Cytokines as potential therapies for human immunodeficiency virus infections

ROBERT W SIDWELL PhD, JOHN D MORREY PhD, REED P WARREN PhD

RW SIDWELL, JD MORREY RP WARREN. Cytokines as potential therapies for human immunodeficiency virus infections. Can J Infect Dis 1994;5(Suppl A):28A-35A. Cytokines are attracting increased interest as potential therapies for human immunodeficiency virus (HIV) infections. This attraction has particularly arisen as these cytokines have become more commercially available through recombinant technologies. This review focuses on the effects of these biological response modifiers on preclinical HIV and related retrovirus infections. Cytokines that have particularly been considered for HIV disease control include: interferons-α, -β and -γ; interleukins-2, -3, -4, -6 and -7; tumour necrosis factors-α and -β; and the colony stimulating factors. Efficacy has especially been seen when these cytokines have been used in combination with the more conventional antiviral agents. Due to the many biological functions exerted by cytokines and their interweaving of biological effects with other cytokines, they appear to have the potential to both inhibit as well as enhance viral infections, depending upon how they are used, and caution is therefore urged in their use.

Key Words: Colony stimulating factor, Cytokines, Human immunodeficiency virus, Interferon, Interleukin, Tumour necrosis factor

Les cytokines et leur rôle potentiel dans le traitement des infections au VIH

RÉSUMÉ : Les cytokines font l'objet d'un intérêt croissant à titre de traitement potentiel contre les infections au virus de l'immunodéficience humaine (HIV). Cet intérêt est notamment dû au fait que ces cytokines sont devenues plus accessibles commercialement par l'entremise du génie génétique. Cette revue porte sur les effets des modulateurs de la réponse biologique sur un HIV préclinique et les infections à rétrovirus associées. Ces cytokines, qui ont été particulièrement pressenties dans la lutte contre la maladie au HIV, comprennent les interférons-α, -β et -γ; les interleukines-2, -3, -4, -6 et -7; les facteurs de nécrose tissulaire-α et -β; et les facteurs stimulateurs des colonies. L'efficacité a notamment été observée lorsque ces cytokines ont été utilisées en association avec des antiviraux plus classiques. À cause des nombreuses fonctions biologiques exercées par les cytokines et de leurs effets biologiques complexes en interaction avec d'autres cytokines, elles semblent dotées du pouvoir d'inhiber et d'exacerber les infections virales selon la façon dont elles sont utilisées. Il faut donc faire preuve de beaucoup de circonspection lorsqu'on les emploie.
A BROAD SPECTRUM OF AGENTS HAS BEEN CONSIDERED AS therapies for human immunodeficiency virus (HIV) infections in humans. Among these has been a variety of materials whose primary mechanisms of action are as immunomodulators. Using immunomodulation as an approach for such therapy would seem appropriate since the major manifestation of HIV infection is immunodeficiency.

Among the immunomodulators that have been particularly considered are the cytokines. Balkwill and Burke (1) have offered the following definition for these substances: "Cytokine is one term for a group of protein cell regulators, variously called lymphokines, monokines, interleukins and interferon, which are produced by a wide variety of cells in the body, play an important role in many physiological processes, are involved in the pathophysiology of a range of diseases, and have therapeutic potential."

The cytokines often have major effects on the hematopoietic and immune systems (2), which point to their potential as therapies or for the prevention of infectious complications in the immunosuppressed patient. These same effects, however, if inappropriate to the disease manifestation, may also result in adverse effects that need to be considered.

Recombinant DNA technology has allowed many cytokines to be synthesized efficiently, which enhances their potential as antiviral agents. Of the many cytokines known, research efforts to elucidate their antiviral activity have focused primarily on those listed in Table 1.

This review focuses on the reported nonclinical experiments with these cytokines. The experiments are oriented towards defining the potential of cytokines as therapies for AIDS.

### INTERFERONS

More work has been accomplished with interferons (IFN) than with any other cytokine in studying their potential for therapy of virus diseases. Space does not allow an in-depth review of the vast amount of literature available on IFN; the focus of this review is on the potential of type 1 IFN (α and β) and type 2 IFN (γ) to inhibit HIV infections or those of related retroviruses.

