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Proinflammatory Cytokines in HIV disease – A Review and Rationale for New Therapeutic Approaches

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Abstract

Antiretroviral drugs currently in use for treating HIV-1 infection are very effective at maintaining low viral loads and clinical stability, but have been limited by their inability to eradicate virus in infected individuals, resulting in the need for indefinite therapy. The inability of antiretroviral drug therapy to eliminate HIV-1 infection is thought to be due to incomplete restoration of host immunity to the virus. New strategies to improve control of HIV-1 infection during antiretroviral therapy should target enhancement of host immunity. Proinflammatory cytokines are the central mediators of both innate and adaptive immunity, and modulation of these cytokines has been shown to alter anti-HIV-1 reactivity in vitro. Modulation of proinflammatory cytokines could therefore be utilized in strategies for immunotherapy of HIV-1 infection. The ultimate goal is to find regimens that could more durably suppress viral replication and potentially eliminate the need for indefinite antiretroviral therapy. This review presents what is known about the dysregulation of proinflammatory cytokines in HIV-1 infection, highlighting newly available immune-based therapies that could augment antiretroviral drug therapies. **(AIDS Reviews 2005;7:168-80)**

Key words

HIV-1. Cytokines. IL-1. IL-6. IL-18. TNF-alpha.

Introduction

HIV-1 directly attacks the host's immune system, crippling its ability to use innate and adaptive defenses to control viral infection. For this reason, new strategies for treating HIV-1 could be targeted at altering the host immune response such that it is able to regain its ability to clear pathogenic organisms. Cytokines are essential physiologic mediators of immunologic processes. The function of the cytokine network is quite complex, yet essential to understand in order to devise strategies to alter pathogenic immune responses, not only as therapy for HIV-1 infection, but also for treatment of other chronic infections, cancers, and autoimmune diseases. At every step of cytokine regulation and activity there are multiple levels of complexity which have functional implications. Preclinical studies evaluating individual cytokine levels are imprecise and often non-comparable because of different methods used and parameters measured. Because of this, the best approach to gain a better understanding of the clinical implications of cytokine manipulation *in vivo* is by performing empiric clinical trials based on the best available data.

Measurements of cytokine levels *in vivo* are fraught with difficulty for a myriad of reasons. Cytokines are differentially regulated, with some regulated primarily at the transcriptional level, others regulated at the post-translational modification level, while still others are regulated via soluble cytokine receptors or activator molecules. In most cases a combination of these factors is involved in cytokine function. In addition, systemic cytokine levels may not be an accurate representation of levels found in selected microenvironments, which may be more relevant to physiologic function.

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For these reasons, a series of studies are needed in order to obtain a realistic picture of how cytokines impact on disease states.

For convenience, it is possible to separate cytokines into functional groups, although given the tremendous redundancy of cytokine actions such generalizations are limited. The proinflammatory cytokines include interleukins IL-1β, IL-6, IL-18, and tumor necrosis factor-alpha (TNF-α). The Th1/Th2 CD4+ helper T-cell paradigm suggests that Th1 immunity, mediated by IL-2, IL-12, and IL-15, generally favors cell-mediated immunity, while Th2 immunity, mediated by IL-4, IL-5, and IL-10 generally favors humoral immunity. Finally, the chemokine group including IL-8, MIP-1α, MIP-1β, and RANTES has various immunologic functions, but is mainly involved in chemotaxis of immunologically active cells.

The purpose of this review is to combine the knowledge gained from multiple studies to assemble a cohesive picture of the cytokine milieu in HIV-1 infection. In order to give adequate depth, as well as to highlight a group of cytokines where potential, new, as yet untried therapies are apparent, this review is limited to proinflammatory cytokines. This information will allow us to postulate logical avenues of intervention, i.e. new cytokine-based therapies, for evaluation in clinical trials designed to augment the host's immune response against HIV-1 infection.

Interleukin-1β

Biology

IL-1β is encoded on the long arm of chromosome 2 and is produced mainly by monocytes, macrophages, and dendritic cells (DC) in response to a variety of bacterial products, principally via interactions with toll-like receptors. It is synthesized as an inactive precursor requiring cleavage by IL-1β converting enzyme (ICE), also known as caspase-1 to the active cytokine. Confounding its measurement is the fact that a number of different stimuli induce IL-1β mRNA transcription without resulting in translation of IL-1β precursor. Also, the same cells that produce the majority of IL-1β constitutively produce an IL-1 receptor antagonist (IL-1ra). These facts demonstrate multiple levels of control over IL-1β activity, suggesting the importance of this cytokine for regulation of the inflammatory state. Despite this, mice deficient in IL-1β develop normally and are able to live in non-sterile environments without developing acute phase responses, fevers, anorexia, or secretion of IL-6 in response to noxious stimuli – all reactions normally resulting from the presence of high levels of IL-1β. However, IL-1β-deficient mice are more prone to infection by influenza A virus than wild-type mice and suffer higher mortality rates, but this deficiency is not necessarily lethal in such cases[1].

