Modulation of HIV-1 Transcription by Cytokines and Chemokines

K.F.T. Copeland*

Molecular Medicine Program, Ottawa Health Research Institute, Ottawa, ON, Canada

Abstract: Infection with HIV results in the modulation of circulating levels of many host factors. Several host proteins that are up-regulated in HIV infection have the potential to influence virus replication. More specifically, the transcription of HIV-1 can be modulated *in vivo* by host proteins, including cytokines and chemokines. Cytokines modulate transcription mediated by the HIV-1 long terminal repeat (LTR) via multiple signal transduction pathways with resulting recruitment of numerous transcription factors, including NFκB, C/EBP, AP-1, TCF-1α, NF-IL-6 and ISGF-3. The effects on transcription may vary depending upon the cell type studied and upon the timing of the exposure of infected or transfected cells to cytokines. Furthermore, studies of cytokine mediated activation or inhibition of LTR mediated transcription may also be affected by the presence of the HIV-1 *trans*-activating protein, Tat, which has significant impact upon the redox state of the cell. This review will examine the complexities of the positive and negative control of HIV transcription by cytokines and chemokines.

Keywords: Transcription, HIV-1, LTR, cytokine, chemokine, replication, host protein, inflammatory.

INTRODUCTION

Cytokines are low molecular weight proteins that participate in the regulation of immune responses. Cytokines bind to specific cell membrane receptors resulting in the modulation of gene transcription via the activation of signal transduction pathways. Therefore cytokines exert a variety of effects on cellular function, activation state, proliferation, viability, differentiation and the production of host proteins (including other cytokines). Chemoattractant cytokines (chemokines) bind to cell surface G protein-coupled receptors resulting in the attraction of specific cell types to sites of injury and inflammation in the body. Both cytokines and chemokines may be dysregulated in HIV infection, as demonstrated by the shift from a Th1 to a Th2 phenotype of CD4+ T cells with progression to disease [1]. Cytokines and chemokines may also target other points in the virus life-cycle, including cellular entry, replication and protein maturation. This review will focus on HIV transcription (the effects of cytokines on HIV-1 transcription are summarized in Table **1**). The significance of cytokines and chemokines in HIV infection *in vivo* will also be addressed.

CONTROL OF HIV-1 LTR-MEDIATED TRANSCRIPTION

The long terminal repeat (LTR) of HIV-1 drives the expression of viral genes. The LTR is activated by virally encoded proteins and factors required for cellular transcription. Transcription factors induced by cellular or viral signals bind to regions of the LTR and exert positive and negative effects on HIV transcription. The HIV-1 LTR can be segmented into 3 regions: a) a transcribed LTR region provides an RNA sequence (TAR) which may respond to the viral *trans*-activating protein Tat [2]; b) within the core region flanking the transcriptional start site are the TATA box, Sp-1 sites and two NFκB enhancer elements [3], c) upstream of the NFκB sites are elements which may further modulate transcription including AP-1, NFAT-1, C/EBP and TCF-1α binding sites [4]. The structure of the HIV-1 LTR is outlined in Fig. (**1**).

Retroviral transcription can be modulated by viral proteins, by numerous cellular factors induced during activation and differentiation, and by cytokines and chemokines up-regulated in response to viral infection. The activation of cellular gene expression by cytokines results in the modulation of transcription factors which have diverse effects upon cellular promoters. The HIV LTR contains transcription factor binding sites which are similar to those found in cellular gene promoters and thus LTR-mediated transcription is similarly modulated by cytokines and chemokines. The ability of cytokines to affect HIV transcription may also be cell type-specific and dependant on levels of other cytokines present.

MODULATION OF HIV-1 TRANSCRIPTION BY CYTOKINES

I. Pro-Inflammatory Cytokines

Several inflammatory cytokines are elevated in patients with AIDS, including IL-1, TNFα, TGFβ and IL-6 [5, 6]. TNFα is a pro-inflammatory cytokine which is associated with numerous infectious diseases, including HIV infection, and is associated with AIDS progression. Furthermore, TNFα has been shown to activate virus replication in both chronically infected cell lines and in peripheral blood mononuclear cells (PBMC) derived from HIV-1 infected subjects [7-9]. TNFα has also been shown to increase HIV transcription in ACH-2 T cells [10]. This was further demonstrated by the ability of antibodies to TNFα to inhibit LTR-mediated transcription in primary T cells [11]. Recombinant soluble TNF receptors also cause an inhibition of TNFα-mediated activation of the LTR [12]. Deletion of the NFκB sites in the LTR abolished this enhancement [10], indicating the importance of these sites for TNFα-mediated enhancement of HIV-1 transcription. Therefore,

*Address correspondence to this author at the Ottawa Health Research Institute, 501 Smyth Rd., Ottawa, Ontario, K1H 8L6, CANADA; Tel: (613) 737-8775; Fax: (613) 737-8803; E-mail: kcopeland@ohri.ca

1389-5575/05 $50.00+.00

Table 1. Summary of the Effects of Cytokines on HIV-1 Transcription

			Combined effects		
Cytokine	**T cell**	**Macrophage**	**T cell**	**Macrophage**	**Reference**
Pro-inflammatory					
IL-1				↑ (IL-6, GM-CSF)*	[14, 20]
IL-6				↑ (TNFα, IL-1)	[32]
IL-16	↓	↓			[38-42]
IL-18	↑	↑			[48-50]
TNFα	↑	↑	↓ (IFNγ)	↑(IL-4, IL-10, IL-1, IFNγ)	[10-13]
TGFβ		↑↓		↓ (IL-6)	[24, 25]
IFNγ		↑↓	↓	↑ (IL-6, TNFα)	[29-32]
GM-CSF		↑↓		↑ (IL-6)	[52-63]
Anti-inflammatory					
IL-4	↑	↑		↑↓ (IL-13)	[92-94]
IL-10		↓		↑ (TNFα)	[82-84]
IFNα		↓		↓ (TNFα)	[56, 66]
IFNβ		↓	↓ (TNFα)		[56, 66]

* Cytokines in brackets indicate those that produce an effect in combination with the defined cytokine.
↓ increased transcription
↑ decreased transcription
↑↓ both increased and decreased transcription

TNFα can control HIV-1 replication in primary T cells in an autocrine manner by the regulation of NFκB [11]. In a test of numerous cytokines on different cells of nervous system origin, Swingler *et al.* reported that TNFα activated LTR-mediated transcription in neural cell lines and in primary murine astrocytes [13]. IL-1β activated the promoter in glioblastoma, astrocytoma and astrocyte cells [13].

In co-cultures of thymocytes and thymic epithelial cells (TEC), cytokines secreted in the culture medium include TNFα and IL-1 [14]. Both cytokines induced NFκB activity which upregulated HIV LTR-mediated gene expression. Co-culture of the thymocytes with TEC was required for the support of high replication of HIV in thymocytes. Furthermore, IL-7 secreted by TEC was an important co-factor for NFκB activation by TNFα and IL-1 [14]. IL-7 is a cytokine important in enhancing the survival of mature T cells [15-17]. This is one of many examples of cytokines that rely on another secreted cytokine forefficient function. Westendorp *et al.* have reported that the HIV Tat protein can amplify both the cytotoxic and NFκB activating pathways of TNFα [18]. This is the result of the ability of Tat to decrease the levels of glutathione, thus affecting the redox state of the cells [18]. Glutathione augments IL-2 production and thus the inhibition of glutathione by Tat

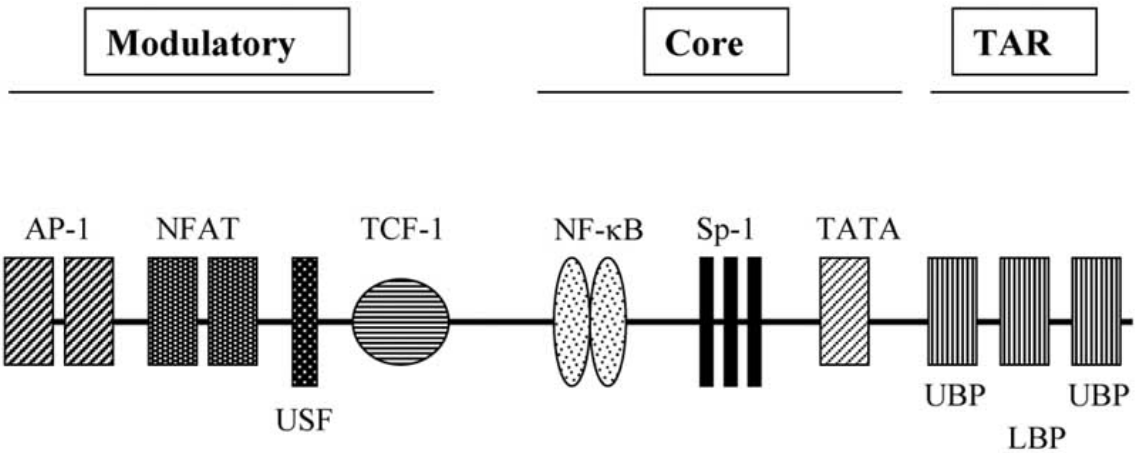

Fig. (1). Structure of the HIV-1 long terminal repeat. Transcription factor binding sites present within modulatory, core and TAR regions are shown.

leads to the promotion of a Th2 response. In the promonocytic cell line U937, TNFα independently stimulated the LTR and this was augmented further by GM-CSF [19]. Similarly, in U1 cells GM-CSF significantly enhanced HIV transcription induced by LPS. This enhancement was blocked by an IL-1 receptor antagonist suggesting that IL-1 mediated the response to LPS and GM-CSF treatment [20].