**Mechanisms of antiviral effect:** All three species of IFN markedly inhibit HIV replication in vitro, as well as inhibiting the replication of other retroviruses (3-11). IFNs exert a spectrum of in vitro effects on these viruses, including inhibition of assembly, maturation and release (12,13). When cells are chronically infected, a late stage of viral morphogenesis is inhibited, which results in either a significant inhibition of virus production or a release of fewer noninfectious particles (4,5,9). In a study in which viral production was slowed, an accumulation of virus particles occurred on the cell surface and in cytoplasmic vacuoles (10). This may be a result of IFN effects on the fluidity of the plasma membrane (11,13). Smith et al (10) showed that inhibition of HIV production by IFN-α in chronically infected cells may have been primarily a result of decreased cell growth since reverse transcriptase and the number of viable cells decreased at similar rates. All IFNs are known to induce 2',5'-A synthetase, which indirectly activates endonucleases that may degrade viral RNA (14), presenting another potential mechanism for their action.

IFNs are significant inhibitors of murine retrovirus disease (15-17). The antiviral efficacy may be attributable to both more traditional antiviral effects, as noted above, and their actions as immunomodulators. IFN-α has been shown to enhance natural killer (NK) cell activity and antibody-dependent cell-mediated cytotoxicity (18). Pertinent to IFNs' immunomodulatory effects are some recent experiments by the present authors using human recombinant IFN-α-A/D (Hoffman La Roche, Inc, New Jersey) against Friend virus (FV) infection in mice. In one experiment, the mice used were (B10.11x1/WySn)F1 hybrids, which can produce high titres of FV-neutralizing antibodies, but are relatively low in FV-specific cytotoxic T lymphocyte activity (19-21). The IFN-α treatment (10,000 U/mouse/day) inhibited splenomegaly and resulted in some reduction of virus titre, particularly in plasma. When the experiment was repeated using (B10.11x1.Balb/c)F1 hybrid mice, which have the ability to produce FV-specific neutralizing antibodies and to have cytotoxic T cell activity (19), 10,000, 5000 and 2500 U/mouse/day of the same IFN-α rendered a significantly enhanced antiviral effect especially manifested in reduced spleen and plasma virus titres. Both mouse strains exhibit a strong NK cell response. These data suggest that the activity of this cytokine may be linked to the cytotoxic T cell response.

The mechanism(s) by which IFNs act synergistically with more standard antiviral drugs is unclear. The differences in specific mechanisms by which virus is being inhibited by the two substances used in combi-

| Table 1: Cytokines studied for efficacy against experimentally induced virus infections |
|---------------------------------|------------------------------------------------------------------|
| Interferon-α                    |                                                                  |
| Interferon-β                   |                                                                  |
| Interferon-γ                   |                                                                  |
| Interleukin-2                  |                                                                  |
| Interleukin-3                  |                                                                  |
| Interleukin-4                  |                                                                  |
| Interleukin-6 (Interferon-β2)   |                                                                  |
| Interleukin-7                  |                                                                  |
| Tumour necrosis factor-α       |                                                                  |
| Tumour necrosis factor-β       |                                                                  |
| Granulocyte-macrophage colony stimulating factor |          |
| Macrophage-specific colony stimulating factor |          |
| Granulocyte-specific colony stimulating factor |          |
TABLE 2

Retrovirus inhibition studies using interferon in combination with other agents

<table>
<thead>
<tr>
<th>Action</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>IFN-α + AZT or PFA synergistically inhibited HIV in PBLs</td>
<td>58,59</td>
</tr>
<tr>
<td>IFN-α + HEPT synergistically inhibited HIV in MT-4 cells and PBLs</td>
<td>60</td>
</tr>
<tr>
<td>IFN-α + protease inhibitor synergistically inhibited HIV in PBLs</td>
<td>61</td>
</tr>
<tr>
<td>IFN-α + AZT synergistically inhibited HIV transmission from U9-111B cells to cocultivated U937 cells</td>
<td>62</td>
</tr>
<tr>
<td>IFN-α + AZT synergistically inhibited Rauscher virus infection in mice</td>
<td>15</td>
</tr>
<tr>
<td>IFN-α + AZT was more effective than IFN-α or AZT alone in reducing FeLV viremia and preventing FeLV in cats</td>
<td>26</td>
</tr>
<tr>
<td>IFN-α + AZT + adoptive transfer of activated lymphocytes reversed established FeLV infection in cats. Antiviral effect of IFN-α alone was limited by production of antibodies to IFN-α</td>
<td>25,28</td>
</tr>
<tr>
<td>IFN-α + AZT failed to protect monkeys from SIV challenge, but reduced virus replication better than AZT alone</td>
<td>25</td>
</tr>
<tr>
<td>IFN-α + D4T synergistically inhibited Friend virus replication in vitro and enhanced the inhibition of spleenomegaly, spleen and plasma virus titres and hematocrit increase in Friend virus-infected mice. Enhanced NK cell activity was seen with the IFN + D4T treatments</td>
<td>23</td>
</tr>
<tr>
<td>IFN-β + AZT synergistically inhibited HIV-1 in HeLa T4 and MT-2 cells</td>
<td>25</td>
</tr>
<tr>
<td>IFN-γ + AZT synergistically inhibited LP-BM5 virus in macrophages</td>
<td>63</td>
</tr>
<tr>
<td>IFN-γ + TNF-α synergistically induced HuT178 and RPM11788 cell resistance to HIV, reduced HIV RNA and p24 synthesis, inhibited production of infectious HIV, killed cells already infected with HIV and inhibited production of HIV mRNA in chronically infected cells</td>
<td>64</td>
</tr>
<tr>
<td>IFN-γ + human lactoferrin synergistically inhibited Friend virus-induced splenomegaly in mice</td>
<td>65</td>
</tr>
</tbody>
</table>

