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Immunology

Interleukin 1 induces expression of the human immunodeficiency virus alone and in synergy with interleukin 6 in chronically infected U1 cells: Inhibition of inductive effects by the interleukin 1 receptor antagonist

(cytokines/NF-κB/latency)

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ABSTRACT **In the present study we have observed that interleukin (IL) 1α or IL-1β directly induced expression of human immunodeficiency virus (HIV) in the latently infected human promonocytic cell line U1. In addition, IL-1 synergized with IL-6, but not with tumor necrosis factor, in the upregulation of virus expression in U1 cells as measured by accumulation of steady-state mRNAs and production of reverse transcriptase activity. The HIV inductive effect of IL-1 was blocked by transforming growth factor β, anti-IL-1 antibodies, or monoclonal antibodies directed to the type 1, but not to the type 2, cell surface receptor for IL-1; the latter actually caused enhancement of the IL-1-mediated effect. Unlike tumor necrosis factor α, IL-1 either alone or in combination with IL-6 did not induce activation of the transcription activating factor NF-κB above the constitutive levels of unstimulated U1 cells. Finally, the IL-1 receptor antagonist effectively blocked IL-1-mediated direct and synergistic inductive effects on virus production. Thus, IL-1 may be an important mediator of HIV expression, and blocking of IL-1 expression and/or its effects may have a potential therapeutic role in the inhibition of HIV expression in infected individuals.**

Human immunodeficiency virus (HIV) infection leads to the functional impairment and progressive depletion of $CD4^+$ T lymphocytes, followed by the emergence of opportunistic infections and neoplasms, which clinically define AIDS (1). The recent demonstration that active HIV replication is present throughout the entire course of HIV disease in the lymphoid organs (2), although in the presence of a larger pool of latently infected cells (3), underscores the concept that HIV disease is characterized by a dynamic state of both microbiological latency and expression (1–3). In addition to $CD4^+$ T lymphocytes, mononuclear phagocytes represent an important target of infection (3–5).

Several studies have demonstrated that both tumor necrosis factor (TNF) α and interleukin (IL) 1 are upregulated as a consequence of *in vitro* (6–9) or *in vivo* (10, 11) HIV infection. At the molecular level, TNF-α and TNF-β upregulate virus production via activation of the cellular transcription factor NF-κB (12, 13), which is normally present as an inactive complex bound to the inhibitory molecule(s) known as I-κB in the cytoplasm of several cell types (reviewed in ref. 14). The HIV-1 promoter contains two tandemly repeated sequences for NF-κB binding, in close proximity to the transcription start site (12–14). In addition to TNF-α and IL-6 (15), IL-1 has also been reported to exert upregulatory effects on HIV replication in primary cultures of monocyte-derived macrophages (16, 17). Furthermore, IL-1, similar to TNF-α, has been shown to activate an HIV-1 long terminal repeat construct transfected in Jurkat and MOLT-4 human T-cell lines (18, 19) as well as in mouse cell lines (12). In this latter study, the inductive effect of IL-1 on the HIV long terminal repeat was correlated with the induction of NF-κB binding activity (12). However, little information is available on the precise molecular mechanisms of virus expression induced by IL-1 in human cells.

In the present study, we have investigated the effect of IL-1 in chronically infected promonocytic U1 cells in the presence or absence of a natural antagonist of IL-1-mediated biological effects, the IL-1 receptor antagonist (IL-1ra).

MATERIALS AND METHODS

Cytokine Induction of HIV Expression from U1 Cells. U1 cells are maintained at 1–3 × 10^5 cells per ml in RPMI 1640 (BioWhittaker) supplemented with 10% (vol/vol) fetal calf serum (HyClone). Stimulation of U1 cells was performed using recombinant (r) IL-1α, rIL-1β, and rIL-6 (R & D Systems) and rTNF-α and recombinant transforming growth factor β (rTGF-β; Genzyme). Neutralization experiments of IL-1 effects on U1 cells were carried out in the presence of IL-1ra, anti-IL-1α or anti-IL-1β polyclonal antibody (R & D Systems), or monoclonal antibody (mAb) directed to the type I (M1 and M4) or the type II (M22) cell surface receptor for IL-1, which were a generous gift of John E. Sims (Immunex). Culture supernatants were collected at various time points after stimulation to be evaluated for the presence of HIV reverse transcriptase (RT) activity.