IL-1β binds to IL-1-receptor type 1 and forms a heterodimer with IL-1 receptor 1 accessory protein which, by increasing binding affinity, results in amplification of the response. These actions result in recruitment of the cytosolic adapter protein MyD88 and activation of IL-1 receptor activating proteins, which ultimately result in translation of enzymes that increase circulating levels of prostaglandin E2, platelet activating factor, nitric oxide, and adhesion molecules – all important mediators of a proinflammatory state[2]. Disrupting cleavage, receptor binding, or MyD88 function can eliminate or diminish IL-1β activity.

Alteration in HIV-1 infection

The few studies of the presence of IL-1β in sera of HIV-1-infected individuals show both decreased[3,4], and increased[5,6] levels in progressive disease. At the same time, increases in IL-1β protein have been demonstrated in specialized compartments such as the cerebrospinal fluid (CSF) and skin[7-9]. Cell-stimulation studies have generally shown that the capacity of peripheral blood mononuclear cells (PBMC) and DC infected with HIV-1 to produce IL-1β in response to activating stimuli such as lipopolysaccharide (LPS) or CD40L is increased in HIV-1 infection in association with higher viral load, albeit with significant interindividual variability[3,6,10-15]. High levels of IL-1β appear to decrease with HAART[16] possibly as a result of increased availability of IL-1β receptor antagonist[17]. Alteration in IL-1β production in response to physiologic stimuli may not be specific to HIV-1 infection, in that *in vitro* studies show that Epstein-Barr virus (EBV) increases IL-1β secretion, while hepatitis C virus (HCV) decreases it[18,19]. In the case of HIV-1 infection, it has been suggested that HIV-1 Tat protein and/or gp120 can act as virulence factors responsible for increasing physiologic IL-1β production in response to activating stimuli. This results in increased numbers of activated cells as targets for HIV-1 infection, and therefore increased HIV-1 replication[7,20].

Role in pathogenesis

HIV-1 replication appears to be increased by the presence of IL-1β, but this effect is complex and ap-

pears to be related to both the presence of additional replication stimuli and the specific viral subspecies[21,22]. IL-1β appears to contribute to increased *in vitro* susceptibility of both naive and memory CD4+ and CD8+ T-cells to apoptosis in the setting of HIV-1 infection via the pro-apoptotic Fas/FasL pathway[23]. Petit, et al. demonstrated that Fas/FasL binding, in the appropriate setting, can result in T-cell proliferation rather than apoptosis, and that this effect was abrogated in the presence of inhibitors of caspase-1 or IL-1β[24]. Also, in CHP100 cells, a neuroblastoma cell line found to be useful for elucidating the neurotoxic role of gp120, addition of gp120 not only results in calcium-dependent cellular toxicity, but also in increased IL-1β levels and decreased IL-1β inhibitor activity. Furthermore, retinoids, naturally occurring regulators of gene transcription and translation, are thought to possess inherent anti-HIV-1 properties, possibly by suppressing Tat and nuclear factor kappa B (NFκB) activity. This activity is both IL-1β and IL-6 dependent[25,26]. Finally, in IL-1β-deficient transgenic mice, LPS-induced increases in HIV-1 replication are blunted, suggesting that IL-1β, in the setting of other physiologic stimuli, augments HIV-1 replication[27].

Downstream IL-1β inhibitors, such as cyclooxygenase-2 (COX-2) inhibitors, block apoptosis of neuronal cells, suggesting that the presence of HIV-1 proteins results in an increase in IL-1β that is detrimental to neuronal tissue[7]. Therefore, while the mechanisms are as yet incompletely defined, inhibitors of IL-1β (such as IL-1ra analogues, caspase-1 inhibitors, and COX-2 inhibitors) seem to curtail detrimental HIV-1-mediated effects in animal and *in vitro* models[28], suggesting that IL-1β contributes to increased HIV-1 replication and pathogenesis *in vivo.*

With respect to the direct toxicity of IL-1β in HIV-1-infected individuals, high levels of IL-1β, in conjunction with TNF-α, decrease transepithelial resistance in mucosal tissues, thereby possibly contributing to HIV-1-associated diarrhea[29], and *in vitro* studies demonstrate that IL-1β directly contributes to neuronal injury, which could possibly contribute to HIV-1-associated encephalopathy[7].

In summary, high IL-1β levels likely facilitate HIV-1 replication by more than one mechanism. Animal and *in vitro* models have shown that HIV-1 proteins increase IL-1β levels as well as those of IL-1β inhibitors. Exogenously administered IL-1β inhibitors rescue neuronal cells *in vitro*. IL-1β modulates the Fas/FasL-mediated pro-apoptotic potential, possibly resulting in the increase in activation-induced cell death observed in HIV-1 infection, and high physiologic levels of IL-1β (acting in combination with other cytokines) probably have direct, detrimental effects with respect to symptoms in late HIV-1 infection. Despite a plurality of interactions between IL-1β, IL-1ra and HIV-1, several points are clear: first, there is an increased capacity of cells to produce IL-1β and IL-1β activity in the setting of chronic HIV-1 infection; second, IL-1β increases HIV-1 replication; and third, inhibitors of IL-1β diminish HIV-1 replication *in vitro*.