TGFβ has a wide array of functions, which may vary depending upon cell type and the state of differentiation of the cell. The cytokine is produced by platelets and bone marrow stromal cells [21]. Li *et al.* have reported that TGFβ enhanced HIV-1 mediated transcription in human T cells and B cells via increased NFκB binding to the promoter [22]. The association of the TGFβ signaling protein SMAD3 with CCAAT/enhancer binding protein (C/EBP) was also associated with increased HIV transcription [23]. In contrast, the association of SMAD4 and C/EBP resulted in decreased transcription in astrocytes [23]. Poli *et al*. also reported that TGFβ suppressed virus replication in IL-6 treated U1 cells at the level of transcription, similarly to retinoic acid [24]. In another study, TGFβ potently suppressed PMA induced HIV transcription in U1 cells [25]. In contrast, TGFβ did not alter HIV transcription induced by TNFα. These effects were not observed in T cells and were independent of the induction of IFNα [25]. The contrasting results likely reflect differences in cell type and the mode of stimulation used in each study.

Increased levels of IFNγ are detected in the serum of HIV-infected individuals [26]. IFNγ has been shown to decrease Tat mediated activation of the LTR in HeLa cells expressing CD4 and to decrease HIV replication in H9 T cells and U937 monocytic cells [27, 28]. In contrast, IFNγ or TNFα had no effect alone on activation of the LTR in mouse macrophages [29]. However, co-exposure of macrophages to IFNγ and either IL-6 or TNFα, led to synergistically increased activation [29]. Indeed, a greater effect was observed when IFNγ was added prior to the addition of IL-6 [29]. However, Poli *et al*. have also reported that while IL-6 can synergize with TNFα in increasing HIV transcription, up-regulation of HIV by IL-6 alone is not controlled at the level of transcription [30]. Whether IL-6-mediated synergism is dependant upon transcription factors up-regulated by TNFα has not been determined. Therefore IL-6 acts at both transcriptional and post-transcriptional levels. Co-stimulation of U1 cells with TNFα and IFNγ resulted in the synergistic induction of HIV RNA synthesis [31]. The synergistic effects of this co-stimulation was further documented by the demonstration that potent induction of HIV RNA expression by IFNγ in U1 cells occurred by a pathway independent of TNFα [31]. This suggests that viral load may be influenced during concurrent infections which may enhance IFNγ expression in the presence of cytokines which are up-regulated in HIV infection. In monocytic cells, IL-1 used in combination with IL-6 resulted in increased binding at the NFκB sites of the LTR at a magnitude similar to that induced by TNFα [32]. Both IL-1 and TNFα are up-regulated *in vivo* in HIV infected individuals [26, 33].

IL-16 is a proinflammatory chemoattractant cytokine produced by CD8+ T cells, bronchial epithelial cells from asthma patients, mast cells and eosinophils [34-37]. IL-16 has been shown to strongly suppress HIV-1 LTR-mediated transcription in T cells [38, 39], monocytic cells and dendritic cells [40]. CD4+ T cells do not secrete cytokines in response to IL-16 treatment [41]. In contrast, monocytes and maturing macrophages secrete TNFα, IL-1β, IL-6 and IL-15 in response to IL-16 [41]. IL-16 treatment of macrophages has also been reported to result in the activation of the SAPK signaling pathway [42].

IL-18 belongs to the Th1 family of cytokines [43-45]. This cytokine is produced primarily by monocytes and macrophages and contributes to immune function by enhancing T cell responses, interferon production and Th1 cell development [46]. Raised serum levels of IL-18 are associated with disease progression in HIV-1-infected patients [47]. IL-18 has been found to inhibit HIV-1 replication in PBMC [48]. In contrast, other reports have indicated that IL-18 enhances HIV-1 replication in both monocytic cells and T cell lines [49, 50]. IL-18 has been shown to enhance HIV transcription in monocytic cells in a TNF dependent manner [50]. The enhancement was also found to depend upon p38 MAPK and resulted from increased binding of NFκB to the HIV promoter [50].

Granulocyte macrophage-colony stimulating factor (GM-CSF), macrophage-colony stimulating factor (M-CSF) and IL-3 are produced by T cells, macrophages, B cells and endothelial cells [51]. All three cytokines are required for the growth of haematopoietic precursors in bone marrow. In addition, these cytokines have been shown to enhance HIV-1 replication in cells of the monocyte/macrophage lineage, bone marrow stem cells and microglia [52-60]. Further, antagonists to M-CSF were reported to inhibit replication of HIV-1 in human macrophages [61]. In contrast, in macrophages differentiated from monocytes, M-CSF and GM-CSF were found to inhibit HIV-1 replication [62, 63]. Fewer studies have been performed examining the effects of these cytokines on HIV-1 transcription. Watanabe *et al*. have shown that GM-CSF enhanced HIV-1 LTR-mediated transcription in the IL-3-dependent mouse cell line, BaF3 [64]. Stimulation of the human erythroleukemia cell line, TF-1, with GM-CSF was found to be associated with increased HIV transcription and increased levels of Bcl-3 [65]. GM-CSF was also shown to induce Bcl-3 resulting in increased nuclear translocation of NFκB [65].

II. Anti-Inflammatory Cytokines

In response to viral infections, type 1 interferons (IFN) are produced. IFNα is produced by leukocytes and IFNβ by fibroblasts. Both IFNα and IFNβ inhibit HIV-1 transcription when added to cultures of macrophages or monocytes following infection [56, 66]. The inhibition resulted from a decrease in LTR-mediated transcription and from degradation of viral RNA [56]. In contrast, other reports have suggested that IFNα and IFNβ do not act at the level of transcription, but rather, inhibit at a posttranslational level [67-69]. Type I interferons (IFNα and IFNβ) have been shown to inhibit HIV-1 transcription through the induction of C/EBP in macrophages [70]. This inhibition required intact C/EBP sites in the LTR and the induction of the interferon-specific transcription factor ISGF-3 [70]. IFNα was also found to inhibit NFκB dependent transcription of HIV-1 induced by TNFα in Jurkat T cells [71]. IFN-τ is a type 1 interferon,

which is expressed in ruminants, has antiviral and antiproliferative properties and is less cytotoxic than IFNα and IFNβ [72, 73]. IFN-τ inhibits HIV transcription more strongly than IFNα in human macrophages [74]. In addition, IFN-τ has inhibitory effects upon reverse transcription [74]. Although IFN-τ is not expressed in human cells, its antiviral activity and low toxicity may identify it as a possible treatment for HIV infection.

IL-10 has been shown to inhibit the production of cytokines by T cells, macrophages and natural killer cells [75, 76]. This anti-inflammatory cytokine is up-regulated in HIV infection and acts to down-regulate cellular immune responses to HIV [5]. IL-10 is produced by cells of the monocyte/macrophage lineage, B cells and CD4+ and CD8+ T cells [76-78]. The production of IL-1, TNFα, IL-8 and IL-6 are suppressed by IL-10 through the inhibition of NFκB [79-81]. In addition to this role, IL-10 also modulates HIV-1 transcription. IL-10 enhances TNFα activation of the HIV LTR in latently infected T cells and in cells of the monocyte/macrophage lineage [82, 83]. This increase in activation of the LTR was also associated with an elevation in endogenous TNFα in both cell types [83]. In another study it was demonstrated that IL-10 and TNFα enhanced the binding of AP-1 and NFκB to the LTR. In the absence of TNFα however, IL-10 has no effect upon HIV replication in monocytic cells and inhibits replication in T cells [82, 83]. In contrast, Kootstra *et al.* reported that IL-10 on its own inhibited HIV replication in primary macrophages but not T cells [84]. The inhibition was not found to be at the level of transcription, but possibly at the level of protein processing [84]. Therefore this provides another example of how the cytokine milieu *in vivo* may markedly change the transcription of HIV-1. The ability of IL-10 to modulate transcription in monocytic cells is also dependent upon the state of differentiation of the cell. IL-10 pre-treatment followed by infection with HIV_{Bal}, inhibited HIV-1 RNA expression in monocytes [85]. However, the inhibition waned as the cells matured into macrophages. In contrast, the inhibition of transcription was not observed in the monocytic cell line THP-1α [85]. It is also important to note that the up-regulation of IL-10 in HIV infection may be a direct result of viral infection as the HIV Tat protein has been reported to induce IL-10 production by monocytes [86]. The Vpr protein of HIV-1 also up-regulates IL-10 in addition to IL-6 and the chemokine IL-8. These effects are due to the activation of NFκB and NF-IL-6 transcription factors, which also results in the increased replication of HIV-1 in monocytic cells [87].