RT Activity Assay. Five microliters of culture supernatants was added in duplicate or triplicate to 25 μl of a mixture containing poly(A) and oligo(dT) (Pharmacia), $MgCl_2$, and ^{32}P-labeled dTTP (Amersham) and incubated for 2 h at 37°C. Six microliters of the mixture was spotted onto DE 81 paper; the paper was air-dried, washed five times in 2× standard saline citrate buffer, and washed two additional times in 95% ethanol as described (20). Next the paper was dried, cut, and assayed for radioactivity in a Beckman LS 7000 scintillation counter. Variability of replicate cultures was always <15%.

Northern Blot Analysis. U1 cells were either unstimulated or stimulated for 24 h or 72 h with cytokines before total RNA was extracted from 2 × 10^7 cells by the guanidine thiocyanate/phenol method with an RNA isolation kit (Stratagene). Ten micrograms of total RNA was loaded per lane on an 0.8%

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Abbreviations: IL, interleukin; IL-1ra, IL-1 receptor antagonist; TNF, tumor necrosis factor; TGF-β, transforming growth factor β; HIV, human immunodeficiency virus; mAb, monoclonal antibody; RT, reverse transcriptase; r, recombinant.

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agarose/formaldehyde gel and transferred to nitrocellulose by Northern blotting. The filters were baked and hybridized for 12 h with a ^{32}P-labeled HIV probe (*Sac* I–*Sac* I) as described (15). Filters were washed and exposed to x-ray film.

NF-κB Mobility Shift Assay. Twenty million U1 cells were harvested at different times after stimulation with various cytokines, washed twice in cold phosphate-buffered saline, and centrifuged. Whole-cell extracts were obtained by three cycles of freezing and thawing, followed by resuspension in buffer [20 mM Hepes, pH 7.9/20% (vol/vol) glycerol/0.42 M NaCl/1.5 mM $MgCl_2$/0.2 mM EDTA/0.5 mM dithiothreitol/0.5 mM phenylmethylsulfonyl fluoride], and vigorous Dounce homogenization of the cell pellet. Cellular debris was pelleted at 10,000 rpm at 4°C in a microcentrifuge. The oligonucleotide (5′ → 3′) NF-κB probe GCTACAAGGGACTTTCCGCTGGGGACTTTCCAGG used in these studies has been described (21). Each oligonucleotide was annealed to its complementary strand and end labeled with [^{32}P]ATP (Amersham) using polynucleotide kinase (New England Biolabs). Comparable amounts of cell extracts (10–15 μg) were incubated in a reaction mixture with 17 μl of a buffer containing 20 mM Hepes at pH 7.9, 20% glycerol, 100 mM KCl, 0.2 mM EDTA, 0.5 mM dithiothreitol, and phenylmethylsulfonyl fluoride plus 1 μl of poly(dI-dC) (5 mg/ml; Pharmacia) for 2 min at room temperature. One microliter of ^{32}P-labeled probe (≈0.5 ng) was then added, and the mixture was incubated at room temperature for 30 min. It was then run on a 5% (30:1) acrylamide gel in 0.5× TBE (0.045 M Tris-borate/0.001 M EDTA) as described (21). Gels were dried and subjected to autoradiography.

RESULTS

IL-1α and IL-1β Induce HIV Expression in U1 Cells Alone and in Synergy with IL-6, But Not with TNF-α. Unstimulated U1 cells are characterized by a state of relative latency and lack of detectable virion production, as measured by the production of RT activity (Fig. 1). Stimulation of U1 cells with IL-1α or IL-1β resulted in the induction of RT activity in the culture supernatants, although to lower levels compared to those triggered by stimulation with TNF-α (Fig. 1). Costimulation of U1 cells with IL-1α or IL-1β and IL-6 resulted in a synergistic upregulation of virus production to levels comparable to those induced by TNF-α (Fig. 1). In contrast, no substantial modification of the levels of virus production occurred when U1 cells were coincubated with IL-1α or IL-1β and TNF-α, as shown in Fig. 1. The lack of synergy between IL-1 and TNF-α was not dependent upon downregulation of the expression of TNF receptors caused by IL-1 (data not shown).

