce NF- κ B in immature T-cells for the expression of IL-2R α , which is essential for T-cell proliferation. In HIV-1-infected T-cells, the HIV-1 LTR can use this transcriptional factor more efficiently because its enhancer consists of a tandemly repeated, rather than a singular NF- κ B site; HIV-1 can therefore be activated by even lower levels of stimuli which are not sufficient for lymphokine production. If the HIV-1 enhancer contains only a single NF- κ B site, the virus adopts a more latent and transcriptionally silent phenotype as seen with HIV-2 and simian immunodeficiency virus (SIV) [125]. Indeed, a virulent strain of SIV was discovered to have a tandemly repeated NF- κ B site in its enhancer, similar to HIV-1 [126]. However, *in vitro* experiments have shown that the continuous presence of TNF is required for optimal HIV activation. This indicates that, although its enhancer is arranged in duplicate, the HIV promoter requires a continuous supply of considerable NF- κ B levels for full activation. As discussed above, the intracellular supply of active NF- κ B is increased upon antigen stimulation via a PK-C-dependent pathway and upon TNF- α or TNF- β stimulation via a PK-C-independent pathway. Sporadic microbial infections will result in temporary supplies of active NF- κ B, although not sufficient to produce large amounts of HIV. However, if this transient viral production is not eliminated by the host, repeated episodes of infection will contribute to the accumulation of viral burden. When the host suffers from opportunistic infections, the supply of NF- κ B for HIV replication becomes unlimited,

precipitating the inevitable destruction of the host. In the absence of TNF, the supply of NF- κ B would be more limited and, hence the pathogenic features of an HIV infection would be profoundly different.

We see TNF as the amplifiers of HIV infection and clinical disease. From another viewpoint, HIV infection is an indirect amplifier of the pathogenic effects of TNF. We therefore propose that AIDS is not established without the influential effects of TNF. In this regard, it can be said that AIDS is as much a TNF disease as it is an HIV disease. Clinically, the careful regulation of the effects of TNF and the prevention of opportunistic infections becomes an important focus. An unresolved issue requiring further study is the mechanism by which TNF can selectively kill HIV-infected T-cells. We believe that knowledge in this regard will provide fruitful information not only for AIDS research and therapy, but also for the original question raised when TNF- α was first discovered: why are malignant cells selectively killed by tumor necrosis factor?

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Table 1. Cytokines and HIV replication.

Cytokine	Source	Effect on HIV replication in			
		T-cell line	Macrophage cell line	PBMC	Primary macrophages
TNF- α	Macrophage, T-cell, B-cell, Keratinocyte	↑ ↑ ↑ ↑	↑ ↑	↑ ↑ ↑ ↑	↑ ↑ ↑
TNF- β	T-cell, B-cell	↑ ↑ ↑ ↑	NT	↑ ↑ ↑	NT
GM-CSF	Macrophage, T-cell	—	↑ ↑ or ↓	↑ ↑ ↑	NT
M-CSF	Fibroblast	NT	NT	NT	↑
IL-1	Macrophage, fibroblast, endothelial cell	↑ or —	—	NT	NT
IL-2	T-cell	—	—	↑	NT
IL-3	T-cell	—	NT	NT	↑ ↑
IL-4	T-cell	—	NT	NT	↑ ↑ ↑
IL-6	Macrophage, T-cell, glia, fibroblast	—	↑ ↑	NT	↑ ↑ ↑
IFN α	B-cell	↓ ↓ ↓	↓ ↓ ↓	↓ ↓ ↓	↓ ↓ ↓
IFN β	Fibroblast, B-cell	↓	↓ ↓ ↓	↓ ↓ ↓	NT
IFN γ	T-cell	↓ or —	↓	↑ or —	↑
PDGF	Platelet	—	NT	NT	NT
TGF- β	Platelet, macrophage, T-cell	—	↓ ↓	NT	↓ ↓

HIV stimulation indices were derived from the results presented in [20,26,27,35]. HIV inhibitory indices are derived from the results in [53-55] and J.A. Levy (personal communication, 1991). ↑-↑ ↑ ↑ ↑, relative HIV stimulation index; ↓-↓ ↓ ↓ ↓, relative HIV inhibition index; —, no activity on HIV. NT, not tested; PBMC, peripheral blood mononuclear cells; TNF, tumor necrosis factor; GM-CSF, granulocyte/macrophage colony-stimulating factor; M-CSF, macrophage colony stimulating factor; IL, interleukin; IFN, interferon; PDGF, platelet-derived growth factor; TGF- β , transforming growth factor β .

