Immunosuppression and HIV infection: A therapeutic challenge

SANDRA H BRIDGES, PHD, MARGARET I JOHNSTON, PHD, JOHN J McGOWAN, PHD

SH BRIDGES, MI JOHNSTON, JJ McGOWAN. Immunosuppression and HIV infection: A therapeutic challenge. Can J Infect Dis 1992;3(Suppl B):55B-59B. The optimal use of biological response modifiers (BRMs) in human immunodeficiency virus (HIV)-related disease depends on knowledge of the molecular basis of the immune deficiencies and dysregulations that occur during the course of the infection; evidence for the role of viral products and cytokines in the suppression of immune function is discussed. Immune-based therapies are currently being explored alone and in combination with drugs targeted to HIV and associated opportunistic infections and malignancies. These therapies include hematopoietic growth factors for the management of drug toxicities, cytokines, antigen- and cell-based therapies, and synthetic immunomodulators. The entry of additional BRMs into clinical trials for HIV-disease can be facilitated by well-designed preclinical studies that address special problems related to the disease, including the need for concomitant therapy for the spectrum of disease manifestations encountered.

Key Words: Human immunodeficiency virus, Immune suppression, Immune-targeted therapies, Immunodeficiency

Immunosuppression et infection à VIH: Un défi thérapeutique

RÉSUMÉ: L'utilisation optimale des modificateurs des réponses biologiques (MRB) pour contrôler les diverses entités pathologiques associées à l'infection à VIH dépend des connaissances que nous avons du fondement moléculaire des immuno-déficiences et perturbations qui surviennent au cours de l'infection. Le présent article traite du rôle des produits vitaux et des cytokines comme agents immunosuppresseurs. Les immunothérapies sont présentement étudiées seules ou associées à d'autres substances qui ciblent les infections à VIH ainsi que les autres infections opportunistes et les néoplasies associées. Ces traitements incluent les facteurs de croissance hématopoïétiques qui contrôlent la toxicité des médicaments, les cytokines, les thérapies à médiation cellulaire et antigénique, et les immunomodulateurs synthétiques. L'introduction de MRB supplémentaires dans les essais cliniques portant sur l'infection à VIH peut être facilitée par des études précliniques bien conçues qui s'attachent à certains problèmes spécifiques liés à la maladie et qui tiennent compte de la nécessité d'instaurer un traitement concomitant des nombreuses manifestations pathologiques observées dans cette maladie.

Basic Research and Development Program, Division of AIDS, National Institute of Allergy and Infectious Diseases, National Institutes of Health (USA), Bethesda, Maryland, USA

Correspondence: Dr Sandra Bridges, NIAID, Control Data Building, 6003 Executive Boulevard, Room 2C12, Bethesda, MD 20892, USA. Telephone (301) 496-8197, Fax (301) 480-5703
The major manifestation of human immunodeficiency virus (HIV-1) infection is immunodeficiency, and thus the development of biological response modifiers (BRMs) as therapeutic agents for HIV infection, specifically as immune modulators, seems appropriate. The challenge is to understand the molecular basis of the deficiencies and dysregulations that occur in the immune system during the course of the infection so that BRMs can be used effectively.

The most apparent component of HIV immunodeficiency is a numerical deficit of CD4+ T lymphocytes; however, functional abnormalities are demonstrable when normal numbers of CD4+ cells are still present (1-3). This article will focus on the latter aspect of the immunodeficiency and point out problem areas, specific to HIV disease, that should be taken into consideration in developing BRMs for HIV-associated immune dysfunction.