The anti-inflammatory cytokine IL-4 is produced by Th2 cells [88]. These cells may be up-regulated *in vivo* in HIV infection [89-91]. IL-4 was shown to stimulate transcription of HIV-1 in PBMC [92]. IL-4 has also been shown to increase HIV transcription 2-3 fold in monocytes cultured on Teflon by stimulating the nuclear translocation of NFκB [93]. Both IL-4 and IL-13 significantly enhanced transcription in monocyte derived macrophages. However, following 3-5 days adherence to plastic this effect was reversed and HIV transcription was inhibited [93]. Similarly, in chronically infected U1 cells, HIV transcription was inhibited by IL-4 and this inhibition was dependent upon the NFκB sites of the LTR [94]. However, IL-4 may also inhibit the replication of macrophage tropic strains of HIV-1 and enhance replication of T cell tropic strains by differentially regulating HIV-1 co-receptors [92, 95-98]. Contrasting reports on the effects of IL-4 on HIV transcription, most notably in cells of the monocyte/macrophage lineage, likely reflect the differentiated state of the different cell lines used. In addition, the state of differentiation and activation may differ depending upon the method of cell culture (ie. adherence to plastic versus teflon) and to the presence of other cytokines or other stimulators [99].

III. Chemokines

In HIV infection, specific chemokines may block the entry of HIV-1 into the cell by binding to their natural receptors. Cellular entry of T-cell tropic isolates of HIV-1 may be blocked by the ligand for CXCR4, stromal cell-derivedfactor-1α (SDF-1α), a member of the α-chemokine family. SDF-1α may block T cell tropic HIV-1 entry by binding to the CXCR4 receptor [100, 101]. The β-chemokine receptors CCR5 [102-104], CCR3 [105, 106] and CCR2b [105] were identified as co-receptors used by macrophage tropic isolates for entry into CD4+ T cells. The natural ligands for CCR5, macrophage inhibitory protein-1α (MIP-1α), MIP-1β and regulated on activation normal T cell expressed and secreted (RANTES), block entry of macrophage tropic HIV-1 [102, 103]. It was later established that individuals carrying a homozygous deletion of 32 base pairs in the co-receptor CCR5 were resistant to HIV-1 infection [107, 108]. Those individuals heterozygous for the deletion are susceptible to infection but demonstrate a slower rate of disease progression [107, 109]. In some cohorts of infected subjects, mutation of a CCR2 allele (64I) has been associated with slower progression to disease [110-114] as has a mutation in the 3' untranslated region of the SDF-1 gene [115]. Several other co-receptors have been identified including CCR8 [116], CXC_3R1 [117], STRL33/Bonzo and GPR15/BOB which mediate entry of HIV-2, SIV and dual tropic strains of HIV [52, 118-120].

In addition to the role played by chemokines in blocking viral entry, the effects of chemokines on LTR-driven transcription have also been previously studied. The chemokine RANTES did not alter HIV-1 LTR-mediated transcription in CD4+ T cell lines [121]. Reports have indicated that the β-chemokines, MIP-1α, MIP-1β and RANTES, have no effect [122] or stimulate [123] HIV-1 replication in macrophages. It has been reported that HIV-1 infection enhanced the production of MIP-1α and MIP-1β by monocytes and that the ability of these chemokines to inhibit or stimulate HIV replication was cell-type dependent [123, 124]. Only one study has demonstrated the suppression of LTR-mediated transcription by entry blocking chemokines. This study reported the suppression of LTR-mediated transcription by the combined action of MIP-1α, MIP-1β and RANTES in Jurkat T cells [125]. This study also demonstrated that the inhibition was not mediated by the NFAT-1 element. However, another study demonstrated that exposure of cells infected with T cell tropic HIV-1 to SDF-1α led to increased replication [126]. In Jurkat T cells SDF-1α activated ERK-1 and ERK-2 while in HeLa cells SDF-1α activated only ERK-2. SDF-1α-dependent HIV-1 expression was sensitive to blocking of ERK activation by pertussis toxin or the MEK inhibitor U0126 [126]. In a separate study, SDF-1α was shown to

activate the JAK/STAT pathway in human T cell lines [127]. In addition, Marechal *et al*. reported that SDF-1α increased the ability of HIV-1 Tat to trans-activate the LTR [128]. Nagira and colleagues have shown that secondary lymphoid chemokine (SLC) [129], also known as 6Ckine [130] or exodus-2 [131], which is expressed in the secondary lymphoid tissues, can enhance HIV promoter activity in HeLa cells [132]. The molecular mechanisms underlying the enhancement have not been determined. Similarly, the chemokine SDF-1α was shown to enhance LTR-mediated transcription of CCR5-tropic HIV-1 in HeLa cells expressing CD4, CCR5 and CXCR4 [128].

Interferonγ-inducible protein-10 (IP-10) is produced by monocytes, activated T cells, endothelial cells and keratinocytes in response to IFNγ treatment [133, 134], resulting in the chemoattraction of PBMC and activated T cells [135]. IP-10 has several functions *in vivo* including antiviral activity, inhibition of angiogenesis and inhibition of the growth of haematopoietic progenitor cells and tumor cells [136-140]. Although IP-10 has antiviral activity [138], it can increase HIV replication in Jurkat T cells, and this enhancement does not occur at the level of transcription [141]. Instead, IP-10 induces an increase in the accumulation of viral DNA [141]. Macrophage derived chemokine (MDC) is a chemoattractant for polarized type II CD4+ and CD8+ T cells [142-144]. MDC inhibits HIV replication in macrophages but not in T cells [145]. The inhibition of HIV by MDC appears to occur at a post entry step which did not involve reverse transcription [145]. The effects of MDC on transcription have not been reported.

MODULATION OF HIV-1 TRANSCRIPTION BY CD8+ T CELL-DERIVED FACTORS

Pertinent to a discussion of the modulation of HIV-1 transcription by cytokines and chemokines is a description of another activity produced by CD8+ T cells. CD8+ T cells produce IFNγ and IL-16, which inhibit HIV-1 transcription in T cells [27, 28, 38, 39]. CD8+ T cells also produce an antiviral factor (CAF) which was originally described by Walker *et al*. in 1986 [146]. The production of CAF by CD8+ T cells was shown to be associated with higher CD4+ cell counts, improved clinical status [147, 148] and long-term non-progression [149, 150]. CAF was later shown not to share an identity with known cytokines or chemokines. While CAF inhibits transcription primarily via the NFκB and COUP elements in the LTR in T cells, in monocytic cells CAF induces an increase in HIV-1 transcription via the same transcription factor binding sites [151-153]. As shown in Table 1 there may be opposite effects upon HIV-1 transcription by different combinations of cytokines depending on cell type. CAF activity could be the result of two cytokines working in concert to produce these opposite effects on LTR-mediated transcription. Several proteins have been suggested to represent CAF activity, including the β-chemokines MIP-1α, MIP-1β and RANTES [154], the α-defensins [155-157], and natural killer enhancing factors (NKEF) A and B [158]. However, while these factors may inhibit HIV-1 replication, the inhibition is not mediated at the level of HIV-1 transcription [122, 155-160].

IMMUNE BASED THERAPY IN HIV INFECTION

In addition to the use of current antiretroviral drugs to treat HIV-1 infection, several cytokines have been tested in clinical trials to determine their effects on viral replication and immune responses *in vivo*. In a trial of GM-CSF, a statistically significant reduction in infection or death was observed in patients receiving GM-CSF than in those receiving placebo [161]. In addition, GM-CSF treatment resulted in better control of virus replication and higher CD4 cell counts. In another clinical trial, GM-CSF was administered to HIV-1 infected patients treated with zidovudine and didanosine [162]. A statistically significant greater decline in HIV-1 RNA levels was observed in patients receiving GM-CSF as compared to patients receiving placebo. In addition, 38% of patients treated with GM-CSF had higher CD4 cell counts than those in the placebo group. There was also a lower frequency of codon mutations associated with zidovudine resistance in the GM-CSF group. GM-CSF treatment was also used in a clinical trial of patients receiving indinavir or ritonavir whose virus replication was stably suppressed [163]. In patients treated with GM-CSF a greater than 0.5 $\log_{10}$ decrease in viral load and a greater than 30% increase in CD4 cell counts was observed as compared to the placebo group.

Several studies have been performed to examine the effects of IFNα on HIV-1 infection. Early trials of IFNα did not report clinical benefits or adverse effects for treatment with this cytokine [164-168]. Treatment of HIV-1 infected patients with an IFNα vaccine, however, did result in lower rates of progression to disease, although these rate changes were not statistically significant [169].

