Recombinant interleukin 4 stimulates human immunodeficiency virus production by infected monocytes and macrophages

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Recombinant interleukin 4 (IL-4) stimulated extracellular (EC) and intracellular (IC) production of human immunodeficiency virus (HIV) from infected human blood-derived monocytes and macrophages when incubated with the cells after but not before virus inoculation. Significant stimulation was observed in 20 of 27 experiments with monocytes (inoculated with HIV immediately after adherence) and 10 of 13 experiments with macrophages (inoculated after 5 days adherence) using a total of 30 normal donors of monocytes and macrophages, and 11 recent isolates of monocytotropic HIV strains (after one passage in mononuclear cells). Marked increases in EC and IC HIV antigen were observed in some experiments, which were comparable with the maximal stimulatory effects of other cytokines such as IL-2. IL-4 also had similar effects on infectious HIV concentration as measured by reverse transcriptase and TCID₅₀ assays. Antibody to IL-4 prevented the stimulatory effect of the cytokine. The proportion of monocytes and macrophages infected by HIV, as determined by in situ hybridization, also increased after incubation with IL-4 for 7 days. The most marked effects were observed with HIV-infected macrophages, for which the proportion of unstimulated infected cells was lower (35 to 45% increasing to 66 to 70% with IL-4 treatment). There was also an increased proportion of cells with high granule concentrations, suggesting that IL-4 increases the intracellular concentration of viral nucleic acids. This was supported by semi-quantitative hybridization experiments showing that total HIV RNA increased in IL-4-stimulated monocytes 48 to 96 h after HIV inoculation. A marked increase in aggregates was observed on day 7 in HIV-infected monocytes treated with IL-4, compared to that in HIV-infected cells alone or IL-4-treated uninfected monocytes. These findings suggest that IL-4 stimulates HIV replication in the early phases of infection and may also facilitate virus transmission by aggregate formation.

Introduction

The importance of human immunodeficiency virus (HIV) infection of monocytes, macrophages and related cells (e.g. microglial cells) in the persistence of infection and the eventual development of the clinical disease is well established (Gartner et al., 1986; Ho et al., 1986; Koenig et al., 1986; Salahuddin et al., 1986; Nicholson et al., 1986; Gendelman et al., 1988). In addition, the marked biological differences between HIV infection of monocyte/macrophages and CD4⁺ lymphocytes are also well described. For example, in contrast to lymphocytes, productive HIV infection of monocyte/macrophages does not require a cellular activation step, is predominantly intracellular (within vacuoles) and is not associated with marked c.p.e. in macrophages (Gartner et al., 1986; Nicholson et al., 1986; Gendelman et al., 1988; Kazazi et al., 1989). Factors that modulate HIV infection of mononuclear phagocytes may influence the local production of virus, the function of infected monocytes/macrophages, or their production of toxic or biologically active molecules. Several cytokines have been reported to influence production of HIV by infected monocytes or monocytoid cell lines, during either initial or chronic infection. Hence, interleukins (ILs) 1, 2, 3 and 6, tumour necrosis factor (TNF)-α, granulocyte–macrophage colony-stimulating factor (GM-CSF) and macrophage colony-stimulating factor (M-CSF) have been reported to augment HIV production by infected monocytes (Koyanagi et al., 1988; Folks et al., 1989; Matsuyma et al., 1989; Nakajima et al., 1989; Poli et al., 1990), whereas interferons (IFNs) γ, α and β, and transforming growth factor (TGF) β have been reported to decrease production (Kornbluth et al., 1990; Poli et al., 1991). Conversely, HIV infection of monocytes has been reported to stimulate production of cytokines such as IL-1, IL-6 and
TNF-α (Poli et al., 1989; Nakajima et al., 1989). The situation has been somewhat confused by a report that some of these findings may have been caused by contamination of the medium with endotoxin (Molina et al., 1990).

