Journal of Cellular Biochemistry 53:336–342 (1993)

Cytokine Involvement in Viral Permissiveness and the Progression of HIV Disease

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Abstract Many viruses have evolved novel means of exploiting host defense mechanisms for their own survival. This exploitation may be best exemplified by the interrelationships between certain viruses and the host cytokine networks. Many viruses, including the human immunodeficiency virus type-1 (HIV-1), rely on the liberation and cellular action of host immune cytokines to expand their host cell range, to regulate their cellular expression, and to maintain their dormant state until the proper extracellular conditions arise. As again exemplified by HIV-1, viruses may also take an active role regulating cytokine expression and cell surface cytokine receptors. Because the viral life cycle, and in particular the HIV-1 life cycle, is so intertwined with cytokine regulatory networks, these networks represent potential points for therapeutic intervention. As our understanding of cellular cytokine pathways involved in viral infection and replication continues to expand, so too will our ability to design rational anti-viral therapies to alter multiple steps along the viral life cycle. © 1993 Wiley-Liss, Inc.*

Key words: permissiveness, HIV-1, tumor necrosis factor, viral activation

By virtue of their need to utilize cellular replication machinery, viruses establish a parasitic relationship with the host cell. In many instances, this relationship goes beyond the replicative capacity of the cell and may result in cytolysis and pathology. Many influences can ultimately affect the host cell range and the ability of viruses to infect and replicate within their host cell. While some of these factors may be virally encoded, other factors are components of normal cellular physiology that viruses exploit to enhance their survival.

An area of extreme medical importance is the mechanisms regulating cellular infection with and expression of the human immunodeficiency virus type-1 (HIV-1). The natural prolonged course of HIV disease [1] suggests that several stages of the viral life cycle at the cellular level may be controlled by extracellular influences and thus may be open to therapeutic intervention. HIV-1 preferentially infects cells expressing the CD4 surface molecule, the demonstrated receptor for this virus [2]. Furthermore, target cells must be in an activated state to permit complete reverse transcription and integration of the virus, the two steps essential for productive infection [1]. Once integrated, HIV-1 may remain dormant until the right combination of extracellular influences are encountered to induce viral replication [3]. An understanding of these influences is essential to the development of novel anti-viral strategies.

ALTERED VIRAL PERMISSIVENESS

Alterations in viral permissiveness can be defined as any physiologic cellular change that allows increased infectivity or increased production of viral antigens from otherwise noninfectable or nonexpressing host cells. These alterations in permissiveness occur on at least four distinct levels (Fig. 1). First, at the membrane level, a change in permissiveness can occur through the increased surface expression of the specific viral receptor or a cytokine receptor that alters viral expression. Second, within a host cell, changes in viral permissiveness can result from a change in the signaling pathways leading to viral expression. Again within the host cell, any change in the nuclear binding proteins required for efficient viral expression will result in a change of viral permissiveness. The fourth

Received June 10, 1993; accepted July 26, 1993.

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change in permissiveness results from intracellular signaling that alters viral RNA transcription, stability, or translation, ultimately allowing progeny virions to be produced.

Events that alter the membrane expression of host cellular proteins could potentially influence each level of viral permissiveness. The most obvious alteration would be a direct increase in the surface expression of a viral receptor. However, increased host expression of other surface proteins could also positively influence viral expression by indirectly altering signaling pathways, activating nuclear proteins, and increasing overall cellular activation or RNA translation.

CYTOKINE-INDUCED CHANGES IN PERMISSIVENESS

As a class of immune modulators, cytokines can alter any or all of the four levels of viral permissiveness, depending upon the cytokine, the virus involved, and the host cell type in question. For those viruses that must utilize a cellular receptor to gain entry into the host cell, cytokines may positively or negatively influence receptor surface expression. This scenario might be most likely with viruses that utilize members of the immunoglobulin supergene family or adhesion molecules as receptors because these surface structures are generally controlled by immune modulation. A partial list of viruses known to use immunoglobulin supergene family members as receptors is presented in Table I. For instance, intercellular adhesion molecule-1 (ICAM-1) has been identified as the surface receptor for rhinovirus infection [4]. Any response that increases the expression of ICAM-1 on susceptible host cells might increase that cell's permissiveness to rhinovirus infection.