IL-2 has also been used as a treatment for HIV-1 infection. Intermittent treatment of patients with CD4 cell counts greater than 200/μl with recombinant IL-2 resulted in increased CD4 cell counts [170]. Following dose escalation in 10 patients, 6 achieved a 50% or greater increase in CD4 cell counts. However, IL-2 treatment was also associated with toxicities in patients with CD4 cell counts of 200/μl or less. A transient increase in viral load was also observed in this patient group suggesting that IL-2 treatment in patients with low CD4 cell counts could lead to increases in viral burden. Another clinical trial demonstrated that long term IL-2 treatment of patients with CD4 cell counts greater than 200/μl resulted in an approximate 2-fold increase in CD4 cell counts [171]. The increased CD4 cell counts were maintained for greater than 2 years with continued IL-2 treatment. Similar effects of IL-2 on CD4 cell counts have been observed in subsequent clinical trials [172-183]. Low dose IL-2 treatment plus HAART resulted in increased NK cell populations and CD4 cell counts compared to HAART treatment alone [177]. The change in CD4 cell count resulted from increased naïve CD4 cells. In another study of IL-2 combined with antiretroviral treatment, in addition to increased CD4 and NK cell numbers, reduced levels of CD8+CD38+ and CD8+HLA-DR+ cells and increased levels of CD8+CD25+ and CD8+CD122+ cells were observed [184]. Therefore increases of cell sub-types, which are associated with a lack of progression to disease, were present and those associated with disease progression were reduced. In an earlier study which examined adoptive therapy of HIV-1 infected patients (with mild to severe disease) with

activated autologous CD8+ T cells and IL-2 infusion, improvement of oral hairy leukoplakia and Kaposi's sarcoma was observed [185].

Only one study has been performed to examine the effects of IL-3 treatment on HIV-1 infection *in vivo* [186]. Increased white blood cell counts and neutrophil counts were observed but CD4 cell counts were unaffected. A transient increase in CD4 cell counts was observed in one study of IL-4 treatment of HIV-1 infection [187]. In this trial of HIV-1 infected patients with Kaposi's sarcoma, 4 of 11 patients had marked decline in plasma HIV-1 RNA after 4 weeks of treatment. Recombinant IL-10 has also been tested for its effects on HIV-1 infection, however, no virological or immunological benefit was demonstrated in treated patients [188]. Therefore the cytokines IL-2 and GM-CSF have shown potential in improving CD4+ cell counts and control of HIV-1 replication *in vivo* and hold promise for future treatment regimens.

SUMMARY

Cytokines have pleiotropic effects on HIV-1 transcription. These effects are complex and require careful interpretation of the experimental conditions. It is important to understand how cytokines affect HIV-1 replication and whether this control is at the level of transcription. This is particularly important in the case of chemokines which may inhibit HIV replication at the level of viral entry with perhaps no effect at the transcriptional level. Our understanding of cytokines and their actions in HIV infection is underscored by their current use *in vivo* to enhance immune responses in individuals on antiretroviral drug treatment.

REFERENCES

[1] Clerici, M.; Shearer, G. M. *Immunol. Today*, **1993**, *14*, 107.
[2] Berkhout, B.; Silverman, R. H.; Jeang, K. T. *Cell*, **1989**, *59*, 273.
[3] Berkhout, B.; Jeang, K. T. *J. Virol.*, **1992**, *66*, 139.
[4] Copeland, K. F.; Heeney, J. L. *Microbiol. Rev.*, **1996**, *60*, 722.
[5] Breen, E. C. *Pharmacol. Ther.*, **2002**, *95*, 295.
[6] Tyor, W. R.; Glass, J. D.; Griffin, J. W.; Becker, P. S.; McArthur, J. C.; Bezman, L.; Griffin, D. E. *Ann. Neurol.*, **1992**, *31*, 349.
[7] Folks, T. M.; Clouse, K. A.; Justement, J.; Rabson, A.; Duh, E.; Kehrl, J. H.; Fauci, A. S. *Proc. Natl. Acad. Sci. USA*, **1989**, *86*, 2365.
[8] Matsuyama, T.; Kobayashi, N.; Yamamoto, N. *AIDS*, **1991**, *5*, 1405.
[9] Mellors, J. W.; Griffith, B. P.; Ortiz, M. A.; Landry, M. L.; Ryan, J. L. *J. Infect. Dis.*, **1991**, *163*, 78.
[10] Duh, E. J.; Maury, W. J.; Folks, T. M.; Fauci, A. S.; Rabson, A. B. *Proc. Natl. Acad. Sci. USA*, **1989**, *86*, 5974.
[11] Munoz-Fernandez, M. A.; Navarro, J.; Garcia, A.; Punzon, C.; Fernandez-Cruz, E.; Fresno, M. *J. Allergy Clin. Immunol.*, **1997**, *100*, 838.
[12] Howard, O. M.; Clouse, K. A.; Smith, C.; Goodwin, R. G.; Farrar, W. L. *Proc. Natl. Acad. Sci. USA*, **1993**, *90*, 2335.
[13] Swingler, S.; Easton, A.; Morris, A. *AIDS Res. Hum. Retro.*, **1992**, *8*, 487.
[14] Chene, L.; Nugeyre, M. T.; Barre-Sinoussi, F.; Israel, N. *J. Virol.*, **1999**, *73*, 2064.
[15] Boise, L. H.; Minn, A. J.; June, C. H.; Lindsten, T.; Thompson, C. B. *Proc. Natl. Acad. Sci. USA*, **1995**, *92*, 5491.
[16] Vella, A. T.; Dow, S.; Potter, T. A.; Kappler, J.; Marrack, P. *Proc. Natl. Acad. Sci. USA*, **1998**, *95*, 3810.
[17] Vella, A.; Teague, T. K.; Ihle, J.; Kappler, J.; Marrack, P. *J. Exp. Med.*, **1997**, *186*, 325.
[18] Westendorp, M. O.; Shatrov, V. A.; Schulze-Osthoff, K.; Frank, R.; Kraft, M.; Los, M.; Krammer, P. H.; Droge, W.; Lehmann, V. *EMBO J.*, **1995**, *14*, 546.
[19] Latham, P. S.; Lewis, A. M.; Varesio, L.; Pavlakis, G. N.; Felber, B. K.; Ruscetti, F. W.; Young, H. A. *Cell. Immunol.*, **1990**, *129*, 513.
[20] Goletti, D.; Kinter, A. L.; Hardy, E. C.; Poli, G.; Fauci, A. S. *J. Immunol.*, **1996**, *156*, 3501.
[21] Massague, J. *Annu. Rev. Cell. Biol.*, **1990**, *6*, 597.
[22] Li, J. M.; Shen, X.; Hu, P. P.; Wang, X. F. *Mol. Cell. Biol.*, **1998**, *18*, 110.
[23] Coyle-Rink, J.; Sweet, T.; Abraham, S.; Sawaya, B.; Batuman, O.; Khalili, K.; Amini, S. *Virol.*, **2002**, *299*, 240.
[24] Poli, G.; Kinter, A. L.; Justement, J. S.; Bressler, P.; Kehrl, J. H.; Fauci, A. S. *Proc. Natl. Acad. Sci. USA*, **1992**, *89*, 2689.
[25] Poli, G.; Kinter, A. L.; Justement, J. S.; Bressler, P.; Kehrl, J. H.; Fauci, A. S. *J. Exp. Med.*, **1991**, *173*, 589.
[26] Fuchs, D.; Hausen, A.; Reibnegger, G.; Werner, E. R.; Werner-Felmayer, G.; Dierich, M. P.; Wachter, H. *J. Acquir. Immune Defic. Syndr.*, **1989**, *2*, 158.
[27] Hartshorn, K. L.; Neumeyer, D.; Vogt, M. W.; Schooley, R. T.; Hirsch, M. S. *AIDS Res. Hum. Retro.*, **1987**, *3*, 125.
[28] Emilie, D.; Maillot, M. C.; Nicolas, J. F.; Fior, R.; Galanaud, P. *J. Biol. Chem.*, **1992**, *267*, 20565.
[29] Warfel, A. H.; Thorbecke, G. J.; Belsito, D. V. *J. Leukoc. Biol.*, **1995**, *57*, 469.
[30] Poli, G.; Bressler, P.; Kinter, A.; Duh, E.; Timmer, W. C.; Rabson, A.; Justement, J. S.; Stanley, S.; Fauci, A. S. *J. Exp. Med.*, **1990**, *172*, 151.
[31] Biswas, P.; Poli, G.; Kinter, A. L.; Justement, J. S.; Stanley, S. K.; Maury, W. J.; Bressler, P.; Orenstein, J. M.; Fauci, A. S. *J. Exp. Med.*, **1992**, *176*, 739.
[32] Poli, G.; Kinter, A. L.; Fauci, A. S. *Proc. Natl. Acad. Sci. USA*, **1994**, *91*, 108.
[33] Roux-Lombard, P.; Modoux, C.; Cruchaud, A.; Dayer, J. M. *Clin. Immunol. Immunopathol.*, **1989**, *50*, 374.
[34] Bellini, A.; Yoshimura, H.; Vittori, E.; Marini, M.; Mattoli, S. *J. Allergy Clin. Immunol.*, **1993**, *92*, 412.
[35] Laberge, S.; Cruikshank, W. W.; Kornfeld, H.; Center, D. M. *J. Immunol.*, **1995**, *155*, 2902.
[36] Lim, K. G.; Wan, H. C.; Bozza, P. T.; Resnick, M. B.; Wong, D. T.; Cruikshank, W. W.; Kornfeld, H.; Center, D. M.; Weller, P. F. *J. Immunol.*, **1996**, *156*, 2566.
[37] Rumsaeng, V.; Cruikshank, W. W.; Foster, B.; Prussin, C.; Kirshenbaum, A. S.; Davis, T. A.; Kornfeld, H.; Center, D. M.; Metcalfe, D. D. *J. Immunol.*, **1997**, *159*, 2904.
[38] Maciaszek, J. W.; Parada, N. A.; Cruikshank, W. W.; Center, D. M.; Kornfeld, H.; Viglianti, G. A. *J. Immunol.*, **1997**, *158*, 5.
[39] Zhou, P.; Goldstein, S.; Devadas, K.; Tewari, D.; Notkins, A. L. *Nat. Med.*, **1997**, *3*, 659.
[40] Truong, M. J.; Darcissac, E. C.; Hermann, E.; Dewulf, J.; Capron, A.; Bahr, G. M. *J. Virol.*, **1999**, *73*, 7008.
[41] Mathy, N. L.; Scheuer, W.; Lanzendorfer, M.; Honold, K.; Ambrosius, D.; Norley, S.; Kurth, R. *Immunol.*, **2000**, *100*, 63.
[42] Krautwald, S. *J. Immunol.*, **1998**, *160*, 5874.
[43] Okamura, H.; Tsutsi, H.; Komatsu, T.; Yutsudo, M.; Hakura, A.; Tanimoto, T.; Torigoe, K.; Okura, T.; Nukada, Y.; Hattori, K.; Akita, K.; Namba, M.; Tanabe, F.; Konishi, K.; Fukudu, S.; Kurimoto, M. *Nature*, **1995**, *378*, 88.
[44] Nakamura, K.; Okamura, H.; Nagata, K.; Komatsu, T.; Tamura, T. *Infect. Immun.*, **1993**, *61*, 64.
[45] Nakamura, K.; Okamura, H.; Wada, M.; Nagata, K.; Tamura, T. *Infect. Immun.*, **1989**, *57*, 590.
[46] Nakanishi, K.; Yoshimoto, T.; Tsutsui, H.; Okamura, H. *Cytokine Growth Factor Rev.*, **2001**, *12*, 53.
[47] Stylianou, E.; Bjerkeli, V.; Yndestad, A.; Heggelund, L.; Waehre, T.; Damas, J. K.; Aukrust, P.; Froland, S. S. *Clin. Exp. Immunol.*, **2003**, *132*, 462.
[48] Choi, H. J.; Dinarello, C. A.; Shapiro, L. *J. Infect. Dis.*, **2001**, *184*, 560.
[49] Klein, S. A.; Klebba, C.; Kauschat, D.; Pape, M.; Ozmen, L.; Hoelzer, D.; Ottmann, O. G.; Kalina, U. *Eur. Cytokine Netw.*, **2000**, *11*, 47.
[50] Shapiro, L.; Puren, A. J.; Barton, H. A.; Novick, D.; Peskind, R. L.; Shenkar, R.; Gu, Y.; Su, M. S.; Dinarello, C. A. *Proc. Natl. Acad. Sci. USA*, **1998**, *95*, 12550.