CD4+ T cells lose the ability to respond to soluble antigen relatively early in the disease process. This has been demonstrated both in vivo, by examining responses of HIV-infected subjects to skin testing with recall antigens, and in vitro, by measuring responses of peripheral blood lymphocytes from HIV-infected donors to recall antigens and mitogenic monoclonal antibodies. For example, purified CD4+ T cells from HIV-infected individuals fail to respond to tetanus toxoid, a recall antigen, and to antibodies directed to the T cell antigen receptor complex (CD3-Ti), but respond normally to T cell mitogens concanavalin A and phytohemagglutinin A (4). Similarly, purified CD4+ T cells from normal donors, infected with HIV in vitro, fail to mobilize calcium after stimulation with anti-CD3, whereas calcium mobilization proceeds normally after stimulation with an antibody directed towards CD2, a signal-transducing cell surface molecule not associated with CD3-Ti (5). These findings suggest that HIV infection uncouples signal transduction through the T cell receptor, but not through other pathways and immune dysfunction is not simply linked to infection since, in the former case when cells are from an HIV-infected subject, the majority of cells are not infected.

**MEDIATORS OF HIV-ASSOCIATED IMMUNE SUPPRESSION**

**Viral gene products:** Several possible mediators of HIV-associated immune suppression are given in Table 1. Considerable attention has been focused on the role of two viral gene products, the envelope protein gp120 and the regulatory protein Tat, in immune suppression.

With regard to the role of gp120 in HIV-associated immune dysfunction, several groups have reported that gp120 inhibits antigen-specific responses of peripheral blood mononuclear cells (6) or CD4+ antigen-specific T cell clones (7-10) from normal donors. Collectively, they demonstrated that μg/mL (or less) concentrations of highly purified native or recombinant gp120 inhibited proliferative responses of tetanus and diphtheria toxoid-specific T cells and cytolytic activity of Epstein-Barr virus-specific T cells. Proliferative responses to anti-CD3 were also inhibited by gp120, but not responses to anti-CD2 or mitogens. In-depth studies revealed that gp120 inhibited 'early' events of the activation program, i.e., intracellular calcium increase, inositol phosphate accumulation and translocation of cytosolic protein kinase C to the cell membrane (8,10). The expression of mRNA for the interleukin (IL)-2 gene was inhibited, as were IL-2 production and surface IL-2 receptor expression (9).

Similarly, the HIV regulatory protein Tat was shown to inhibit antigen-driven proliferative responses but not mitogen-driven responses (11). Highly purified recombinant Tat inhibited tetanus toxoid- and candida-specific responses over the range of 0.1 to 10 μg/mL in a dose-dependent manner. The inhibitory effect could be neutralized by an anti-Tat serum or abolished by oxidation of Tat. In agreement with earlier studies, exogenously added Tat was shown to enter cells since it transactivated the HIV long terminal repeat-linked chloramphenicol acetyl transferase gene in several transfected cell lines.

The mechanism(s) by which these viral gene products mediate suppressive effects in vitro is not known. The envelope protein gp120 interacts with CD4 on the surface of T cells and monocytes/macrophages. In the case of T cells, CD4 is linked closely to the T cell antigen receptor complex and likely plays a role in signal transduction through that complex; the interaction of gp120 with CD4 may prevent critical associations at the cell surface that are required for T cell activation (12,13) or alternatively, may transmit a negative signal (14,15). The mechanism by which Tat inhibits T cell proliferation is less clear. It has been suggested that Tat may interfere with signal transduction via the T cell antigen receptor, with antigen processing or presentation, or function indirectly by inducing cytokine mediators (11).

**Cytokines:** A great deal of interest in the role of cytokines in HIV-associated immune suppression has been engendered by observations of increased levels of several cytokines in HIV-infected subjects. The cytokines most studied in relation to HIV infection are IL-6, tumour necrosis factor-α and transforming growth factor-β.

Elevated plasma levels of IL-6 are seen in HIV-infected individuals.
fected individuals (16,17). At a cellular level, peripheral blood mononuclear cells from infected donors produce elevated levels of IL-6 as reflected in mRNA and release of biologically active material in vitro in the absence of exogenous stimulators (16). In normal subjects, IL-6 is produced by a variety of cell types, including T cells, B cells and macrophages; live or inactivated HIV, when used as a stimulus in vitro, induces IL-6 from monocytes/macrophages, but not T cells (18). Studies with fractionated peripheral blood mononuclear cells from HIV-infected subjects suggest that both monocytes/macrophages (16) and B cells (19) contribute to the overproduction.