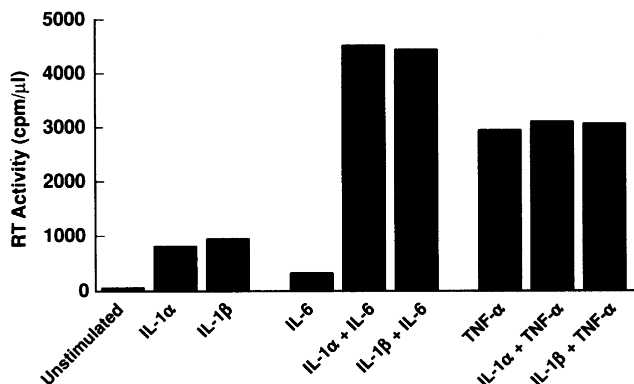

FIG. 1. IL-1α and IL-1β directly stimulate HIV expression in U1 cells and synergize with IL-6, but not with TNF-α. U1 cells were stimulated with rIL-1α, rIL-1β, rIL-6, rTNF-α, or the combination of the two forms of IL-1 with either IL-6 or TNF-α (each at 1 ng/ml). Synergy in induction of HIV expression occurred between IL-1α or IL-1β and IL-6, whereas no synergy occurred between IL-1α or IL-1β and TNF-α, even when a broad range of concentrations of the two cytokines was tested. The results were obtained after a 72-h stimulation and are representative of five independent experiments.

IL-1β Synergizes with IL-6 in the Induction of HIV mRNA Expression in U1 Cells. We have previously demonstrated that IL-6, unlike TNF-α, was a poor inducer of HIV transcription and mRNA accumulation in U1 cells (15), as shown in Fig. 2. Similarly, stimulation of U1 cells with IL-1β caused minimal expression of HIV messages, in contrast to the inductive effect exerted by TNF-α (Fig. 2). However, a strong synergistic induction of accumulation of HIV mRNAs was observed in U1 cells costimulated with IL-1β and IL-6 for 72 h, to levels comparable to those induced by TNF-α (Fig. 2). No synergy at the viral mRNA level was observed between IL-1β and TNF-α (data not shown). These findings were consistent with our observations of production of RT activity (Fig. 1). No evidence was obtained that the observed synergy between IL-1 and IL-6 involved the production of endogenous TNF-α (which is inducible in U1 cells after phorbol ester stimulation; ref. 22), in that TNF-α was not detectable in the cultures in the presence of IL-1 and IL-6, and addition of anti-TNF-α to U1 cells costimulated with IL-1 and IL-6 did not affect the levels of virus expression (data not shown).

IL-1 and IL-6 Do Not Activate NF-κB in U1 Cells. TNF-α inductive effects on HIV expression in U1 cells are correlated with the activation of NF-κB (Fig. 3) as shown in a variety of other cells (12–14). Induction of NF-κB by TNF-α was detectable as early as 5 min poststimulation of U1 cells and was observed after stimulation with as little as 1 unit of TNF-α per ml (data not shown). In contrast, no clear evidence of increased NF-κB DNA binding activity over the levels already present in unstimulated conditions was ob-

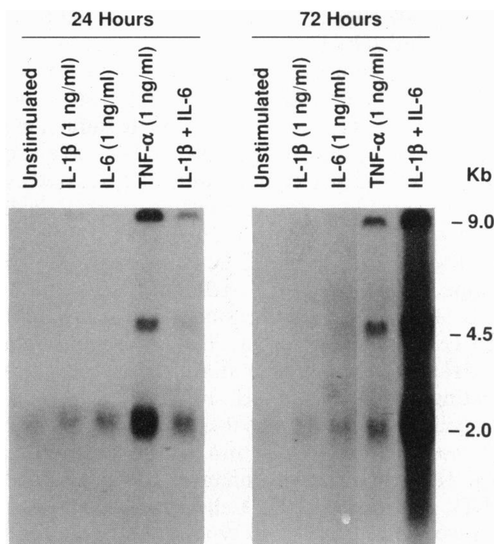

FIG. 2. Synergistic accumulation of steady-state HIV mRNAs in U1 cells costimulated with IL-1β and IL-6. Unstimulated U1 cells show the presence of fully spliced 2-kb viral messages, whereas stimulation with TNF-α induced both spliced and unspliced HIV mRNAs. Low levels of unspliced 9-kb mRNA were sometimes observed after stimulation with IL-1β (1 ng/ml), particularly after 24 h, whereas IL-6 alone (1 ng/ml) had very modest effects on viral messages, as reported (15). However, costimulation of U1 cells with IL-1 and IL-6 resulted in the synergistic induction of HIV mRNAs at levels slightly less (24 h) or even greater (72 h) than those induced by TNF-α. No synergy was observed between IL-1β and TNF-α by Northern blot analysis of HIV mRNAs.