[51] Arai, K. I.; Lee, F.; Miyajima, A.; Miyatake, S.; Arai, N.; Yokota, T. *Ann. Rev. Biochem.*, **1990**, *59*, 783.

[52] Pohlmann, S.; Krumbiegel, M.; Kirchhoff, F. *J. Gen. Virol.*, **1999**, *80 (Pt 5)*, 1241.

[53] Bergamini, A.; Perno, C. F.; Dini, L.; Capozzi, M.; Pesce, C. D.; Ventura, L.; Cappannoli, L.; Falasca, L.; Milanese, G.; Calio, R. *Blood*, **1994**, *84*, 3405.

[54] Folks, T. M.; Justement, J.; Kinter, A.; Dinarello, C. A.; Fauci, A. S. *Science*, **1987**, *238*, 800.

[55] Gendelman, H. E.; Orenstein, J. M.; Martin, M. A.; Ferrua, C.; Mitra, R.; Phipps, T.; Wahl, L. A.; Lane, H. C.; Fauci, A. S.; Burke, D. S. *J. Exp. Med.*, **1988**, *167*, 1428.

[56] Gendelman, H. E.; Baca, L.; Turpin, J. A.; Kalter, D. C.; Hansen, B. D.; Orenstein, J. M.; Friedman, R. M.; Meltzer, M. S. *AIDS Res. Hum. Retro.*, **1990**, *6*, 1045.

[57] Koyanagi, Y.; O'Brien, W. A.; Zhao, J. Q.; Golde, D. W.; Gasson, J. C.; Chen, I. S. *Science*, **1988**, *241*, 1673.

[58] Schuitemaker, H.; Kootstra, N. A.; Koppelman, M. H.; Bruisten, S. M.; Huisman, H. G.; Tersmette, M.; Miedema, F. *J. Clin. Invest.*, **1992**, *89*, 1154.

[59] Si, Q.; Cosenza, M.; Zhao, M. L.; Goldstein, H.; Lee, S. C. *Glia*, **2002**, *39*, 174.

[60] Kitano, K.; Abboud, C. N.; Ryan, D. H.; Quan, S. G.; Baldwin, G. C.; Golde, D. W. *Blood*, **1991**, *77*, 1699.

[61] Kutza, J.; Crim, L.; Feldman, S.; Hayes, M. P.; Gruber, M.; Beeler, J.; Clouse, K. A. *J. Immunol.*, **2000**, *164*, 4955.

[62] Kedzierska, K.; Maerz, A.; Warby, T.; Jaworowski, A.; Chan, H.; Mak, J.; Sonza, S.; Lopez, A.; Crowe, S. *AIDS*, **2000**, *14*, 1739.

[63] Matsuda, S.; Akagawa, K.; Honda, M.; Yokota, Y.; Takebe, Y.; Takemori, T. *AIDS Res. Hum. Retro.*, **1995**, *11*, 1031.

[64] Watanabe, S.; Murakami, T.; Nakamura, T.; Morimoto, C.; Arai, K. *Biochimie*, **2002**, *84*, 633.

[65] Zhang, M. Y.; Harhaj, E. W.; Bell, L.; Sun, S. C.; Miller, B. A. *Blood*, **1998**, *92*, 1225.

[66] Gendelman, H. E.; Baca, L. M.; Turpin, J.; Kalter, D. C.; Hansen, B.; Orenstein, J. M.; Dieffenbach, C. W.; Friedman, R. M.; Meltzer, M. S. *J. Immunol.*, **1990**, *145*, 2669.

[67] Yamamoto, J. K.; Barre-Sinoussi, F.; Bolton, V.; Pedersen, N. C.; Gardner, M. B. *J. Interferon Res.*, **1986**, *6*, 143.

[68] Smith, M. S.; Thresher, R. J.; Pagano, J. S. *Antimicrob. Agents Chemother.*, **1991**, *35*, 62.

[69] Ho, D. D.; Hartshorn, K. L.; Rota, T. R.; Andrews, C. A.; Kaplan, J. C.; Schooley, R. T.; Hirsch, M. S. *Lancet*, **1985**, *1*, 602.

[70] Honda, Y.; Rogers, L.; Nakata, K.; Zhao, B. Y.; Pine, R.; Nakai, Y.; Kurosu, K.; Rom, W. N.; Weiden, M. *J. Exp. Med.*, **1998**, *188*, 1255.

[71] Hottiger, M. O.; Felzien, L. K.; Nabel, G. J. *EMBO J.*, **1998**, *17*, 3124.

[72] Pontzer, C. H.; Yamamoto, J. K.; Bazer, F. W.; Ott, T. L.; Johnson, H. M. *J. Immunol.*, **1997**, *158*, 4351.

[73] Dereuddre-Bosquet, N.; Clayette, P.; Martin, M.; Mabondzo, A.; Fretier, P.; Gras, G.; Martal, J.; Dormont, D. *J. Acquir. Immune Defic. Syndr. Hum. Retrovirol.*, **1996**, *11*, 241.

[74] Rogez, C.; Martin, M.; Dereuddre-Bosquet, N.; Martal, J.; Dormont, D.; Clayette, P. *J. Virol.*, **2003**, *77*, 12914.

[75] Vieira, P.; de Waal-Malefyt, R.; Dang, M. N.; Johnson, K. E.; Kastelein, R.; Fiorentino, D. F.; deVries, J. E.; Roncarolo, M. G.; Mosmann, T. R.; Moore, K. W. *Proc. Natl. Acad. Sci. USA*, **1991**, *88*, 1172.

[76] Fiorentino, D. F.; Bond, M. W.; Mosmann, T. R. *J. Exp. Med.*, **1989**, *170*, 2081.

[77] Del Prete, G.; De Carli, M.; Almerigogna, F.; Giudizi, M. G.; Biagiotti, R.; Romagnani, S. *J. Immunol.*, **1993**, *150*, 353.

[78] O'Garra, A.; Stapleton, G.; Dhar, V.; Pearce, M.; Schumacher, J.; Rugo, H.; Barbis, D.; Stall, A.; Cupp, J.; Moore, K. *Int. Immunol.*, **1990**, *2*, 821.