A direct role for IL-6 in HIV-related immune suppression has not been demonstrated; however, the overproduction of IL-6 may contribute to the pathogenesis of HIV disease. IL-6 has been shown to upregulate HIV expression in acutely and chronically infected cell lines of monocytic lineage and synergizes with tumour necrosis factor-α in the induction of latent HIV expression (20). Moreover, plasma levels of IL-6 reflect the stage of HIV disease (17). Because of its demonstrated ability to function as a growth factor for myeloma cells (21), it has been suggested that IL-6 may contribute to the polyclonal B cell activation that characterizes the HIV disease process. Recently, it has been shown that Epstein Barr virus-transformed B lymphoblasts genetically engineered to produce IL-6 constitutively have increased clonogenic potential and are tumourigenic in nude mice (22). As will be discussed later, the potential of BRMs to impact negatively on disease course is a special concern in HIV infection.

Tumour necrosis factor-α shares many of the negative characteristics of IL-6 as a potential therapeutic for HIV infection. Added exogenously to chronically infected cell lines of monocytic and T lymphocytic origin, it up-regulates HIV expression; when produced endogenously as a result of cell activation by PMA, tumour necrosis factor-α up-regulates HIV expression, tumour necrosis factor-α mRNA and biologically active tumour necrosis factor-α (23). Similar enhancement of expression has been observed in primary macrophages (24) and purified CD4+ cells (25) infected in vitro with HIV and treated with exogenous tumour necrosis factor-α. Additionally, it has been shown that tumour necrosis factor-α antagonizes the inhibitory effect of zidovudine on HIV-1 replication in vitro (26). Thus, with the current level of information on interactions between this cytokine, HIV replication and zidovudine, tumour necrosis factor-α would not appear to be a good candidate for use in HIV infection.

The presence of endotoxin, mycoplasma, endogenous cytokines or other extraneous material in media, serum or virus stocks can affect the activation state of cells in vitro. Therefore, in vitro enhancement of HIV expression should not necessarily contraindicate the

![Image](image.png)

**TABLE 2**

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Rationale for use</th>
<th>Proposed indication</th>
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</thead>
<tbody>
<tr>
<td>IFN-α plus AZT</td>
<td>Antiviral, antiproliferative</td>
<td>AIDS with Kaposi’s sarcoma</td>
</tr>
<tr>
<td>IFN-β plus AZT</td>
<td>Antiviral, antiproliferative</td>
<td>AIDS with Kaposi’s sarcoma</td>
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<tr>
<td>IFN-γ plus AZT</td>
<td>Immunomodulator</td>
<td>AIDS with history of opportunistic infection</td>
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<tr>
<td>TNF-α alone</td>
<td>Antiviral/Immunomodulator</td>
<td>AIDS-related complex</td>
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<tr>
<td>TNF-α plus IFN-γ</td>
<td>Antiviral/Immunomodulator</td>
<td>AIDS-related complex</td>
</tr>
<tr>
<td>IL-2/IL-2-PEG plus AZT</td>
<td>Immunomodulator (T and NK cells)</td>
<td>All stages of HIV infection</td>
</tr>
<tr>
<td>GM-CSF/G-CSF plus AZT or mBACOD/ABVD</td>
<td>Management of hematopoietic toxicity</td>
<td>AIDS-related complex, AIDS-associated malignancies</td>
</tr>
</tbody>
</table>

*ABVD: Doxorubicin, bleomycin, vinblastine, dacarbazine; AZT: Zidovudine; GM-CSF: Granulocyte Macrophage colony-stimulating factor; IFN: Interferon; IL: Interleukin; mBACOD: Cyclophosphamide, doxorubicin, vincristine, bleomycin, dexamethasone, methotrexate, folic acid; NK: Natural killer; PEG: Polyethylene glycol; TNF: Tumour necrosis factor*
use of a particular BRM, but rather serve as a stimulus for careful monitoring of effects on viral parameters in vivo.