Cytokines may also alter the intracellular events that control viral expression. Signaling via a specific cytokine receptor generally induces an intracellular cascade that involves second messenger kinases and phosphatases and ultimately affects gene expression [5]. As a direct or indirect consequence of these signaling events, viral expression may be altered.

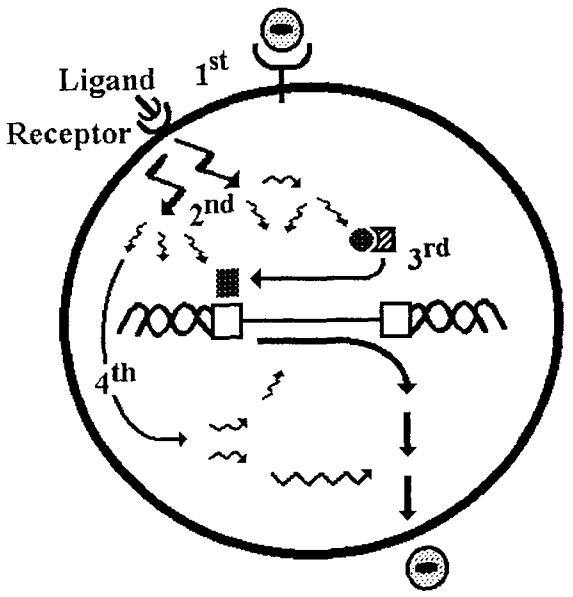

Fig. 1. Multiple levels may alter viral permissiveness. The first level indicates a change in the surface expression of the viral receptor itself or of an accessory molecule necessary to enhance viral entry or replication. The change at the second level of permissiveness would alter the complement of second messenger molecules necessary to induce viral replication. Similarly, a change at the third level of permissiveness alters the stability or expression of DNA binding proteins that enhance viral replication. Finally, the fourth level of permissiveness results in an alteration of viral RNA processing or translation to enhance viral production. Each level of permissiveness may be a target of cytokine action to influence viral expression.

EFFECTS OF CYTOKINES ON HIV-1 EXPRESSION

We have been applying the concepts of cytokine-induced alterations of viral permissiveness to the study of HIV-1 expression. In the context of HIV-1 infection at the cellular level, cytokines may alter any of the four levels of permissiveness in either a positive or negative manner. Numerous recombinant cytokines have been examined for influential effects upon HIV-1 expression, and different cytokines have been identified that influence several steps along the viral life cycle [6–10]. For instance, interleukin-2 (IL-2) supports the activation of resting peripheral T lymphocytes and permits a productive HIV infection. Interleukin-6 (IL-6) increases viral production by enhancing viral RNA translation. On the other hand, transforming growth factor-beta (TGF-β) and interferon-alpha (IFN-α) antagonize HIV production by reducing viral protein translation and mature virion release, respectively. We have been examining the multiple effects of the cytokine tumor necrosis factor-alpha (TNF-α) on HIV-1 infected cells and the ways in which HIV-1 regulates its own expression through the TNF-α network [11–13].

TNF-α has its most dramatic inductive effect on HIV-1 expression on chronically infected cells

TABLE I. Viruses That Use Immunoglobulin Superfamily Members as Surface Receptors

Virus family	Representative	Receptor	Natural ligand	Reference
Retroviridae	HIV-1	CD4	HLA-D	2
Picornaviridae	Rhinovirus	ICAM-1	LFA-1	4
Coronaviridae	MHV	mmCGM-1	?	29
Papovaviridae	SV40	HLA-A/B/C	TCR-CD8	30

in the postintegrative phase of the viral life cycle. Chronically infected cell lines can be developed if the cells that survive an acute cytopathic HIV-1 infection are cloned and expanded [3]. Among these populations, clonal cell lines that harbor an integrated but dormant HIV-1 provirus can be identified. These chronically HIV-1 infected cell populations presumably mimic the in vivo HIV-1 infected reservoir of cells that do not express HIV-1 until they encounter the right combination of external stimuli. Thus far, TNF-α has appeared to be nearly universal in its ability to stimulate HIV-1 expression from resting chronically infected cells of several different lineages.