[79] Wang, P.; Wu, P.; Anthes, J. C.; Siegel, M. I.; Egan, R. W.; Billah, M. M. *Blood*, **1994**, *83*, 2678.

[80] Wang, P.; Wu, P.; Siegel, M. I.; Egan, R. W.; Billah, M. M. *J. Biol. Chem.*, **1995**, *270*, 9558.

[81] Wang, P.; Wu, P.; Siegel, M. I.; Egan, R. W.; Billah, M. M. *J. Immunol.*, **1994**, *153*, 811.

[82] Finnegan, A.; Roebuck, K. A.; Nakai, B. E.; Gu, D. S.; Rabbi, M. F.; Song, S.; Landay, A. L. *J. Immunol.*, **1996**, *156*, 841.

[83] Rabbi, M. F.; Finnegan, A.; Al-Harthi, L.; Song, S.; Roebuck, K. A. *J. Acquir. Immune Defic. Syndr. Hum. Retrovirol.*, **1998**, *19*, 321.

[84] Kootstra, N. A.; van 't Wout, A.; Huisman, H. G.; Miedema, F.; Schuitemaker, H. *J. Virol.*, **1994**, *68*, 6967.

[85] Naif, H. M.; Chang, J.; Ho-Shon, M.; Li, S.; Cunningham, A. L. *AIDS Res. Hum. Retro.*, **1996**, *12*, 1237.

[86] Yamina, B.; Xavier, C.; Marc, M.; Catherine, L.; Abdallah, B.; Bahraoui, E. *J. Soc. Biol.*, **2001**, *195*, 319.

[87] Roux, P.; Alfieri, C.; Hrimech, M.; Cohen, E. A.; Tanner, J. E. *J. Virol.*, **2000**, *74*, 4658.

[88] O'Garra, A. *Immunity*, **1998**, *8*, 275.

[89] Lee, B. N.; Lu, J. G.; Kline, M. W.; Paul, M.; Doyle, M.; Kozinetz, C.; Shearer, W. T.; Reuben, J. M. *Clin. Diagn. Lab. Immunol.*, **1996**, *3*, 493.

[90] Clerici, M.; Hakim, F. T.; Venzon, D. J.; Blatt, S.; Hendrix, C. W.; Wynn, T. A.; Shearer, G. M. *J. Clin. Invest.*, **1993**, *91*, 759.

[91] Clerici, M.; Shearer, G. M. *Immunol. Today*, **1994**, *15*, 575.

[92] Valentin, A.; Lu, W.; Rosati, M.; Schneider, R.; Albert, J.; Karlsson, A.; Pavlakis, G. N. *Proc. Natl. Acad. Sci. USA*, **1998**, *95*, 8886.

[93] Naif, H. M.; Li, S.; Ho-Shon, M.; Mathijs, J. M.; Williamson, P.; Cunningham, A. L. *J. Immunol.*, **1997**, *158*, 501.

[94] Goletti, D.; Kinter, A. L.; Coccia, E. M.; Battistini, A.; Petrosillo, N.; Ippolito, G.; Poli, G. *Cytokine*, **2002**, *17*, 28.

[95] Suzuki, Y.; Koyanagi, Y.; Tanaka, Y.; Murakami, T.; Misawa, N.; Maeda, N.; Kimura, T.; Shida, H.; Hoxie, J. A.; O'Brien, W. A.; Yamamoto, N. *J. Virol.*, **1999**, *73*, 316.

[96] Wang, J.; Harada, A.; Matsushita, S.; Matsumi, S.; Zhang, Y.; Shioda, T.; Nagai, Y.; Matsushima, K. *J. Leukoc. Biol.*, **1998**, *64*, 642.

[97] Galli, G.; Annunziato, F.; Mavilia, C.; Romagnani, P.; Cosmi, L.; Manetti, R.; Pupilli, C.; Maggi, E.; Romagnani, S. *Eur. J. Immunol.*, **1998**, *28*, 3280.

[98] Jourdan, P.; Abbal, C.; Noraz, N.; Hori, T.; Uchiyama, T.; Vendrell, J. P.; Bousquet, J.; Taylor, N.; Pene, J.; Yssel, H.; Nora, N. *J. Immunol.*, **1998**, *160*, 4153.

[99] Bennett, S.; Breit, S. N. *J. Leukoc. Biol.*, **1994**, *56*, 236.

[100] Bleul, C. C.; Farzan, M.; Choe, H.; Parolin, C.; Clark-Lewis, I.; Sodroski, J.; Springer, T. A. *Nature*, **1996**, *382*, 829.

[101] Oberlin, E.; Amara, A.; Bachelerie, F.; Bessia, C.; Virelizier, J. L.; Arenzana-Seisdedos, F.; Schwartz, O.; Heard, J. M.; Clark-Lewis, I.; Legler, D. F.; Loetscher, M.; Baggiolini, M.; Moser, B. *Nature*, **1996**, *382*, 833.

[102] Deng, H.; Liu, R.; Ellmeier, W.; Choe, S.; Unutmaz, D.; Burkhart, M.; Di Marzio, P.; Marmon, S.; Sutton, R. E.; Hill, C. M.; Davis, C. B.; Peiper, S. C.; Schall, T. J.; Littman, D. R.; Landau, N. R. *Nature*, **1996**, *381*, 661.

[103] Dragic, T.; Litwin, V.; Allaway, G. P.; Martin, S. R.; Huang, Y.; Nagashima, K. A.; Cayanan, C.; Maddon, P. J.; Koup, R. A.; Moore, J. P.; Paxton, W. A. *Nature*, **1996**, *381*, 667.

[104] Alkhatib, G.; Combadiere, C.; Broder, C. C.; Feng, Y.; Kennedy, P. E.; Murphy, P. M.; Berger, E. A. *Science*, **1996**, *272*, 1955.

[105] Doranz, B. J.; Rucker, J.; Yi, Y.; Smyth, R. J.; Samson, M.; Peiper, S. C.; Parmentier, M.; Collman, R. G.; Doms, R. W. *Cell*, **1996**, *85*, 1149.

[106] Choe, H.; Farzan, M.; Sun, Y.; Sullivan, N.; Rollins, B.; Ponath, P. D.; Wu, L.; Mackay, C. R.; LaRosa, G.; Newman, W.; Gerard, N.; Gerard, C.; Sodroski, J. *Cell*, **1996**, *85*, 1135.

[107] Dean, M.; Carrington, M.; Winkler, C.; Huttley, G. A.; Smith, M. W.; Allikmets, R.; Goedert, J. J.; Buchbinder, S. P.; Vittinghoff, E.; Gomperts, E.; Donfield, S.; Vlahov, D.; Kaslow, R.; Saah, A.; Rinaldo, C.; Detels, R.; O'Brien, S. J. *Science*, **1996**, *273*, 1856.

[108] Liu, R.; Paxton, W. A.; Choe, S.; Ceradini, D.; Martin, S. R.; Horuk, R.; MacDonald, M. E.; Stuhlmann, H.; Koup, R. A.; Landau, N. R. *Cell*, **1996**, *86*, 367.

[109] Huang, Y.; Paxton, W. A.; Wolinsky, S. M.; Neumann, A. U.; Zhang, L.; He, T.; Kang, S.; Ceradini, D.; Jin, Z.; Yazdanbakhsh, K.; Kunstman, K.; Erickson, D.; Dragon, E.; Landau, N. R.; Phair, J.; Ho, D. D.; Koup, R. A. *Nat. Med.*, **1996**, *2*, 1240.

[110] Smith, M. W.; Dean, M.; Carrington, M.; Winkler, C.; Huttley, G. A.; Lomb, D. A.; Goedert, J. J.; O'Brien, T. R.; Jacobson, L. P.; Kaslow, R.; Buchbinder, S.; Vittinghoff, E.; Vlahov, D.; Hoots, K.; Hilgartner, M. W.; O'Brien, S. J. *Science*, **1997**, *277*, 959.

[111] Smith, M. W.; Carrington, M.; Winkler, C.; Lomb, D.; Dean, M.; Huttley, G.; O'Brien, S. J. *Nat. Med.*, **1997**, *3*, 1052.

[112] Rizzardi, G. P.; Morawetz, R. A.; Vicenzi, E.; Ghezzi, S.; Poli, G.; Lazzarin, A.; Pantaleo, G. *Nat. Med.*, **1998**, *4*, 252.

[113] Kostrikis, L. G.; Huang, Y.; Moore, J. P.; Wolinsky, S. M.; Zhang, L.; Guo, Y.; Deutsch, L.; Phair, J.; Neumann, A. U.; Ho, D. D. *Nat. Med.*, **1998**, *4*, 350.

[114] Anzala, A. O.; Ball, T. B.; Rostron, T.; O'Brien, S. J.; Plummer, F. A.; Rowland-Jones, S. L. *Lancet*, **1998**, *351*, 1632.