Future directions

Given the safety and availability of IL-1 receptor antagonists, it seems logical to consider trials of IL-1β inhibitors in animal models and, if promising, continuing to look at possible antiviral effects in infected subjects.

Anakinra (Kineret®, Amgen, Thousand Oaks, CA) is currently the only FDA-approved IL-1β receptor antagonist. Administered parenterally, it has been shown to be safe and effective when used in the setting of rheumatoid arthritis[30,31]. It would be logical to evaluate this agent in *in vitro* and/or animal models in order to determine whether blocking IL-1β could decrease viral replication. However, if one prudently assumes that the effects of IL-1β are only partially responsible for facilitating HIV translation and replication, the effect, at least at the systemic level, might be minimal. If so, a reasonable approach may be to use combination therapy to eliminate both of the proviral mechanisms hypothesized by Poli, et al.[32]. Other strategies could include the use of orally active caspase-1 inhibitors, such as pralnacasan[33], that could decrease levels of activated IL-1β (and IL-18), potentially decreasing the toxic systemic effects of higher than normal IL-1β (i.e. diarrhea and potentially encephalopathy) while decreasing systemic proinflammatory effects of IL-1β.

Interleukin-6

Biology

IL-6 is produced by monocytes, macrophages and T-lymphocytes in response to cytokine or antigenic stimulation. It mediates B-cell terminal differentiation and maturation as well as antibody synthesis by activated B-cells at the mRNA level. Acting synergistically with IL-1β and TNF-α, IL-6 is involved in T-cell activation, growth, and differentiation, possibly through its effect of increasing expression of IL-2 receptors on T-cells and thymocytes. It is thought to play a stimulatory role in cytotoxic T-lymphocyte (CTL) differentiation

and function. Additionally, IL-6 is involved in hematopoiesis, and acts with IL-1β and TNF-α as a hepatocyte-stimulatory factor to induce production of acute phase proteins resulting in fevers, anorexia, and myalgia. Finally, IL-6 may have a stimulatory role in osteoclast development and, as estrogen is known to suppress levels of IL-6, is likely involved in age-related osteoporosis[34].

IL-6 activity is regulated at multiple levels, including transcriptional, posttranslational modification and by the presence of a soluble IL-6 receptor (IL-6Rα), that not only antagonizes it but also alters its activity in a cell type specific way. It is also regulated by selective expression of membrane-bound IL-6 receptors. Activity is upregulated by TNF-α and IL-1β and downregulated by IL-4, IL-6, and IFN-γ via soluble mediators. It exerts its activity via numerous intracellular enzyme cascades, but predominantly through the Janus kinase/signal transducer and activator of transcription (JAK/STAT) pathway[2,34].

Alteration in HIV-1 infection

Many studies have demonstrated that IL-6 expression and secretion are abnormally high in HIV-1-infected subjects[35]. HIV-1 infection directly increases IL-6 secretion by monocytes and macrophages. The presence of IL-6 alone, as well as in conjunction with IL-1β and TNF-α, facilitate HIV-1 replication and secretion *in vitro*[36]. Additionally, CD40L stimulation of macrophages *in vitro* results in IL-6 (and IL-1β) secretion in HIV-1-infected but not uninfected macrophages[14]. This is not a direct effect of HIV-1 infection on IL-6 receptor expression, but rather the process of nonspecifically stimulated macrophages resulting in increased cytokine expression and secretion, which in turn increases HIV-1 replication. It remains a matter of some controversy as to what mechanism IL-6 uses to increase HIV-1 replication. While IL-1β and TNF-α induce HIV-1 replication in infected T-cells, it appears that IL-6 induces HIV-1 replication predominantly, if not exclusively, in macrophages[37]. Further, a number of IL-6 responsive nuclear factors, including C/EBP (CCAAT enhancer binding protein) and mitogen-activated protein kinase (MAPK) have been implicated in increasing HIV-1 replication when activated, and many of these are triggered by pro-inflammatory cytokine stimulation[38-43]. In primate studies, infection with pathogenic SIV, but not *nef*-deleted nonpathogenic virus, results in prolonged increases in IL-6 production[44].

Role in pathogenesis

IL-6 both increases HIV-1 replication in latently infected macrophages[36] and alters the function of infected macrophages, likely rendering them suboptimally functional[14,45]. It also plays a prominent role in immune reconstitution in the setting of HAART[46] and, via a complex system of neuroendocrine actions, likely has a significant role in HIV-1-associated lipodystrophy syndromes[47-50]. Additionally, IL-6 overproduction could be involved in HIV-1-related hypergammaglobulinemia seen early in infection[51,52] and has been implicated as a cofactor, along with TNF-α, in HIV-1-related encephalopathy[53,54].