Treating chronically infected cells with TNF-α alters both the second and third levels of permissiveness. Signal transduction from the TNF receptors (TNFRs) involves activating a complex series of protein kinases that make up the second level of permissiveness and are just now being dissected [14–16]. It has been repeatedly observed that signaling from TNFRs is independent of protein kinase-C. Recent information suggests that TNFRs trigger an intracellular sphingomyelinase to generate ceramide as the second messenger effector molecule [17,18]. Ceramide may then act at the third level of permissiveness to release nuclear factor-κB (NF-κB) from its cytoplasmic inhibitor and allow transposition to the nucleus. Active NF-κB within the nucleus could then bind to the enhancer region within the HIV-1 5′-long terminal repeat to induce viral transcription [8].

Cytokine induction of HIV-1 expression may influence other aspects of cellular physiology because of the added effects of viral proteins themselves. For instance, in the chronically HIV-infected cell system that we have examined most extensively, an HL-60 promyelocyte-derived clone known as OM-10.1 [11–13], the cells maintained in normal culture medium retain their surface expression of the CD4 molecule. However, as a direct consequence of viral activation after TNF-α treatment, these cells quickly down-modulate the surface CD4 receptor [11,12]. This down-modulation results, at least in part, from direct intracellular complexing between the HIV envelope precursor and its natural ligand, the CD4 molecule [9]. However, the viral protein Nef can also directly participate in down-modulating the CD4 receptor [19]. This surface CD4 down-modulation may be an important survival adaptation for HIV-1 because it could prevent the continued superinfection that has been postulated to lead to cell death due to an intracellular accumulation of unintegrated viral DNA [1]. Preventing this cytolytic cycle would permit the chronically infected cell to survive the acute burst of viral production, revert to a state of dormancy once the extracellular stimuli has been removed, and remain capable of virus production when another burst of extracellular stimulus is encountered [11].

Cytokine induction of HIV expression from chronically infected cells in vivo may be best exemplified by the microenvironment of the lymph node, recently identified as an important reservoir for HIV [20] where a large proportion of latently infected cells reside [21]. Antigens draining into the lymphoid compartments will induce an immune response, including the liberation of immunomodulating cytokines. The continued presence of these cytokines within the confined microenvironment of the lymph node would activate HIV expression from cells harboring an integrated but dormant virion and promote transmission to other cells within the node. Therefore, repeated immune stimulation by subclinical opportunistic infections may accelerate HIV disease progression. Therapeutic blocking of cytokine induced viral expression remains an important theoretical approach to preventing the perpetuating cycle of HIV activation and transmission to new target cells.

EFFECT OF HIV-1 EXPRESSION ON TNF INTERACTIONS

An area of continued controversy is the role of HIV-1 in regulating its own expression by altering the production of certain cytokines, includ-

ing TNF-α. Levels of circulating cytokines, especially TNF-α and IL-6, are elevated in patients with HIV disease, although this elevation may be caused by opportunistic infections. HIV infection of some cell types, as well as individual structural components of HIV, has been found to directly induce cytokine expression, again especially TNF-α. However, HIV-induced cytokine expression has not been a universal observation and may be strictly controlled by cell type [12].

While the controversy regarding the influences of HIV on TNF-α production remains unresolved, it is clear that this cytokine can function in an autocrine and paracrine manner to increase HIV-1 expression in chronically infected cells [8,12]. Because one response of promyelocytes to TNF-α treatment is to produce newly synthesized TNF-α, we have used the chronically infected OM-10.1 cell system to investigate further whether autocrine and paracrine HIV activation could result from exogenous TNF-α stimulation. Our data fully confirmed this possibility in that newly synthesized TNF-α could sustain HIV-1 expression during limited suboptimal exogenous TNF-α stimulation [12].