[115] Winkler, C.; Modi, W.; Smith, M. W.; Nelson, G. W.; Wu, X.; Carrington, M.; Dean, M.; Honjo, T.; Tashiro, K.; Yabe, D.; Buchbinder, S.; Vittinghoff, E.; Goedert, J. J.; O'Brien, T. R.; Jacobson, L. P.; Detels, R.; Donfield, S.; Willoughby, A.; Gomperts, E.; Vlahov, D.; Phair, J.; O'Brien, S. J. *Science*, **1998**, *279*, 389.

[116] Rucker, J.; Edinger, A. L.; Sharron, M.; Samson, M.; Lee, B.; Berson, J. F.; Yi, Y.; Margulies, B.; Collman, R. G.; Doranz, B. J.; Parmentier, M.; Doms, R. W. *J. Virol.*, **1997**, *71*, 8999.

[117] Combadiere, C.; Salzwedel, K.; Smith, E. D.; Tiffany, H. L.; Berger, E. A.; Murphy, P. M. *J. Biol. Chem.*, **1998**, *273*, 23799.

[118] Zhang, L.; He, T.; Huang, Y.; Chen, Z.; Guo, Y.; Wu, S.; Kunstman, K. J.; Brown, R. C.; Phair, J. P.; Neumann, A. U.; Ho, D. D.; Wolinsky, S. M. *J. Virol.*, **1998**, *72*, 9307.

[119] Owen, S. M.; Ellenberger, D.; Rayfield, M.; Wiktor, S.; Michel, P.; Grieco, M. H.; Gao, F.; Hahn, B. H.; Lal, R. B. *J. Virol.*, **1998**, *72*, 5425.

[120] Morner, A.; Bjorndal, A.; Albert, J.; Kewalramani, V. N.; Littman, D. R.; Inoue, R.; Thorstensson, R.; Fenyo, E. M.; Bjorling, E. *J. Virol.*, **1999**, *73*, 2343.

[121] Garzino-Demo, A.; Arya, S. K.; Devico, A. L.; Cocchi, F.; Lusso, P.; Gallo, R. C. *AIDS Res. Hum. Retro.*, **1997**, *13*, 1367.

[122] Copeland, K. F.; Leith, J. G.; McKay, P. J.; Rosenthal, K. L. *AIDS Res. Hum. Retro.*, **1997**, *13*, 71.

[123] Schmidtmayerova, H.; Nottet, H. S.; Nuovo, G.; Raabe, T.; Flanagan, C. R.; Dubrovsky, L.; Gendelman, H. E.; Cerami, A.; Bukrinsky, M.; Sherry, B. *Proc. Natl. Acad. Sci. USA*, **1996**, *93*, 700.

[124] Schmidtmayerova, H.; Sherry, B.; Bukrinsky, M. *Nature*, **1996**, *382*, 767.

[125] Handen, J. S.; Rosenberg, H. F. *FEBS Lett.*, **1997**, *410*, 301.

[126] Montes, M.; Tagieva, N. E.; Heveker, N.; Nahmias, C.; Baleux, F.; Trautmann, A. *Eur. Cytokine Netw.*, **2000**, *11*, 470.

[127] Vila-Coro, A. J.; Rodriguez-Frade, J. M.; Martin De Ana, A.; Moreno-Ortiz, M. C.; Martinez, A. C.; Mellado, M. *FASEB J.*, **1999**, *13*, 1699.

[128] Marechal, V.; Arenzana-Seisdedos, F.; Heard, J. M.; Schwartz, O. *J. Virol.*, **1999**, *73*, 3608.

[129] Nagira, M.; Imai, T.; Hieshima, K.; Kusuda, J.; Ridanpaa, M.; Takagi, S.; Nishimura, M.; Kakizaki, M.; Nomiyama, H.; Yoshie, O. *J. Biol. Chem.*, **1997**, *272*, 19518.

[130] Hedrick, J. A.; Zlotnik, A. *J. Immunol.*, **1997**, *159*, 1589.

[131] Hromas, R.; Kim, C. H.; Klemsz, M.; Krathwohl, M.; Fife, K.; Cooper, S.; Schnizlein-Bick, C.; Broxmeyer, H. E. *J. Immunol.*, **1997**, *159*, 2554.

[132] Nagira, M.; Sato, A.; Miki, S.; Imai, T.; Yoshie, O. *Virol.*, **1999**, *264*, 422.

[133] Luster, A. D.; Ravetch, J. V. *J. Exp. Med.*, **1987**, *166*, 1084.

[134] Luster, A. D.; Unkeless, J. C.; Ravetch, J. V. *Nature*, **1985**, *315*, 672.

[135] Taub, D. D.; Lloyd, A. R.; Conlon, K.; Wang, J. M.; Ortaldo, J. R.; Harada, A.; Matsushima, K.; Kelvin, D. J.; Oppenheim, J. J. *J. Exp. Med.*, **1993**, *177*, 1809.

[136] Strieter, R. M.; Kunkel, S. L.; Arenberg, D. A.; Burdick, M. D.; Polverini, P. J. *Biochem. Biophys. Res. Commun.*, **1995**, *210*, 51.

[137] Sarris, A. H.; Broxmeyer, H. E.; Wirthmueller, U.; Karasavvas, N.; Cooper, S.; Lu, L.; Krueger, J.; Ravetch, J. V. *J. Exp. Med.*, **1993**, *178*, 1127.

[138] Mahalingam, S.; Farber, J. M.; Karupiah, G. *J. Virol.*, **1999**, *73*, 1479.

[139] Kanegane, C.; Sgadari, C.; Kanegane, H.; Teruya-Feldstein, J.; Yao, L.; Gupta, G.; Farber, J. M.; Liao, F.; Liu, L.; Tosato, G. *J. Leukoc. Biol.*, **1998**, *64*, 384.

[140] Angiolillo, A. L.; Sgadari, C.; Taub, D. D.; Liao, F.; Farber, J. M.; Maheshwari, S.; Kleinman, H. K.; Reaman, G. H.; Tosato, G. *J. Exp. Med.*, **1995**, *182*, 155.

[141] Lane, B. R.; King, S. R.; Bock, P. J.; Strieter, R. M.; Coffey, M. J.; Markovitz, D. M. *Virol.*, **2003**, *307*, 122.

[142] D'Ambrosio, D.; Iellem, A.; Bonecchi, R.; Mazzeo, D.; Sozzani, S.; Mantovani, A.; Sinigaglia, F. *J. Immunol.*, **1998**, *161*, 5111.

[143] Bonecchi, R.; Bianchi, G.; Bordignon, P. P.; D'Ambrosio, D.; Lang, R.; Borsatti, A.; Sozzani, S.; Allavena, P.; Gray, P. A.; Mantovani, A.; Sinigaglia, F. *J. Exp. Med.*, **1998**, *187*, 129.

[144] Sallusto, F.; Lanzavecchia, A.; Mackay, C. R. *Immunol. Today*, **1998**, *19*, 568.

[145] Cota, M.; Mengozzi, M.; Vicenzi, E.; Panina-Bordignon, P.; Sinigaglia, F.; Transidico, P.; Sozzani, S.; Mantovani, A.; Poli, G. *Proc. Natl. Acad. Sci. USA*, **2000**, *97*, 9162.

[146] Walker, C. M.; Moody, D. J.; Stites, D. P.; Levy, J. A. *Science*, **1986**, *234*, 1563.

[147] Gomez, A. M.; Smaill, F. M.; Rosenthal, K. L. *Clin. Exp. Immunol.*, **1994**, *97*, 68.

[148] Mackewicz, C. E.; Ortega, H. W.; Levy, J. A. *J. Clin. Invest.*, **1991**, *87*, 1462.

[149] Scala, E.; D'Offizi, G.; Rosso, R.; Turriziani, O.; Ferrara, R.; Mazzone, A. M.; Antonelli, G.; Aiuti, F.; Paganelli, R. *J. Immunol.*, **1997**, *158*, 4485.

[150] Cao, Y.; Qin, L.; Zhang, L.; Safrit, J.; Ho, D. D. *N. Engl. J. Med.*, **1995**, *332*, 201.

[151] Copeland, K. F.; McKay, P. J.; Rosenthal, K. L. *AIDS Res. Hum. Retro.*, **1995**, *11*, 1321.

[152] Copeland, K. F.; McKay, P. J.; Rosenthal, K. L. *AIDS Res. Hum. Retro.*, **1996**, *12*, 143.

[153] Maslove, D. M.; Ni, L. W.; Hawley-Foss, N. C.; Badley, A. D.; Copeland, K. F. *Clin. Exp. Immunol.*, **2001**, *125*, 102.

[154] Cocchi, F.; DeVico, A. L.; Garzino-Demo, A.; Arya, S. K.; Gallo, R. C.; Lusso, P. *Science*, **1995**, *270*, 1811.

[155] Mackewicz, C. E.; Yuan, J.; Tran, P.; Diaz, L.; Mack, E.; Selsted, M. E.; Levy, J. A. *AIDS*, **2003**, *17*, F23.

[156] Chang, T. L.; Francois, F.; Mosoian, A.; Klotman, M. E. *J. Virol.*, **2003**, *77*, 6777.