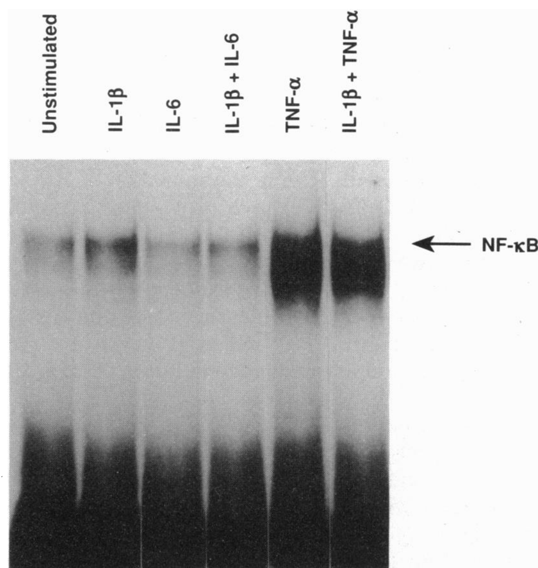

FIG. 3. TNF-α, but not IL-1β, IL-6, or costimulation with IL-1β and IL-6, activates NF-κB in U1 cells. Unstimulated U1 cells show the presence of a constitutive NF-κB DNA binding activity that was blocked by an excess of cold NF-κB probe (data not shown). TNF-α, but not any of the other stimuli tested, induced increased levels of NF-κB DNA binding activity in U1 cells after a 30-min incubation. These results were confirmed when either total cell or nuclear extracts were prepared at different time points up to 20 h after cell stimulation with the indicated cytokines.

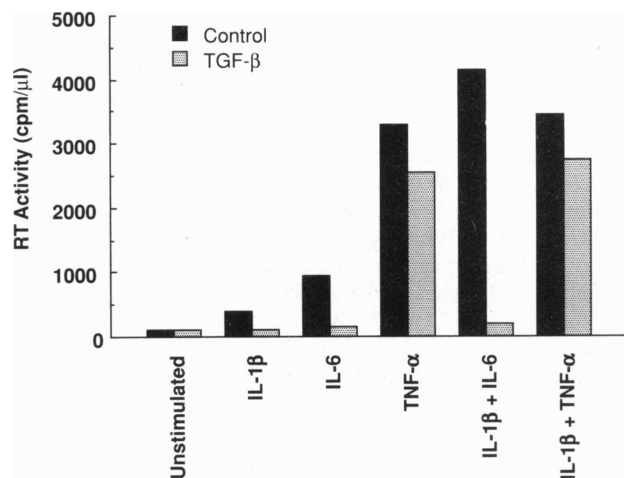

FIG. 4. TGF-β suppresses IL-1β-mediated direct and synergistic induction of HIV expression in U1 cells. TGF-β (1 ng/ml) blocked virus production induced by IL-1β (1 ng/ml), IL-6 (1 ng/ml), and IL-1β plus IL-6. However, TGF-β did not substantially affect TNF-α-mediated induction of HIV expression, in either the presence or absence of IL-1.

served after stimulation with either IL-1 alone, IL-6 alone, or IL-1 plus IL-6 (Fig. 3).

Thus, despite the fact that costimulation of U1 cells with IL-1 and IL-6 induced levels of HIV production of the same magnitude as those caused by TNF-α stimulation (Figs. 1 and 2), these effects were not correlated with the activation of NF-κB (Fig. 3), suggesting that the upregulation of virus expression induced by IL-1 in the presence or absence of IL-6 may affect one or more posttranscriptional levels, as described in U1 cells after stimulation with IL-6 alone (15).

TGF-β Suppresses Direct and Synergistic Induction of Virus Production by IL-1, But Not by TNF-α. We have previously reported that TGF-β, a potent immunosuppressive cytokine, could selectively block the expression of HIV in U1 cells stimulated with IL-6 or phorbol 12-myristate 13-acetate, but not with TNF-α (23). When U1 cells were stimulated with IL-1β alone, IL-6 alone, or IL-1β plus IL-6 in the presence of TGF-β, a complete suppression of viral production was observed (Fig. 4). In contrast, TGF-β did not reduce the levels of HIV production after stimulation with TNF-α (Fig. 4), indicating that TNF-α and IL-1 induce expression of HIV from U1 cells via distinct signal-transduction pathways.