The TNF-α cytokine network can take on several additional levels of complexity (Fig. 2). Because TNF is initially expressed as a membrane-bound monomer before being shed and assembling into the bioactive trimer, the membrane-bound precursor may serve as a signaling protein when it interacts with the TNFRs on an adjacent cell. This juxtacrine interaction may signal the receptor-bearing cell, the cell expressing membrane-bound TNF, or both. Furthermore, the TNFRs may be shed from activated cells to signal distant cells via membrane-bound TNF; this signaling is known as a retrocrine interaction [3]. In our OM-10.1 model of autocrine TNF induction, a series of additional experiments was performed to investigate the role of soluble TNFRs in regulating TNF-induced HIV expression. We found that soluble TNFRs not only reduced the amount of autocrine TNF being synthesized but also reduced HIV expression from activated cells [12]. Therefore, retrocrine signaling via membrane-bound cytokines

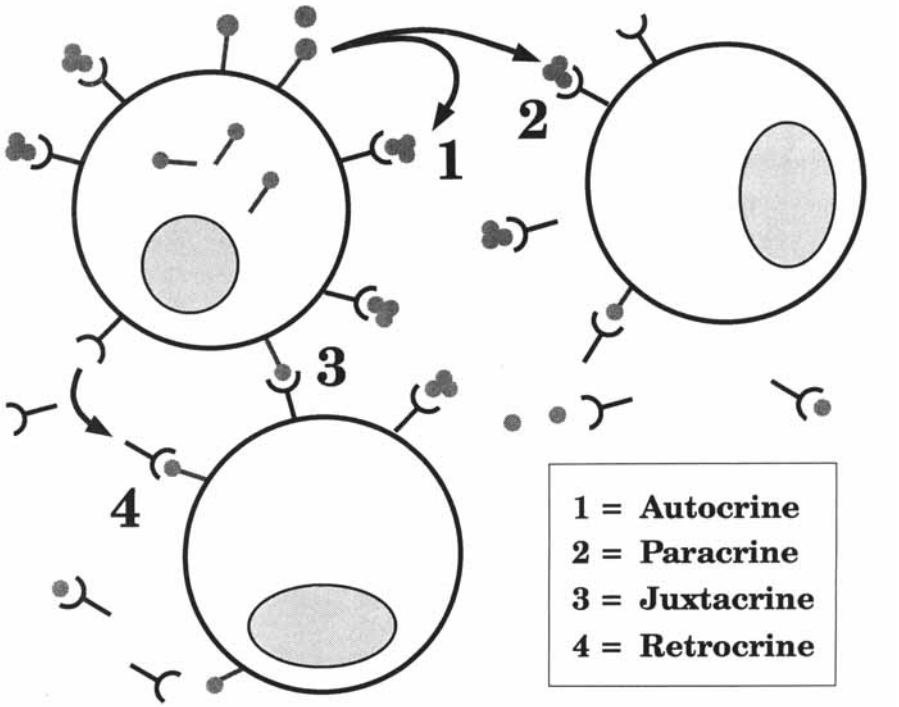

Fig. 2. Proposed signaling pathways for regulation of TNF-α responses. Upon transcription, TNF-α is expressed as a membrane-bound molecule that is liberated by proteolytic action. The active trimeric form of TNF-α assembles and can act back upon the secreting cell (autocrine) or upon a distant cell (paracrine) to induce a response. TNF receptors can also participate in modifying TNF-α responses by either contacting membrane-bound TNF-α of an adjacent cell (juxtacrine) or, as a soluble receptor, binding membrane-bound TNF-α of a distant cell (retrocrine). The consequences of these latter two interactions remain to be fully elucidated. (Reproduced from Butera and Folks, 1993, with permission.)

and soluble receptors may serve the important immunoregulatory role of restricting viral responsiveness to extracellular stimuli.

The added complexity of the TNF cytokine network encouraged us to examine the interrelationships between HIV-1 expression and the cellular receptors for TNF. Two separate receptors for TNF have been identified, a 55 kD TNFR (TR55) and a 75 kD TNFR (TR75) [14–16]. To date, most cellular responses to TNF-α treatment, including cellular proliferation, lysis of virally infected cells, and induction of NF-κB, have been attributed to signaling events mediated by TR55 [16]. Although TR75 has been implicated in TNF-induced cytolysis [22], the role of TR75 may be mainly that of a higher affinity "sink" to shuttle soluble TNF to TR55 during periods when exogenous TNF is at low concentrations [16]. With these observations in mind, we were not surprised to find that signaling solely through TR55 could maximally induce HIV-1 expression from OM-10.1 promyelocytes even though these cells express both TNFRs [13]. This observation must be conservatively interpreted until its universality to other chronically infected systems can be evaluated. However, at least in our promyelocytic model of latent HIV-1 infection, TR55 appears to be the major signaling receptor for TNF-induced viral activation.