The most compelling new data to emerge regarding the role of IL-6 in HIV-1 disease were from a study by Bacsi, et al. In an *in vitro* syncytiotrophoblast model of mother-to-child-transmission, blocking IL-6 with monoclonal antibodies (MAb) resulted in a 35-fold decrease in replication and secretion of HIV-1. Anti-TNF-α MAb alone resulted in a 12-fold decrease, while addition of both together resulted in complete cessation of detectable HIV-1 replication and secretion[55]. In summary, IL-6 levels are increased in HIV-1 infection, and these increased levels are detrimental to infected hosts. The mechanism whereby higher than normal IL-6 levels facilitate HIV-1 replication in macrophages remains unknown, and clearly more study is required.

Future directions

Several anti-IL-6 agents are currently available and have been used with some success in clinical trials of rheumatoid arthritis, multiple myeloma, and Crohn's disease, among others[56-60]. High-affinity, chimeric, anti-IL-6 MAb have been used in trials of multiple myeloma. Tenidap (Pfizer), a novel anti-inflammatory agent known to decrease levels of certain cytokines (including IL1β, IL-6, TNF-α, and IL-8) demonstrated efficacy in clinical studies in rheumatic disorders, but failed to win FDA approval because of concerns that it caused a decrease in bone mineral density consistent with its known role in osteoclast stimulation. Tenidap decreases HIV-1 replication in chronically HIV-1-infected monocyte cell lines, but this may be an IL-6-independent effect[61]. It is logical to hypothesize that anti-IL-6 therapy, in conjunction with other cytokine-blocking modalities, could decrease HIV-1 replication *in vivo* and, as IL-6 seems to act on HIV-1 replication on cells of the monocyte lineage rather than the T-cell lineage, represents a complementary mechanism. Efficacy test-

ing would be a logical next step in animal models. If these results showed promise, safety testing in the HIV-1-infected populations would be reasonable. Additional studies evaluating the mechanism whereby IL-6 increases HIV-1 replication in monocytes are needed.

Interleukin-18

Biology

IL-18, previously called IFN-γ-inducing factor, is a pleiotropic, proinflammatory cytokine with structural homology to IL-1β that has numerous functions. IL-18 induces activation of T-cells and natural killer (NK) cells and uniquely induces secretion of either a Th1 or Th2 cytokine profile, depending on the presence or absence of IL-12. IL-18 is probably essential for the cytolytic activity of T-cells and NK cells. Some or all of these actions result in induction of iNOS and COX-2 (effector functions responsible for physiologic cell killing) activity, which are thought to be important physiologically for *in vivo* antimicrobial action against cryptococcus, yersinia, and leishmaniasis as well as antitumor activity. In mouse studies, IL-18 has a protective effect against a variety of bacterial, viral, and fungal infections, and depletion results in more severe disease. The mechanism for this, while not fully defined, is thought to be primarily IFN-γ mediated. IL-18 activity is regulated at both the transcriptional level and at the posttranslational processing level largely by caspase-1, the same enzyme responsible for activation of inactive IL-1β[62].

Regulation of IL-18 is largely via receptor expression; IL-18 receptor (IL-18R) is expressed at very low levels on naive T-cells, and at high levels on activated Th1, IL-12 stimulated T-cells, but not Th2 CD4+ T-cells. NK cells express IL-18R both constitutively and via an IFN-γ-inducible mechanism. Another mode of IL-18 regulation is the production of soluble IL-18 binding protein which binds and effectively antagonizes it. IL-18 exerts its activity in large part via the MyD88 and NFκB intracellular intermediates, resulting in upregulated transcription of other cytokine and cytokine receptor mRNA[1,62,63].

Alteration in HIV-1 disease

Serum IL-18 levels are increased in HIV-1 disease, and there are conflicting data as to whether this effect is pronounced or diminished during disease progression[64-67]. Most *in vivo* studies suggest that, as with other proinflammatory cytokines, IL-18 levels are increased with increasing viremia and decreased by HAART[67].