Anti-Type I IL-1 Receptor mAbs Suppress, Whereas an Anti-Type II Receptor mAb Enhances, IL-1-Mediated Induction of HIV Expression in U1 Cells. Differential distribution exists between the type I and type II receptors for IL-1, in that T lymphocytes and fibroblasts are known to express high levels of type I molecules, whereas B lymphocytes and phagocytic cells express high levels of type II receptors (24–27). Furthermore, it is believed that as few as 10 or less type I receptor molecules per cell are required for IL-1 to transduce a signal in cells expressing high levels of type II receptors, in that anti-type I-specific but not anti-type II-specific mAbs could block IL-1-mediated effects (25, 27). Two mAbs (M1 and M4) directed against the type I IL-1 receptor and one mAb (M22) recognizing the type II receptor (24–26) were added to U1 cells 30 min before IL-1β. Complete suppression of IL-1-mediated induction of virus production was observed in the presence of both mAbs directed against the type I cell surface molecule, whereas the anti-type II mAb did not suppress viral production in these culture conditions (Fig. 5*A*). An enhancing effect of anti-type II mAb was observed in U1 cells stimulated with suboptimal concentrations (200 pg/ml) of IL-1β (Fig. 5*B*), consistent with the recently proposed model that type II IL-1 receptors are shed from the cell surface under certain stimulatory conditions and act as "receptor decoy" molecules, blocking the effect of free IL-1 (26). Similar results were obtained when IL-1α instead of IL-1β was used (data not shown).

The IL-1ra Blocks the Direct and Synergistic Induction of HIV Expression by IL-1. The family of IL-1-related molecules is unique among other cytokines in that one member, known as IL-1ra, binds with comparable affinity to type I and type II IL-1 receptors, without causing any of the multiple biological effects induced by IL-1α or IL-1β under similar experimental conditions (reviewed in refs. 27 and 28). Unlike IL-1, incubation of U1 cells with IL-1ra did not induce HIV expression even at concentrations up to several hundredfold greater than those of IL-1 (Fig. 6*A*). However, a short preincubation (30 min) of U1 cells with IL-1ra prior to stimulation with IL-1α or IL-1β caused a significant suppression of HIV expression (Fig. 6*A*). This effect was specific in that no interference of IL-1ra was observed when U1 cells were preincubated with IL-1ra (250 ng/ml) and then stimulated with several inducers of virus production, including IL-6, granulocyte/macrophage colony-stimulating factor, TNF-α, and phorbol 12-myristate 13-acetate (data not shown). Furthermore, IL-1ra effectively inhibited HIV expression induced after costimulation of U1 cells with IL-1 and IL-6 (Fig. 6*B*). Thus, IL-1ra, as previously described in a variety of other model systems (27, 28), does not activate virus production in infected cells that are sensitive to the upregulatory effect of IL-1, but it effectively counteracts IL-1-mediated induction of HIV expression.

DISCUSSION

In the present study we have demonstrated that IL-1 alone and in synergy with IL-6 induces HIV expression in chronically infected promonocytic U1 cells. Unlike TNF-α, the direct and synergistic effects of IL-1 on HIV expression appeared to be independent of the activation of NF-κB, although substantial increases in the levels of steady-state