We further extended our examination of the interrelationships between HIV expression and the TNFRs by evaluating surface levels of these receptors on resting and TNF-treated OM-10.1 cells as well as on similarly treated uninfected parental HL-60 promyelocytes [13]. Expression of TR55, the major signaling receptor, was not influenced by HIV expression, and surface levels on TNF-induced OM-10.1 cells were nearly identical to those of TNF-treated uninfected HL-60 cells. However, the surface expression of TR75 on HIV-expressing OM-10.1 cells dramatically increased within 24 h of viral activation. This up-regulation of TR75 expression appeared to be a direct consequence of HIV expression and was not observed in any of the similarly treated uninfected HL-60 subclones examined (n = 6).

Therefore, HIV-1 has not only adapted to regulate its expression by becoming entwined in normal cytokine networks but has also acquired an ability to alter cytokine networks by modulating specific receptors. The specific up-regulation of TR75 may not immediately appear to be beneficial for regulating HIV expression from dormancy, especially if TR55 serves as the major signaling component for HIV activation. However, if TR75 does serve as a high-affinity sink, an up-regulation of this receptor during HIV activation would ensure continued viral expression when exogenous TNF is limited.

The mechanism by which HIV induces a specific up-regulation of TR75 remains to be elucidated. Our preliminary studies clearly indicate that HIV expression does not result in an increase of mRNA encoding TR75. Therefore, other post-transcriptional effects on TR75 must be considered. It has been well established that both TNFRs can be shed from the surface of activated cells [14,15]. An HIV-specific mechanism to prevent TR75 shedding and allow for the surface accumulation of this molecule remains a plausible explanation.

The contribution of HIV regulatory proteins in controlling the surface expression of cytokine receptors must also be considered. In addition to the viral structural proteins, HIV encodes several well-characterized regulatory proteins that influence viral replication and normal cellular physiology. The HIV Tat regulatory protein has multiple effects on cellular responses including an induction of TNF and IL-6 expression. Recently, a down-modulation of TR75 was observed when Tat was transfected into Raji B-cells [23]. Several possible explanations exist for the apparent discrepancy between these observations and those we made with the chronically infected OM-10.1 cells. One explanation may be cell type specific differences (B-cell vs. promyelocytic culture systems), as we have not universally observed TR75 up-regulation in chronically HIV-infected T-cell lines. Another may be the effect of Tat alone in the transfected Raji cells vs. the presence of all the HIV gene products in our model of chronic infection. The involvement of the HIV regulatory protein Tat in TNF receptor modulations awaits further elucidation.

The HIV Nef regulatory protein also deserves consideration for its role in TNF receptor modulation. Nef is myristylated and membrane associated and has been implicated in altering surface expression of cellular membrane proteins, including the CD4 receptor, and the induction of NF-κB. The ability to alter cellular signaling pathways leading to viral activation may help explain why Nef was indispensable for pathogen-

esis in an animal model of HIV disease [24]. Therefore, virally encoded regulatory proteins are active at several levels of normal cellular physiology for the benefit of the HIV life cycle, and the involvement of these proteins in cytokine immunoregulation must be further appreciated.

INVOLVEMENT OF CYTOKINE NETWORKS IN HIV DISEASE PROGRESSION

The "cytokine network" theory predicts that multiple cytokines have overlapping functions, synergize or antagonize the actions of each other, and influence the expression of other cytokines along the network [25]. Immunomodulating cytokines that regulate multiple facets of the immune response include TNF, IL-1β, IL-2, IL-6, IL-8, IL-10, and the newly described IL-13 [25,26]; individually these cytokines are clearly implicated in regulating HIV expression. However, the control of HIV disease progression in vivo will involve a complex array of self-regulating cytokines functioning in a network fashion.