Role in pathogenesis

IL-18 appears to decrease HIV-1 replication in some cells, while increasing replication in others[67-71]. Evidence suggests that IL-18 decreases HIV-1 replication, at least in early disease, by virtue of its ability, in conjunction with IL-12, to polarize acute immunologic responses toward a Th1-type response. IL-18 augments the cytolytic capacity of CD8+ T-cells. Both of these effects are beneficial for effective cell-mediated control over HIV-1 infection. *In vitro* studies show that these effects are largely mediated by IL-18 via stimulation of IFN-γ production[69]. The stimulatory effect of IL-18 on HIV-1 replication is likely also mediated by increasing expression of cellular attachment factors, CXCR4, and TNF-related apoptosis-inducing ligand (TRAIL)[67] as well as by increasing expression of proinflammatory cytokines including, IL-1β, IL-6, IL-8, and TGF-β[64,71,72]. Notably, IL-18 exerts some of its physiologic actions via the NFκB pathway. The HIV-1 long terminal repeat (LTR) incorporates a highly conserved binding site for human NFκB and a correlation has been noted in several studies between activation of NFκB, and stimulation of transcription of LTR reporter constructs[73-76]; therefore, increased levels of IL-18 could result in an increase in HIV-1 replication through the NFκB pathway[21,77,78]. As a result of its known ability to stimulate Th1 immunity, IL-18 has been studied as a vaccine adjuvant in preclinical HIV-1 vaccine studies[79,80].

Future directions

The increase of Th1 activity by IL-18 is dependent on the presence of IL-12. IL-12 is generally considered the cytokine most important for promotion of a Th1 response and, with the goal of augmenting HIV-1-specific Th1 immunity, short-term trials using IL-12 have been undertaken in the SIV-macaque model with variable success[81,82]. Currently, IL-12 is being studied as an adjuvant in HIV and other therapeutic vaccine strategies[83,84]. While IL-18 is thought to augment the ability of IL-12 to stimulate Th1-type immunity, it is not essential for Th1 activation. IL-18 appears to be important for potent innate immunity against a variety of pathogens, but this effect is dependent on the production of other cytokines, notably IFN-γ. Possibly, the increase in HIV-1 replication seen in the presence of IL-18 may

in part be due to activation of the NFκB cascade, but this effect is only one of many pathways IL-18 may use to increase circulating HIV-1.

Studies using IL-18 or IL-18 inhibitors for a variety of disease states are currently in their infancy, with the majority of studies being performed in murine models. These studies are targeted at controlling a variety of tumors[85-88], autoimmune diseases including experimental neuritis and multiple sclerosis, infectious agents[89-93], and graft-versus-host disease[94-96].

Given the complexity of IL-18 effects, particularly with regards to increasing NFκB activity and thereby potentially increasing the replicative capacity of HIV-1, increasing IL-18 levels in the setting of HIV-1 infection seems likely to worsen disease, as well as leading to significant toxicity. Systemically decreasing IL-18 levels could potentially decrease HIV-1 replication, but would likely have additional effects, such as shifting the CD4+ T-cell population away from the Th1 phenotype, that are less desirable. A more logical strategy, given the complexity of IL-18 actions, would be to target downstream effects of IL-18 that have been implicated in increasing HIV-1 replication, such as the NFκB or IFN-γ pathway. Also, since IL-18 is activated by caspase-1, the same enzyme responsible for the activation of IL-1β, antagonists of caspase-1 would decrease not only IL-1β activity, but also decrease IL-18 activity.

Tumor necrosis factor-alpha

Biology

TNF-α is produced by many types of immune cells in response to a number of stimuli, including antibody cross-linking, LPS, and numerous viral infections, among others. Because of its generally proinflammatory effects when present systemically, expression is tightly regulated at all levels. TNF-α activity is mediated by either of two receptors: TNF receptor 1 (TNF-R1) or TNF receptor 2 (TNF-R2), which are ubiquitously present, both on nucleated cell surfaces and in soluble form. The soluble forms of TNF-R1 and TNF-R2 (sTNF-R) generally act to attenuate the activity of TNF-α, but may simultaneously act as a reservoir for available TNF-α. TNF-α binds membrane-bound TNF-R resulting in trimerization of membrane-bound TNF-R, and initiates cell-type dependent, intracellular signaling cascades, many of which are NFκB dependent, resulting in a huge array of biological effects. TNF-α, depending on the target cell type, can mature and activate antigen-presenting cells (APC), induce IL1β, IL-8, GM-CSF, M-CSF, and IFN-γ from monocytes, activate CTL, and/or induce apoptosis of mature T-cells. Some or all of these actions may result clinically in fever, hypotension, anorexia, and capillary leakage among other events. TNF-α can also act as a potent inhibitor of IL-12[97].

Clinically, pathologic overproduction of TNF-α has been implicated in a variety of disease states including: septic shock, overwhelming meningococcemia, cerebral malaria, autoimmune diseases such as rheumatoid arthritis and myasthenia gravis, as well as some cancers including hairy cell leukemia and colorectal cancer. TNF-α is posited to be essential for killing tumor cells and has been studied as a therapy for certain types of cancers. Currently, therapeutic use for TNF-α is limited by its toxicity, but trials continue with the goal of finding TNF-α variants which preserve their antitumor activity while minimizing dose-limiting toxicity[2,98].