AZT Zidovudine; D4T 2',3'-didehydro-2',3'-dideoxythymidine; FeLV Feline leukemia virus; HEPT 1-(2-hydroxyethoxy) -1-(methylene-6-(phenylthio)thymine; HIV Human immunodeficiency virus; IFN Interferon; NK Natural killer; PBLs Peripheral blood lymphocytes; PFA Phosphonoformate; SIV Simian immunodeficiency virus

The NK cell activity in the animals, and, in combination with D4T, this NK cell activity was further increased (23). Earlier reported studies by Sidwell et al (17) and by Black et al (24) indicated that NK cell activity may be an important factor in the in vivo retrovirus disease inhibition exerted by biological response modifiers.

Potential as therapies for HIV infections: The future of IFNs as therapies for AIDS appears to be in their use in combination with more specific anti-HIV therapies. Table 2 summarizes the retrovirus experiments with the combinations that have been described to date. Only in the study by Fazely et al (25), using the combination of IFN-α and AZT against simian immunodeficiency virus infections in rhesus monkeys, was the treatment not considered effective. In this study, however, the combination treatment did appear to depress the level of virus replication more effectively than either substance used alone. The studies of the effects of IFN-α in cats infected with feline leukemia virus (FeLV) have been quite revealing (26-28): the combination of IFN-α with AZT was absolutely required for a significant antiviral effect to be seen, and the inclusion of adoptive transfer of activated lymphocytes was required to reverse an established FeLV infection. Also of considerable importance was the observation that the development of neutralizing antibody to the recombinant human IFN-α appeared to limit the antiviral efficacy of this cytokine (26,27). Such an effect has also been reported in human patients receiving IFN-α for treatment of neoplastic disease (26).

INTERLEUKINS

Interleukins (ILs) have been defined as "proteins produced by leukocytes which function during inflammatory responses by acting on leukocytes or other targets" by the International Union of Immunological Societies Nomenclature Committee (29). The number of ILs discovered has increased steadily, with at least 11 known to date. Of the ILs, IL-2 has been studied most extensively for antiviral activities. Human recombinant IL-2 is now available commercially and has been used in most of the studies cited.

Mechanism of antiviral effects: IL-2, a 13 to 15 kDa glycoprotein derived from CD4+ and CD8+ T lymphocytes in response to mitogenic or antigenic stimuli, is required for the development of various cell-mediated immune functions that are known to be deficient in AIDS patients. These functions include support of the proliferation and differentiation of T and B lymphocytes and the enhancement of NK cell activity (30). IL-2 production is defective in patients infected with HIV (31).

Potential for therapy of HIV infections: Table 3 summarizes the retrovirus inhibitory effects of the ILs. The Zeidner et al study (26) employing IL-2 alone or in combination with AZT in treating FeLV in cats was run using the same conditions as the previously cited study with IFN-α and AZT. Using the latter combination, a synergistic effect was seen in enabling the animals to
resist the viral challenge, whereas when IL-2 was used alone, no protection was rendered, and using IL-2 in combination with AZT appeared to be less effective than using AZT alone. The most potent action of IL-2 appears to be its ability to restore the immunological responses of peripheral blood lymphocytes from AIDS patients (32,33). NK cell activity was consistently the immune parameter that was most strongly affected by recombinant human IL-2 treatment, an observation also made by Mead et al (34) in a phlebovirus infection model in which marked immunosuppression occurs. Sayers et al (35) also demonstrated activation of NK cells following treatment with human recombinant IL-2 in mice. This potential to restore AIDS-related immunosuppression is provocative: caution should be used, however, in extrapolating in vitro data to potential in vivo efficacy, especially when one must consider pharmacokinetics and potential toxicity of IL-2. Adverse effects associated with IL-2 were recently reviewed (2).