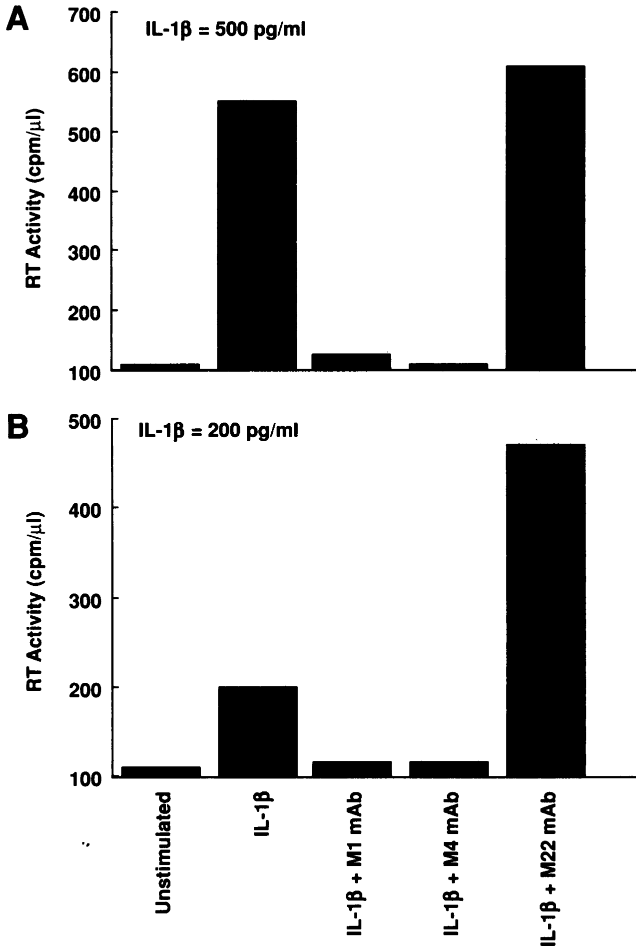

FIG. 5. Differential effect of anti-IL-1 type I and type II receptor mAbs on IL-1β-induced HIV expression. (*A*) Blocking of type I receptors with either M1 or M4 mAb suppressed IL-1-mediated expression of HIV. No effect of the M22 anti-type II receptor mAb was observed when optimal concentrations of IL-1β (0.5–5 ng/ml) were used. (*B*) Enhancement of IL-1-mediated induction of HIV was observed in the presence of M22 when suboptimal concentrations of IL-1 were used. All three anti-IL-1 receptor mAbs were used at 10 μg/ml. The results are representative of three independent experiments.

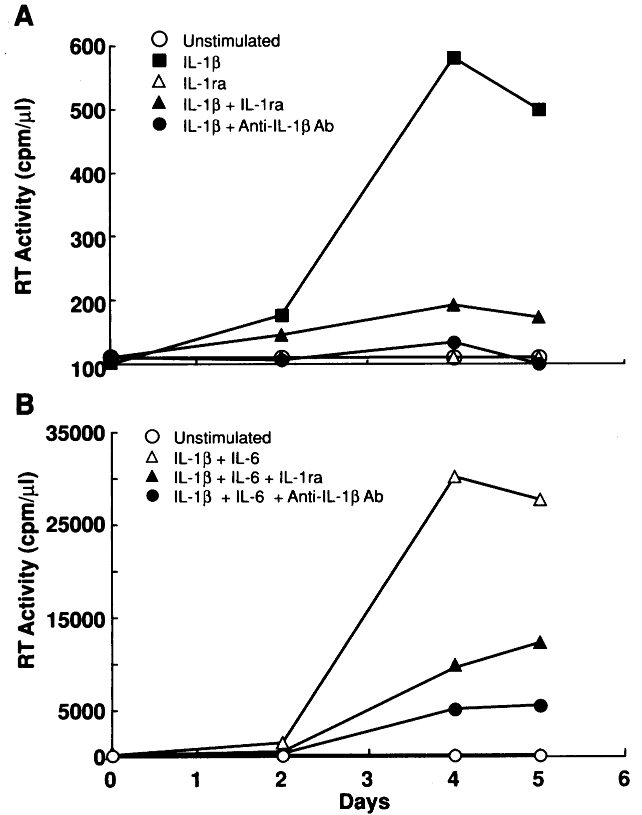

FIG. 6. IL-1ra suppresses IL-1-mediated direct and synergistic induction of HIV expression in U1 cells. (*A*) Treatment of U1 cells with IL-1ra did not induce virus production over the baseline undetectable levels. A 30-min preincubation with IL-1ra suppressed the direct inductive effect of IL-1β (as well as of IL-1α). (*B*) IL-1ra inhibits HIV production synergistically induced by costimulation with IL-1β and IL-6. The efficacy of IL-1ra is shown in comparison to that of a polyclonal anti-IL-1β neutralizing antiserum.

viral mRNA and RT activity production were observed. IL-1 induction of HIV production in U1 cells was mediated by type I cell surface IL-1 receptors. Finally, TGF-β and IL-1ra blocked the IL-1-mediated direct and synergistic induction of virus expression from U1 cells stimulated with IL-1α or IL-1β.