Alteration in HIV-1 infection

It is generally accepted that TNF-α levels, as well as those of sTNF-R, are increased in HIV-1, and that these levels increase with disease progression[11,99-101]. However, a recent study reported that in a cross section of HIV-1-infected volunteers, TNF-α levels were initially high, but then decreased dramatically late in disease[102], possibly as a result of exhaustion of TNF-α-producing cells. TNF-α levels have been directly correlated with HIV-1 RNA levels and inversely correlated with CD4+ T-cell counts[99,103]. There is debate, however, as to whether TNF-α levels, as well as those of sTNF-R, are altered by antiretroviral therapy. For example, significantly higher levels of TNF-α and TNF-R have been noted in HIV-1-infected as compared to HIV-1-uninfected subjects, but the addition of HAART did not significantly reduce TNF-α levels among adherent subjects with good HIV-1 RNA and CD4+ T-cell responses[104]. Other studies have shown that, in subjects initiating HAART, decreases in TNF-α producing CD4+ and CD8+ T-cells precede decreases in total CD4+ and CD8+ cell counts as well as changes in viral load. This suggests an important pathogenic role for TNF-α in viral replication as well as potentially a means of monitoring response to treatment or predicting failure of HIV-1 therapy[105,106].

Role in pathogenesis

HIV-1 induces TNF-α expression, and exogenous TNF-α enhances HIV-1 replication; a positive correla-

tion between increased levels of TNF-α and increased plasma HIV-1 viral load has been repeatedly demonstrated, both *in vivo* and *in vitro*[99,107-111]. Studies performed in transgenic mice deficient in TNF-α and IL-1β have shown these cytokines to be essential for HIV-1 replication *in vivo*[112]. Recently, Capini, et al. showed that some HIV-1-infected slow progressors possess naturally occurring anti-TNF-α antibodies[113]. Clinical studies using TNF-α administration for Kaposi's sarcoma in HIV-1-infected subjects, or MS-8209 (an amphotericin B derivative known to increase TNF-α levels) have confirmed that HIV-1 replication is increased in the presence of increased TNF-α levels[114,115]. Conversely, it has been repeatedly shown that TNF-α, via TNF-R2, is capable of decreasing HIV-1 replication in monocyte-derived macrophages, purified microglial cell cultures and mixed brain cell cultures, possibly via decreasing expression or activity of beta chemokine receptors[116-120].

The mechanisms by which TNF-α increases HIV-1 replication are incompletely understood, and there likely is variation in this ability between strains of HIV-1[121]. One mechanism whereby TNF-α increases HIV-1 replication is by stimulating the proinflammatory signaling cascade via TNF-R1 activating NFκB, resulting in induction of HIV-1 replication in previously latent cells[122]. A circuit has been described in *in vitro* culture systems in which HIV-1, NFκB, and TNF-α each exert positive feedback upon the other, thereby amplifying virus replication and inducing changes in cellular gene expression in a manner which is independent of HIV-1 replication[123]. TNF-α produced by macrophages in the presence of HIV-1 is thought to be instrumental in inducing T-cell anergy[124].

The TNF-α upregulation of CXCR4 expression may represent another mechanism whereby it increases HIV-1 replication[110]. Although this upregulation increases susceptibility to X4 tropic virus, there is concurrent downregulation of CCR5 in some cell populations, mediated partially by TNF-α, possibly in conjunction with IL-13, which results in decreasing HIV-1 replication1[17,125]. Additionally, it has been shown that in humans TNF-α is involved in the development of lymphoid follicles, HIV-1 trapping and, consequently, early host immune responses[126]. Physiologic levels of TNF-α, acting in synergy with IL-1β, have been shown to be important mediators for permitting mother-to-child transmission. In studies by Vidricaire, et al., trophoblasts, a major component of the placental/infant barrier, are generally poorly permissible to productive HIV-1 infection because of compartmentalization of virus within the cells[127]. This can be overcome by addition of physiologic levels of TNF-α and IL-1β, dramatically increasing viral transcription by increasing LTR-mediated viral replication. In addition to the role of TNF-α in increasing replication in chronically infected patients with HIV-1, there is likely a macrophage dependent, *nef*-mediated, anti-apoptotic effect whereby productively infected CD4+ T-lymphocytes, which physiologically should be targeted for apoptosis in order to eliminate infection, are maintained in the circulation, possibly contributing to the pool of latently infected T-cells[120,128].

In vitro and *in vivo*, mycobacteria can induce TNF-α expression, resulting in increased HIV-1 replication in the setting of coinfection with HIV-1 and contributing to acceleration of both disease processes[129-132]. Altered TNF-α levels in HIV-1-infected patients possibly contribute to HIV-1-associated diarrheal disease[29,133]. TNF-α levels in the CSF are higher in HIV-1-infected patients with encephalopathy, suggesting a role for TNF-α in the pathogenesis of HIV-1-associated encephalopathy[134-136].