A study of interest regarding IL-2 was reported by Finberg et al (36). Previously, it was shown that the binding of gp120 from HIV leads to IL-2 receptor expression on monocytes, and cross-linking of CD4 leads to IL-2 receptor expression in T cells. In the Finberg et al study, a genetically engineered fusion toxin consisting of a fragment of diphtheria toxin, DAB486, was bound to IL-2 sequences, which resulted in a selective killing of HIV-infected T cells. Two doses of the cytotoxin were used, and both markedly reduced the number of infected cells while not appreciably affecting uninfected T cells. The DAB486 IL-2 has reportedly been used safely in patients with IL-2 receptor expression malignancies (36). This unique approach of using targeted toxins appears to have promise as a means of treating HIV-infected individuals.

The final study (37) cited in Table 3 involves the use of recombinant human IL-7, a 25 kDa glycoprotein identified as a growth factor that stimulates the proliferation of B cell precursors (38) as well as thymocytes and mature T lymphocytes, and has a variety of other biological activities (39). In the study cited, the splenomegaly as well as the number of splenic focus-forming virus units and viral messenger RNA induced by the Fv complex in mice were significantly reduced by subcutaneous IL-7 treatment. Mean survival time of the animals was significantly increased. IL-7 treatment also restored NK cell activity, IL-6 and IFN-γ to normal levels in the infected animals. The treatments were apparently reasonably well tolerated.

**TUMOUR NECROSIS FACTOR**

Tumour necrosis factors (TNFs) are proteins produced by activated macrophages and lymphocytes and a number of other cells that destroy tumour tissue in a tumour-bearing animal (40). TNFs have recently undergone considerable scrutiny as a potential antiviral substance.

### TABLE 3

**Retrovirus inhibition studies using interleukins**

<table>
<thead>
<tr>
<th>Action</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>rIL-2 used alone moderately reduced circulating FeLV viral core protein in cats: in combination with AZT, viral core protein was markedly reduced, but less than with AZT alone</td>
<td>26</td>
</tr>
<tr>
<td>rIL-2 enhanced the NK cell and CMV-specific cytotoxic lymphocyte activities of PBLs taken from AIDS patients</td>
<td>32</td>
</tr>
<tr>
<td>rIL-2 increased in vitro the NK cell and mitogen and alloantigen dependent proliferative responses of PBLs taken from AIDS patients</td>
<td>33</td>
</tr>
<tr>
<td>IL-2 fused to DAB486 toxin selectively killed HIV-1-infected cells and inhibited production of viral protein and infectious virus</td>
<td>36</td>
</tr>
<tr>
<td>IL-6 induced HIV expression alone and synergistically with TNF-α in infected mononcytic cells</td>
<td>39</td>
</tr>
<tr>
<td>IL-4 was weakly inhibitory, and IL-2 and IL-6 were not inhibitory to HIV p24 antigen production in macrophage cultures</td>
<td>3</td>
</tr>
<tr>
<td>IL-6 increased HIV p24 expression in U1 cells. IL-3, IL-8 had no effect</td>
<td>66</td>
</tr>
<tr>
<td>rIL-7 inhibited the murine disease induced by the Friend virus; NK cell activity, IL-6 and IFN-γ levels were restored to normal</td>
<td>37</td>
</tr>
</tbody>
</table>

**Mechanisms of antiviral effects:** As with the other cytokines, TNF has a wide spectrum of biological activities. TNF produced by macrophages and monocytes is designated TNF-α; TNF-β is lymphotoxin material produced by activated lymphocytes. TNF-α and TNF-β have similar biological properties.

This cytokine is not generally considered to be primarily an antiviral mediator, but has been shown to exert antiviral activity both by suppressing viral replication and by eliminating HIV-infected cells. Wong et al (41) have noted that TNF may function indirectly in antiviral defence by making infected cells more easily recognized by cytotoxic T cells. They cite the induction of viral antigen expression reported by Folks et al (42) and by Yagi et al (43), which results in an increase in viral antigens on the surfaces of latently infected cells. Wong et al (41) reason that the appearance of these antigens, concomitant with increased major histocompatibility complex class 1 expression, which is also stimulated by TNF (44), "signal the immune system that the host cell should be destroyed". In line with these observations are also those of Polli et al (39), who reported that the combination of TNF-α and IL-6 synergistically induced HIV expression. This latter group and others (45-47) have shown that TNF-α may work in activating HIV expression through activation of NF-kB, a
COLONY STIMULATING FACTOR

Colony stimulating factors (CSFs) are proteins that stimulate the in vitro clonal growth of bone marrow cells (51). The name reflects the early observation that CSFs promote the formation of granulocyte or monocyte colonies in semi-solid medium. There are four so-called ‘established’ CSFs, namely, multipoietin/L-3, granulocyte-macrophage CSF (GM-CSF), macrophage-specific CSF (M-CSF) and granulocyte-specific CSF (G-CSF).