IL-1 is a cytokine produced by activated macrophages and other cell types during inflammatory reactions in conjunction with TNF-α and IL-6 (reviewed in ref. 27). A number of studies have described that increased levels of IL-1β are present either in the cerebrospinal fluid (29) or in the tissues of infected individuals, including lymph nodes (11), skin (30), and bronchoalveolar epithelium and lining fluid (31). Furthermore, peripheral blood mononuclear cells isolated from HIV-infected individuals have shown normal (32) or increased (10) production of IL-1 both in unstimulated conditions and after lipopolysaccharide stimulation. Increased *ex vivo* production of IL-1 and IL-6 has also been observed in lung alveolar macrophages isolated from HIV-infected individuals compared to cells obtained from uninfected volunteers (33). Productive infection *in vitro* of primary monocyte-derived macrophages and neoplastic monocytic cells as well as exposure of these cells to inactivated HIV or HIV envelope proteins or peptides resulted in the upregulation of IL-1β expression (6–9).

Just as HIV may upregulate the production of IL-1, IL-1 may induce the expression and replication of HIV. In this regard, previous studies have demonstrated that IL-1 enhanced the ability of HIV to replicate in primary monocyte-derived macrophages (16, 17). At the molecular level, IL-1 has been reported to activate the HIV-1 promoter in a number of cell lines transfected with an LTR-CAT construct, including mouse cell lines (12), MOLT-4 (18) and Jurkat (19, 34) human CD4$^+$ T-cell lines, and human astrocytic cells (35). IL-1 transcriptional effects on HIV expression have been correlated with the activation of NF-κB. In the present study, we have observed a clear synergistic effect of IL-1 on HIV mRNA accumulation and RT activity in the presence of IL-6. However, in contrast to previous studies, we have not been able to correlate either IL-1-mediated direct or synergistic induction of HIV with the activation of NF-κB, although NF-κB was readily activated in the same experimental conditions by TNF-α. These observations suggest that, at least in the U1 cell system, IL-1 and TNF-α may activate HIV expression via distinct molecular pathways. In support of this hypothesis, TGF-β, which had been previously described as a selective suppressor of phorbol 12-myristate 13-acetate, but not of TNF-α-mediated activation of HIV expression (23), fully suppressed IL-1-mediated direct and synergistic induction of viral production.

Suppression of IL-1-induced virus production was obtained with mAbs directed against the type I, but not the type II, IL-1 receptor, in agreement with previous studies indi-

cating that type I receptors transduce the IL-1-associated signal even in those cells where type II receptors are much more abundant than type I molecules (24–27). In contrast, anti-type II IL-1 receptor mAb caused enhancement of IL-1-induced HIV production under certain experimental conditions, a finding consistent with the recently proposed model that type II IL-1 receptors act as receptor decoys, blocking the IL-1 present in the extracellular environment (26).

IL-1ra was first identified as a natural antagonist of the binding of IL-1 to cell surface receptors. This molecule is secreted by several activated cell types, particularly mononuclear phagocytes, and does not cause any of the recognized IL-1-associated effects even when administered *in vivo* or used *in vitro* at concentrations several orders of magnitude higher than IL-1 (27, 28). IL-1ra has been given to both experimental animals and humans as a safe antagonist of excessive or inappropriate production of IL-1 associated with a variety of pathological conditions, ranging from rheumatoid arthritis to septic shock, in the absence of any substantial side effects (27, 28). Of interest is the fact that in addition to blocking IL-1-mediated induction of virus expression in U1 cells, we have recently observed that IL-1ra could also inhibit HIV replication in primary peripheral blood mononuclear cells acutely infected with HIV (unpublished results).

In conclusion, we have observed that IL-1 is an important mediator of HIV expression *in vitro*. The demonstration that a natural antagonist of cytokine function (i.e., IL-1ra) exerts anti-HIV effects *in vitro* may have important implications in the design of therapeutic strategies to control HIV replication and disease progression in infected individuals via manipulation of the cytokine network.

Note. During the preparation of this manuscript, Granowitz *et al.* (36) reported similar findings of IL-1 induction of HIV expression in U1 cells via the type 1 receptor, with blocking of induction by IL-1ra, at the Clinical Research Meeting in Washington, DC on May 1, 1993.

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