Table 2. Immunologic inducers of transcriptional factor NF- κ B

<u>Inducers</u>	<u>Pathway</u>	<u>Reference</u>
Phorbol ester(TPA)	PK-C-dependent	Ghosh et al.[78]
Tumor necrosis factor α / β	PK-C-independent	Hamamoto et al.[30] Meichle et al.[71]
Interleukin-1	PK-A-dependent?	Shirakawa et al.[79]
α -CD3/ α -CD28	PK-C-dependent	Tong-starksen et al.[67] Gruters et al.[81]

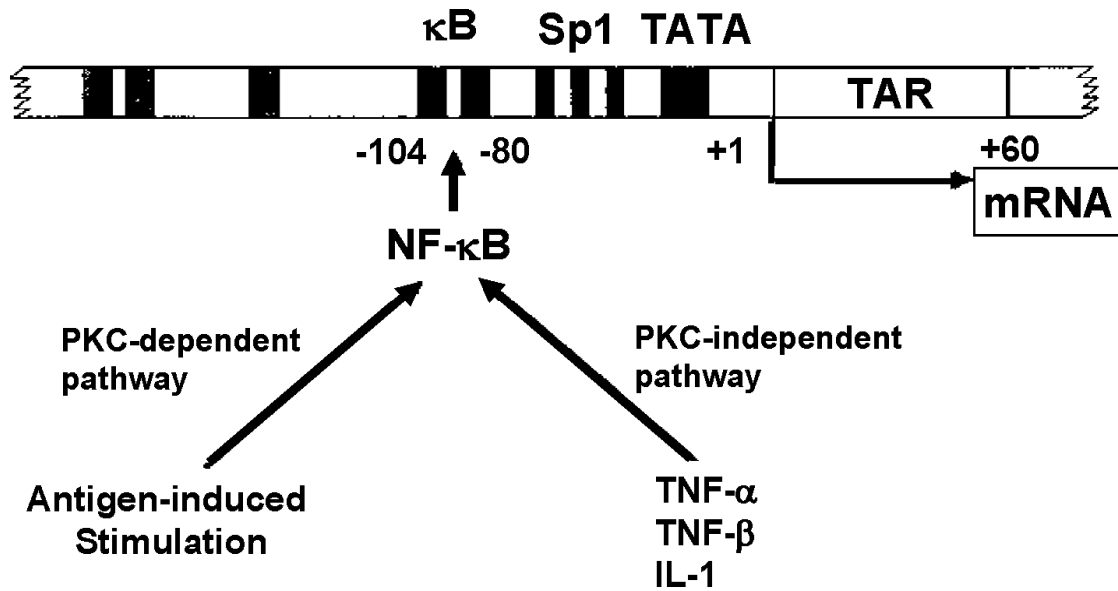


Fig 1. Functional regulatory regions in the long terminal repeat of HIV-1 gene and pathways of transcriptional factor NF- κ B activation by immunologic stimuli. The antigen-induced stimulation pathway is protein kinase-C(PK-C) dependent. In contrast, stimulation by tumor necrosis factor (TNF)- α/β and interleukin (IL)-1 appears to be independent of the PK-C pathway. κ B, NF- κ B binding site; Sp1, Sp1 binding site; TAR, Tat responsive element.