[157] Zhang, L.; Yu, W.; He, T.; Yu, J.; Caffrey, R. E.; Dalmasso, E. A.; Fu, S.; Pham, T.; Mei, J.; Ho, J. J.; Zhang, W.; Lopez, P.; Ho, D. D. *Science*, **2002**, *298*, 995.

[158] Geiben-Lynn, R.; Kursar, M.; Brown, N. V.; Addo, M. M.; Shau, H.; Lieberman, J.; Luster, A. D.; Walker, B. D. *J. Biol. Chem.*, **2003**, *278*, 1569.

[159] Leith, J. G.; Copeland, K. F.; McKay, P. J.; Richards, C. D.; Rosenthal, K. L. *AIDS*, **1997**, *11*, 575.

[160] Zhang, L.; Lopez, P.; He, T.; Yu, W.; Ho, D. D. *Science*, **2004**, *303*, 467.

[161] Angel, J. B.; High, K.; Rhame, F.; Brand, D.; Whitmore, J. B.; Agosti, J. M.; Gilbert, M. J.; Deresinski, S. *AIDS*, **2000**, *14*, 387.

[162] Brites, C.; Gilbert, M. J.; Pedral-Sampaio, D.; Bahia, F.; Pedroso, C.; Alcantara, A. P.; Sasaki, M. D.; Matos, J.; Renjifo, B.; Essex, M.; Whitmore, J. B.; Agosti, J. M.; Badaro, R. *J. Infect. Dis.*, **2000**, *182*, 1531.

[163] Skowron, G.; Stein, D.; Drusano, G.; Melbourne, K.; Bilello, J.; Mikolich, D.; Rana, K.; Agosti, J. M.; Mongillo, A.; Whitmore, J.; Gilbert, M. J. *J. Infect. Dis.*, **1999**, *180*, 1064.

[164] Katabira, E. T.; Sewankambo, N. K.; Mugerwa, R. D.; Belsey, E. M.; Mubiru, F. X.; Othieno, C.; Kataaha, P.; Karam, M.; Youle, M.; Perriens, J. H.; Lange, J. M. *Sex. Transm. Infect.*, **1998**, *74*, 265.

[165] Hulton, M. R.; Levin, D. L.; Freedman, L. S. *J. Acquir. Immune Defic. Syndr.*, **1992**, *5*, 1084.

[166] Kaiser, G.; Jaeger, H.; Birkmann, J.; Poppinger, J.; Cummins, J. M.; Gallmeier, W. M. *AIDS*, **1992**, *6*, 563.

[167] Sperber, S. J.; Gocke, D. J.; Haberzettl, C. A.; Pestka, S. *AIDS*, **1993**, *7*, 693.

[168] Weber, R.; Bonetti, A.; Jost, J.; Vogt, M. W.; Spacey, B.; Siegenthaler, W.; Luthy, R. *Infection*, **1991**, *19*, 395.

[169] Gringeri, A.; Musicco, M.; Hermans, P.; Bentwich, Z.; Cusini, M.; Bergamasco, A.; Santagostino, E.; Burny, A.; Bizzini, B.; Zagury, D. *J. Acquir. Immune Defic. Syndr. Hum. Retrovirol.*, **1999**, *20*, 358.

[170] Kovacs, J. A.; Baseler, M.; Dewar, R. J.; Vogel, S.; Davey, R. T., Jr.; Falloon, J.; Polis, M. A.; Walker, R. E.; Stevens, R.; Salzman, N. P.; Metcalf, J. A.; Masur, H.; Lane, H. C. *N. Engl. J. Med.*, **1995**, *332*, 567.

[171] Kovacs, J. A.; Vogel, S.; Albert, J. M.; Falloon, J.; Davey, R. T., Jr.; Walker, R. E.; Polis, M. A.; Spooner, K.; Metcalf, J. A.; Baseler, M.; Fyfe, G.; Lane, H. C. *N. Engl. J. Med.*, **1996**, *335*, 1350.

[172] Amendola, A.; Poccia, F.; Martini, F.; Gioia, C.; Galati, V.; Pierdominici, M.; Marziali, M.; Pandolfi, F.; Colizzi, V.; Piacentini, M.; Girardi, E.; D'Offizi, G. *Clin. Exp. Immunol.*, **2000**, *120*, 324.

[173] Arno, A.; Ruiz, L.; Juan, M.; Jou, A.; Balague, M.; Zayat, M. K.; Marfil, S.; Martinez-Picado, J.; Martinez, M. A.; Romeu, J.; Pujol-Borrell, R.; Lane, C.; Clotet, B. *J. Infect. Dis.*, **1999**, *180*, 56.

[174] Caggiari, L.; Zanussi, S.; Bortolin, M. T.; D'Andrea, M.; Nasti, G.; Simonelli, C.; Tirelli, U.; De Paoli, P. *Clin. Exp. Immunol.*, **2000**, *120*, 101.

[175] Davey, R. T., Jr.; Chaitt, D. G.; Albert, J. M.; Piscitelli, S. C.; Kovacs, J. A.; Walker, R. E.; Falloon, J.; Polis, M. A.; Metcalf, J. A.; Masur, H.; Dewar, R.; Baseler, M.; Fyfe, G.; Giedlin, M. A.; Lane, H. C. *J. Infect. Dis.*, **1999**, *179*, 849.

[176] Davey, R. T., Jr.; Murphy, R. L.; Graziano, F. M.; Boswell, S. L.; Pavia, A. T.; Cancio, M.; Nadler, J. P.; Chaitt, D. G.; Dewar, R. L.; Sahner, D. K.; Duliege, A. M.; Capra, W. B.; Leong, W. P.; Giedlin, M. A.; Lane, H. C.; Kahn, J. O. *JAMA*, **2000**, *284*, 183.

[177] Lalezari, J. P.; Beal, J. A.; Ruane, P. J.; Cohen, C. J.; Jacobson, E. L.; Sundin, D.; Leong, W. P.; Raffanti, S. P.; Wheeler, D. A.; Anderson, R. D.; Keiser, P.; Schrader, S. R.; Goodgame, J. C.; Steinhart, C. R.; Murphy, R. L.; Wolin, M. J.; Smith, K. A. *HIV Clin. Trials*, **2000**, *1*, 1.

[178] Losso, M. H.; Belloso, W. H.; Emery, S.; Benetucci, J. A.; Cahn, P. E.; Lasala, M. C.; Lopardo, G.; Salomon, H.; Saracco, M.; Nelson, E.; Law, M. G.; Davey, R. T.; Allende, M. C.; Lane, H. C. *J. Infect. Dis.*, **2000**, *181*, 1614.

[179] Pandolfi, F.; Pierdominici, M.; Marziali, M.; Livia Bernardi, M.; Antonelli, G.; Galati, V.; D'Offizi, G.; Aiuti, F. *Clin. Immunol.*, **2000**, *94*, 153.

[180] Zanussi, S.; Simonelli, C.; Bortolin, M. T.; D'Andrea, M.; Crepaldi, C.; Vaccher, E.; Nasti, G.; Politi, D.; Barzan, L.; Tirelli, U.; De Paoli, P. *Clin. Exp. Immunol.*, **1999**, *116*, 486.

[181] Starr, S. E.; McFarland, E. J.; Muresan, P.; Fenton, T.; Pitt, J.; Douglas, S. D.; Deveikis, A.; Levin, M. J.; Rathore, M. H. *AIDS*, **2003**, *17*, 2181.

[182] Miller, K. D.; Spooner, K.; Herpin, B. R.; Rock-Kress, D.; Metcalf, J. A.; Davey, R. T., Jr.; Falloon, J.; Kovacs, J. A.; Polis, M. A.; Walker, R. E.; Masur, H.; Lane, H. C. *Clin. Immunol.*, **2001**, *99*, 30.

[183] Farel, C. E.; Chaitt, D. G.; Hahn, B. K.; Tavel, J. A.; Kovacs, J. A.; Polis, M. A.; Masur, H.; Follmann, D. A.; Lane, H. C.; Davey, R. T., Jr. *Blood*, **2004**, *103*, 3282.

[184] Larsen, C. S.; Ostergard, L.; Moller, B. K.; Buhl, M. R. *Scand. J. Infect. Dis.*, **2000**, *32*, 153.

[185] Klimas, N.; Patarca, R.; Walling, J.; Garcia, R.; Mayer, V.; Moody, D.; Okarma, T.; Fletcher, M. A. *AIDS*, **1994**, *8*, 1073.

[186] Scadden, D. T.; Levine, J. D.; Bresnahan, J.; Gere, J.; McGrath, J.; Wang, Z.; Resta, D. J.; Young, D.; Hammer, S. M. *AIDS Res. Hum. Retro.*, **1995**, *11*, 731.

[187] Tulpule, A.; Joshi, B.; DeGuzman, N.; Espina, B. M.; Mocharnuk, R.; Prakash, O.; Templeton, D.; Levine, A. M.; Gill, P. S. *Ann. Oncol.*, **1997**, *8*, 79.

[188] Angel, J. B.; Jacobson, M. A.; Skolnik, P. R.; Giordano, M.; Shapiro, L.; LeBeaut, A.; Greaves, W.; Fuchs, A. C. *AIDS*, **2000**, *14*, 2503.