An important mechanism for the stimulation of APC effector function is to effectively present appropriate antigens and stimulate T-cells. Vpu, one of the intrinsic HIV-1 proteins, appears to block TNF-α-induced antigen processing, effectively inhibiting its physiologic antiviral activity against HIV-1 infection. Vpu appears to inhibit NFκB-dependent antigen degradation, thus limiting the physiologic activity of TNF-α to stimulate antigen processing and presentation[137]. Finally, *in vitro* experiments examining HIV-1 pathogenesis using a recombinant plasmid encoded Vpr show that this promotes HIV-1 replication by a TNF-α-dependent mechanism[138]. In summary, TNF-α is capable, via TNF-R1, of increasing HIV-1 replication *in vivo* and *in vitro*. TNF-α, while in some cases inhibiting HIV-1 replication in monocyte systems via downregulation of CCR5 receptors, is concurrently capable of upregulating CXCR4 receptors, thus increasing susceptibility to X4 virus strains. Finally, it is largely held that TNF-α in particular, and proinflammatory cytokines in general, increase in activity as HIV-1-disease progresses, probably until immunologic defects are so pronounced that cells are no longer capable of cytokine production.

Previous clinical trials

A number of agents chosen for their ability to modify TNF-α levels have been evaluated for a variety of HIV-1-related therapies. First, TNF-α itself has been used in the pre-HAART era as therapy for HIV-1-as-

sociated Kaposi's sarcoma. This trial resulted in increased HIV-1 p24 levels, providing further evidence that raising TNF-α levels increases HIV-1 replication[115]. Thalidomide and pentoxifylline, both weak TNF-α inhibitors, have been studied in small clinical trials for HIV-1 alone and for HIV-1-related opportunistic infections with variable success at decreasing TNF-α levels or decreasing viral load[99,139-147]. Based on the limited success of these trials, rolipram, a related phosphodiesterase 4 (PDE4) inhibitor known to decrease TNF-α levels, has been tested in several *in vitro* systems. In these studies it was successful in decreasing HIV-1 p24 levels, although whether this effect was based on inhibition of TNF-α, PDE4, or more likely a combination of both, remains to be elucidated[148-151]. Topotecan, a topoisomerase I inhibitor FDA approved for the treatment of ovarian cancer, has been shown *in vitro* to decrease HIV-1 replication in a topoisomerase-deficient cell line, thus indicating antiviral activity independent of its known antineoplastic mechanism, and possibly related to its demonstrated TNF-α blocking ability[152]. Another TNF-α inhibitor, S9a, inhibited HIV-1 replication *in vitro* in acute and chronically infected T-cell lines via downregulation of the TNF-α promoter and consequent NFκB inhibition[153].

In vitro studies performed by Skolnik, et al.[154] have shown that thiazolidinediones such as rosiglitazone (PPAR), currently being studied as treatment for HIV-1-associated lipodystrophy, inhibit both HIV-1 replication and TNF-α production. These data suggest that the decrease in HIV-1 replication is TNF-α dependent. It seems likely that at least some of the decrease in HIV-1 replication seen in *in vitro* infected PBMC, U1 cells, and alveolar macrophages is due to decreased TNF-α. Most recently, a phase I clinical trial designed to test the safety and tolerability of CPI-1189 (a synthetic benzamide with purported anti-TNF-α effects) in 42 HIV-1-infected subjects with cognitive-motor impairment demonstrated safety, but no clinical efficacy or alteration in CNS TNF-α levels[155]. One clinical trial evaluated the TNF-α MAb (cA2), administered in two parenteral doses of 10 mg/kg given at a 14-day interval in six HIV-1-infected subjects. In this trial, cA2 was safe, well tolerated, and achieved a decrease of blood TNF-α level, but the study was of insufficient size to demonstrate CD4 or HIV-1 RNA changes with 42 days of follow-up[156].

Future directions

A number of agents that specifically decrease systemic TNF-α levels are now on the market and currently approved for the treatment of a variety of autoimmune diseases. Infliximab, a chimeric, humanized, anti-TNF MAb, is currently approved for the treatment of methotrexate refractory rheumatoid arthritis and Crohn's disease. It has demonstrated efficacy for other autoimmune diseases and is undergoing evaluation for a diverse group of conditions including sarcoidosis and graft-versus-host disease[157]. Two additional anti-TNF-α agents (etanercept and adalimumab) are also FDA approved for rheumatoid arthritis. These drugs, although generally safe and well tolerated, are associated with increased risk of opportunistic infections including reactivation tuberculosis[158]. New generations of orally active anti-TNF-α therapies that more specifically target steps of the TNF-α secretion and activation cycle are in development[159]. These may have potential as therapy for HIV-1 infection and might act synergistically with currently available therapies, but at present are accompanied by high risk for HIV-1 infected individuals. Optimally, an agent that was specific for the TNF-R1 without impacting TNF-α action at TNF-R2 would deserve serious consideration as an anti-HIV-1 therapy.