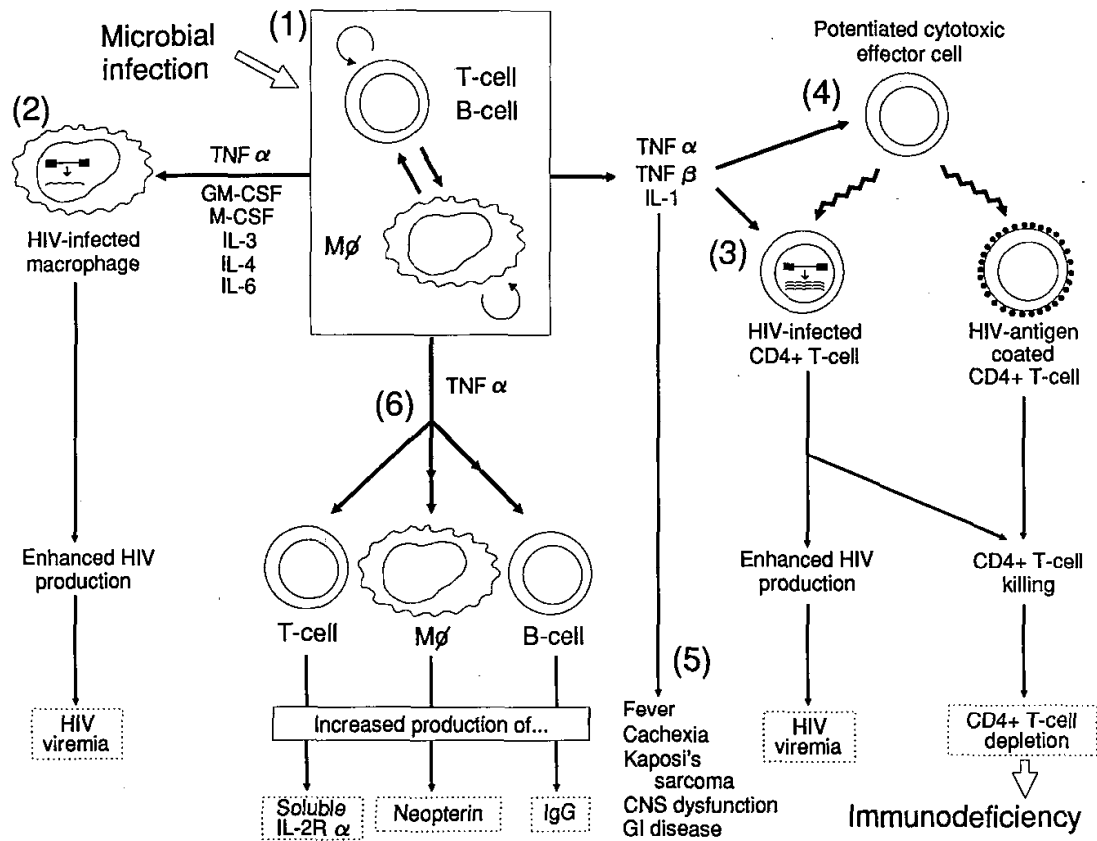


Fig. 2. Cytokine networks and a possible mechanism of AIDS pathogenesis. (1) Following microbial infections (e.g., HIV), cytokines such as tumor necrosis factor (TNF)- α , TNF- β , granulocyte/macrophage colony-stimulating factor (GM-CSF), macrophage colony-stimulating factor (M-CSF), interleukin (IL)-1, IL-3, IL-4, and IL-6, which have stimulatory effects on HIV, are produced from various types of cells, including T-cells, B-cells and macrophages. In addition, IL-2 induced by IL-1 activates T-cells, which will produce TNF- α , TNF- β , IL-3, and IL-4. (2) In macrophages infected by HIV, these cytokines stimulate HIV production, which increases HIV burden in the body. (3) In T-cells infected by HIV, TNF and IL-1 enhance the replication of HIV and TNF selectively kill infected cells, causing HIV viremia and

CD4+ T-cell depletion. (4) TNF and IL-1 also potentiate cytotoxic effector functions, which will not only kill infected CD4+ T-cells but also non-infected CD4+ T-cells covered with HIV antigen, resulting in CD4+ T-cell depletion and immunodeficiency. (5) In addition, increased TNF can result in clinical features observed in AIDS. (6) TNF- α augments the production of soluble IL-2R α , neopterin and immunoglobulin (Ig) G from T-cells, B-cells and macrophages, respectively. Neopterin and IgG may be produced by IFN γ and IL-6, respectively, which are induced by TNF- α . Laboratory findings detected in the development of AIDS are represented by the dotted boxes.