**Mechanisms of antiviral effects:** Like other cytokines, CSFs have a variety of immunological actions, but are best known for their dependency on the presence of other cytokines (51). GM-CSF has a significant effect on bone marrow progenitor cells; this includes increasing the production of granulocytes, monocytes, eosinophils and megakaryocytes (2). The cytokine also increases the number of mature polymorphonuclear leukocytes and monocytes in the peripheral blood, and stimulates the function of mature phagocytic cells (2). These immunological effects have engendered considerable interest in the material as a potential treatment for a variety of infectious diseases. G-CSF and M-CSF also have immunomodulatory effects, but to a lesser extent and in a narrower spectrum than GM-CSF.

**Potential for therapy of HIV infections:** Studies have reported on most of the established CSFs regarding their effect on HIV infections (Table 5), but GM-CSF appears to have the most significant activity. The enhancement of production of bone marrow precursor cells cited above has led to GM-CSF being studied as a supplementary drug in the treatment of AIDS, since it may counteract the narrow suppression due to HIV infection or occurring as a result of AZT therapy (52). In vitro studies performed by Kaplan et al (53) demonstrated that GM-CSF enhanced both spontaneous and stimulated production of reactive oxygen intermediates in monocytes taken from AIDS patients. This effect essentially doubled the cytotoxic action of monocytes against *Candida albicans*. These results suggest that GM-CSF may have the ability to enhance the immune defence of AIDS patients against some types of infection.

Like TNF, the reported effects of this group of cytokines appear to be somewhat conflicting, with both inhibition and enhancement of in vitro HIV replication seen. Since T lymphocytes are among the cells that produce GM-CSF, Perno et al (52) have speculated that this cytokine may play a central role in the progression of HIV infection to AIDS. GM-CSF, produced by T cells in response to antigenic stimulation, may also enhance HIV replication in macrophages leading to the spread of virus to more T cells. As noted in Table 5, however, there are some studies (54,55) in which inhibition of HIV was seen with GM-CSF. The study by Hammer et al (54) measured the replication of laboratory strains of the virus in alveolar macrophages, with inhibition of virus occurring.

The synergistic enhancement of the anti-HIV effects
of AZT and related drugs by GM-CSF (52,54,57) provides an additional potential use for this cytokine. This effect may be a result of GM-CSF increasing cellular levels of the triphosphate metabolite of these drugs (52,56,57) due to an induced increase in thymidine kinase. Cells stimulated with GM-CSF also have been shown to have higher levels of intracellular AZT, which would suggest that GM-CSF may also enhance cell entry of AZT and related drugs.

As with TNF, much additional work with GM-CSF particularly needs to be done.

**SUMMARY**

Cytokines are gaining much increased attention as possible therapies for AIDS. A major boost in these considerations has been the increasing availability of large amounts of purified materials provided through advances in genetic engineering. The cytokines that have been most intensively studied as antiviral therapies include the IFNs, the ILS (particularly IL-2 but also IL-4, IL-6 and IL-7), TNF and the CSFs (particularly GM-CSF and to a lesser extent G-CSF and M-CSF). In vitro antiviral studies have been run with all of these cytokines; in vivo experiments have been primarily limited to the IFNs and ILS. The greatest promise of the cytokines appears to be when they are used in combination with more standard antiviral agents or with selected other cytokines. The targeted cytokine/toxin therapy using IL-2 has yielded results indicating a considerable potential as an AIDS therapy. Caution is urged in the application of the experimental data cited to the clinical situation, due to the many diverse biological properties of these regulatory substances and the interweaving of their effects with other cytokines.

**REFERENCES**

15. Ruprecht RM, Chou TC, Chippy F, et al. Interferon-alpha and 3'-azido-3'-deoxythymidine are highly synergistic in


39. Ruddle NH, Turetskaya RL. Tumor necrosis factor beta.
Cytokines as potential therapies for HIV infections