Summary

In the past decade there has been important progress in the development of clinically relevant cytokine-based therapies. New cytokine-based therapies with increased specificity have demonstrated effectiveness for both autoimmune and neoplastic diseases. The goal of specifically targeting defined defects induced by a pathogenic process is valid, but despite recent progress, currently impractical. Additional research is needed to better understand exactly what cytokines are disrupted and how addition or subtraction of these cytokines would alter the immunologic environment in each disease state. Ultimately, however, while animal models can show us gross effects of altering the cytokine milieu, it is only in human trials that we can truly observe how alteration of specific cytokine functions will affect the clinical status of a patient.

Fortunately, a number of potentially useful cytokine modifying agents are currently FDA approved and have been shown to be safe in different patient populations (Table 1). The TNF-α antagonists adalimumab, infliximab, and etanercept are currently FDA approved for rheumatoid arthritis and are being regularly used to treat Crohn's disease. These agents are too broadly active and potentially toxic, particularly with regard to the possibility of reactivation tuberculosis, to be used

Table 1. Main features of selected cytokines potentially useful as HIV therapy

Immune modulator	Action	Alteration in HIV	Selected inhibitors	Status	References
IL-1β	Fevers, anorexia, secretion of IL-6, increase circulating levels of prostaglandin E2, platelet-activating factor, nitric oxide and adhesion molecules	Probably increased, increasing with disease progression and decreasing with HAART. Decreased in very late stage disease.	Anakinra	FDA approved for RA, no studies in HIV	30,31,160,161
IL-6	T-cell activation and maturation; stimulates acute phase protein production resulting in fevers, anorexia, myalgia	Increased	Specific monoclonal antibodies	In clinical trials for multiple myeloma	56-60
			Tenidap	Failed to win FDA approval for treatment of RA because of decreasing bone mineral density	61
IL-18	IFNγ inducing factor, activates T and NK cells,	increased			
TNF-α	Stimulates APC; induces IL-1β, IL-8, GM-CSF, M-CSF, IFN-γ; activates CTL; induces apoptosis of mature T-cells; induces fever, hypotension, anorexia, capillary leakage; inhibits IL-12 activity	Increased, increases with disease progression	Etanercept = TNF-α binding protein,	FDA approved for a variety of autoimmune disorders	156,157,159
			Infliximab = Chimeric monoclonal antibody		
			Adalimumab = Anti-TNF-α antibody		
			Thalidomide	FDA approved for erythema nodosum leprosum, off-label indication for refractory aphthous ulcers and weight loss associated with HIV	139-145
			Topotecan	FDA approved for a variety of malignancies	152
			Thiazolidinediones	FDA approved for diabetes; in trials for HIV-associated lipodystrophy	154
Phospho-diestera-se E4	Proinflammatory mediator - activity increased by IL-18	Unknown	Cilomilast Roflumilast	Pending FDA approval for either COPD or asthma, awaiting additional efficacy studies. No studies in HIV.	150,162
			Rolipram	Pending FDA approval for depression, awaiting further comparative efficacy trials. No studies in HIV.	151
Caspase-1	Cleaves and activates IL-1 and IL-18	Unknown	Pralnacasan	In development for treatment for IBD	33,163,164
NFκB	Activation stimulates translation of proinflammatory enzymes and, in HIV stimulates translation of HIV from proviral DNA	Likely increased	Calagualine – derived from fern leucomostas, IκB phosphorylation inhibitor of NFκB (similar to salicylates)	Herbal remedy used in South America to treat psoriasis and inflammatory disorders. Blocks TNF-α induced activation of NFκB. No known studies in HIV.	165,166
			N-acetyl Cysteine – antioxidant inhibitor of NFκB	Has demonstrated safety in small trials in HIV-infected individuals. FDA approved for a variety of conditions	167-169

initially in HIV-1. Agents that decrease TNF-α activity downstream of its total effect, such as NFκB inhibitors, or agents that block TNF-R1 while sparing TNF-R2, while not ideal, may be a logical place to start anti-TNF-α trials in HIV-1 disease. NFκB inhibitors are both more and less specific than TNF-α inhibitors in that they block some but not all of the effects of TNF-α, while blocking other mediators, some perhaps unknown, that act via this pathway. The appeal of NFκB lies in the fact that HIV-1 is known to increase its own replication via this pathway and that blocking it could potentially directly decrease HIV-1 replication. Additionally, NFκB inhibitors decrease effects of both IL-18 and TNF-α that result in increased HIV-1 replication.

Because of the tremendous evolutionary redundancy within the cytokine system, it seems unlikely that altering levels of any one cytokine could dramatically decrease pathogenic cytokine effects. For this reason, it is logical to theorize that the combination of an NFκB inhibitor with an IL-1β inhibitor, and/or an IL-6 inhibitor or specific TNF-α inhibitor may decrease HIV-1 replication and possibly even decrease HIV-1 reservoirs. Although the safety of new agents must be assessed by initial studies of individual drugs, combination therapy may ultimately hold the most promise for the future.

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