Transforming growth factor-β is an example of a cytokine with potent immunosuppressive activity that is elevated in HIV infection and acquired immune deficiency syndrome (AIDS). Recent studies show that peripheral blood mononuclear cells from HIV-infected donors spontaneously release transforming growth factor-β and that the level of secretion correlates with the impairment of antigen-specific responses in vitro; further, if antibodies to transforming growth factor-β are added to the cultures, the impaired responses can largely be reversed (27). The immunosuppressive activity of transforming growth factor-β extends to B lymphocytes as well. The proliferative response of purified B cells from normal donors to mitogen is impaired by supernatants from peripheral blood mononuclear cells from HIV-infected donors and by purified transforming growth factor-β; this is reversible by anti-transforming growth factor-β (28).

**Gene products of opportunistic pathogens:** A direct role for opportunistic pathogens has not been defined, although it seems likely that gene products from the various organisms associated with HIV infection will be found to contribute to the observed immune suppression. Simultaneous infection with a particular opportunistic pathogen has not been shown to be a constant feature of HIV disease.

**Immune Targeted Therapies Investigated**

Tables 2 and 3 list immune-based therapies that have been explored in clinical trials by the Division of AIDS (DAIDS), National Institute of Allergy and Infectious Diseases (NIAID), National Institutes of Health (NIH) since 1987. A suggested mechanism of action or rationale for use is given for each therapy. Currently, BRMs are being used in combination with zidovudine because most BRMs would not be expected to impact directly on the virus infection. Since a detailed description of AIDS therapies is beyond the scope of this review, the reader is referred to the AIDS/HIV Treatment Directory (29) for further information on specific drugs and combinations of drugs.

What has been the outcome of the various trials using the listed BRMs? Many of the trials are ongoing; others are completed and the data are being analyzed. In a trial evaluating an IL-2/zidovudine combination, an IL-2 dose was established at which there was an acceptable level of toxicity associated with positive changes in immune parameters (30). However, continuous infusion was used to maximize bioavailability, a strategy that is not easily extended to large numbers of patients. In order to simplify the logistics of administration, a polyethylene glycolated form of IL-2 (PEG-IL-2) is being evaluated in a second trial; it can be given less frequently since it remains in the circulation longer (31).

**Development of BRMs for HIV Infection**

Some of the practical issues to be considered in developing BRMs for HIV infection are: development of a rationale for use that is relevant to HIV disease; evaluation of BRMs in combination with antivirals or other drugs; assessment of the potential of the BRM to activate or enhance HIV expression, and identification of a parameter related to efficacy that is measurable in the clinic. Information on some or all of these points can be obtained at the preclinical level, in vitro and/or in vivo in animal models. First, an immune defect should be targeted and a rationale developed for use of a BRM that might address that deficiency. As an example, HIV-infected individuals have decreased intracellular glutathione levels (31). Since glutathione has been shown to be important in T cell activation and/or proliferation (33,34) and T cell function clearly is compromised in HIV-infected individuals, a rationale has been developed for drugs that might increase intracellular thiol levels; these drugs include N-acetyl cysteine and glutathione ester.

Currently some BRMs are being evaluated for their ability to reverse toxic effects of antiretroviral drugs and of chemotherapeutic agents being used for AIDS-associated lymphomas (Table 2). Granulocyte macrophage colony stimulating factor (GM-CSF) is being used to treat the bone marrow suppression that results from zidovudine treatment. In vitro, GM-CSF alone enhances HIV expression in purified monocytes/macrophages by several 100-fold (35). However, the antiviral activity of zidovudine is increased when combined with GM-CSF, i.e., the combination is synergistic. This example illustrates the need to evaluate potential BRM candidates alone, in order to assess their potential to activate or enhance HIV expression, and in combination with an antiretroviral, to look for positive or negative interactions.

Finally, in developing BRMs for clinical use for HIV infection or other indications, it is important to identify a parameter related to efficacy that can be measured in the clinic.

**References**

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