While the complexity of the cytokine network cannot be mimicked in vitro, agonistic and antagonistic activities among cytokines with respect to HIV expression have been observed [8]. TNF and IL-6 synergize to increase HIV expression by enhancing viral transcription and translation, respectively. As stated before, TGF-β and IFN-α repress TNF-induced HIV expression. While IL-10 and IL-13 suppress acute HIV infections, it remains uncertain whether IL-10 and IL-13 will also antagonize cytokine-induced viral expression as might be predicted by the network theory. During the progression of HIV disease, an alteration in the production of any of the cytokines along the network might have profound effects on HIV expression at a cellular level. Until the vast complexity of cytokine networks is partially unraveled, our understanding of the interactions between immunomodulating cytokines and HIV expression will remain incomplete.

A shift in the immune cell populations that secrete immunomodulating cytokines could also alter HIV disease progression. A growing body of evidence suggests that an important event in the pathogenesis of HIV disease involves a conversion in the subclass of helper T-lymphocytes (T_H) responding to HIV infection [27]. From murine systems, T_H cells can be functionally divided into T_H1 and T_H2 subclasses based on their production of immunomodulating cytokines. By convention, T_H1 cells produce mainly IL-2 and IFN-γ to augment cell-mediated immune responses while T_H2 cells secrete IL-4, IL-5, IL-6, and IL-10 to promote the humoral arm of the immune response. In keeping with the cytokine network theory, T_H cells also appear to be self-regulating. IFN-γ produced by T_H1 cells can down-regulate T_H2 cells and, reciprocally, IL-10 produced by T_H2 cells can down-regulate T_H1 cell function.

The conversion from a predominantly T_H1 cell response to a predominantly T_H2 cell response may explain some of the functional deficiencies observed in the cellular immune response during HIV disease progression [27,28]. The progressive T-cell dysfunction that accompanies HIV disease is characterized by an initial loss of responsiveness to recall antigens, followed by a loss of allo-antigen responsiveness, and finally a loss of responsiveness to mitogenic stimulation. Furthermore, the changing cytokine profile observed during progression of HIV disease (i.e., the loss of IL-2 and IFN-γ production followed by a peak of IL-4 and then IL-10 production) suggests that a conversion from T_H1 cell to T_H2 cell predominance is associated with HIV pathogenesis [27]. The mechanism controlling the conversion to T_H2 cell predominance is probably multifactorial, but HIV may play a direct and active role in this process by altering the production of immunoregulatory cytokines.

In addition to the contribution to HIV pathogenesis, the conversion to a T_H2 cell predominance has important implications for resistance to HIV infection, immunotherapy for HIV disease, and vaccine development. Individuals who have been exposed to HIV but did not become infected demonstrate a strong T_H1 cell responsiveness, suggesting that T_H1 cell function is important in preventing or controlling HIV infection [27]. This raises the possibility of preventing the progression of HIV disease by immunotherapy designed to enhance T_H1 cell function or down-regulate T_H2 cells. Anti–IL-4 and anti–IL-10 immunotherapy has been proposed specifically for this purpose. Finally, vaccines designed to augment T_H1 cell responsiveness may be more effective at preventing HIV infection than vaccines designed to stimulate broad neutralizing antibodies. An understanding of vaccine approaches permitting a selective enhancement of

T_H1 cell function is just being gained from murine systems.

FUTURE PROSPECTS

A major and immediate goal of the scientific and medical community is to apply our basic knowledge of virus-host interactions into areas where real therapeutic benefits can be realized. Although this goal applies to all viral diseases of human consequence, none are more urgent than HIV disease. New insights must be gained into cytokine-induced alterations of viral permissiveness that influence the host range and relative level of viral expression. Furthermore, the interrelationships between cytokine receptor expression and viral pathogenesis must be better appreciated. With this understanding, potential therapeutic approaches involving soluble cytokine receptors or other cytokine antagonists can be implemented to shift the influence of cytokine actions away from viral permissiveness. Immunotherapy in viral diseases targeting cytokine action is an area that continues to hold great promise as a more complete understanding of viral immunology continues to take shape.

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