IL-4 is a cytokine which has received little attention. It has potent biological effects on monocytes, reducing initial adherence of the cells to the extracellular matrix (Elliott et al., 1991), increasing major histocompatibility complex (MHC) class II expression (Littman et al., 1989), stimulating formation of giant cells (McInnes & Rennick, 1988), and enhancing G-CSF and M-CSF production (Wieser et al., 1989). Despite this, studies of the activity of IL-4 in HIV infection of monocytes and macrophages have been limited to one brief report (Novak et al., 1990).

In this paper we compare the effects of IL-4 on intracellular (IC) and extracellular (EC) HIV production by HIV-infected, human blood-derived monocytes and macrophages with those of IL-1α, -2 and -3, IFN-γ and GM-CSF.

Methods

Isolation of monocytes and macrophages. Aliquots (150 ml) of blood were obtained from healthy HIV seronegative volunteers. Monocytes were separated as described previously (Kazazi et al., 1989). Briefly, after Ficoll-Hypaque separation, blood mononuclear cells were separated into eight fractions by countercurrent elutriation in a Beckman J-21 centrifuge fitted with a JE6B elutriation rotor (Beckman Instruments). Fractions 5, 6, 7 and 8 contained esterase-positive monocytes, with fraction 7 giving the highest yield (approximately 90% purity). T lymphocytes were depleted from individual or pooled fractions (6, 7 and 8) by complement-dependent cytotoxicity using an anti-CD3 monoclonal antibody (MAb) (OKT3; Ortho Diagnostics) (Cunningham & Merigan, 1984). Residual contamination with T or B lymphocytes was less than 1% as determined by flow cytometry. Of the residual cells, 98 to 99% were positive for non-specific esterase (Yam et al., 1971) (Sigma kit no. 180).

In some experiments, blood-derived monocytes and macrophages were also obtained by detachig blood-derived mononuclear cells or macrophages adherent to foetal calf serum (FCS)-coated plastic (polystyrene) Petri dishes with 0.2% EDTA. Pure populations of macrophages were obtained by allowing the monocytes to differentiate and enlarge for 5 days in cluster well plates (Nunc); contaminating T or B lymphocytes detached during this period (Cunningham & Merigan, 1984; Kazazi et al., 1989). These cultures were compared with cultures of monocytes purified by centrifugal elutriation and complement-dependent cytotoxicity, grown on plastic or in Teflon jars (Savillex) and allowed to mature for 5 days.

Monocyte/macrophage growth medium (RPMI plus 10% human serum and FCS) and purified water Milli Q (Millipore) used in its preparation regularly tested negative for the presence of endotoxin by the limulus lysate assay (Sigma). Batches of FCS and human serum that were retested immediately before use. More importantly, plastic-adherent macrophages did not produce IL-1 or macrophage procoagulant activity spontaneously under normal (HIV-uninfected) growth conditions (Ryan & Geczy, 1988).

Quantification of cell surface markers. Blood mononuclear cells or purified monocytes were stained singly or doubly with MAbs directly conjugated to either fluorescein isothiocyanate or phycoerythrin, and quantified by immunofluorescence or flow cytometry (Epics Coulter) (Cunningham & Merigan, 1984; Kazazi et al., 1989). MAbs to CD14 (anti-LeuM3), CD3 (OKT3), CD4 (anti-Leu3a/b and OKT4) and CD8 (anti-Leu2a) were obtained from Becton-Dickinson or Ortho Diagnostics.

Viruses. Forty recent, low (one) passage HIV isolates from Australian patients with asymptomatic HIV infection, AIDS-related complex or AIDS were tested for their ability to replicate in human monocytes. Eleven strains, which produced a spectrum of low to high EC HIV concentration, were chosen for use in this study.

Recombinant cytokines. TNF-α, GM-CSF, IL-3 and IFN-α were obtained from Genzyme; IL-1α and IL-1β were from Boehringer Mannheim, and IL-2 and IL-4 from Amersham. Cytokines were used at the following concentrations: TNF-α at 10, 20 and 50 ng/ml, GM-CSF at 500, 1000 and 1500 units (U)/ml, IL-1α and IL-1β at 10, 50 and 100 ng/ml, IL-3 at 1, 5 and 10 ng/ml, IL-2 at 1, 5 and 10 ng/ml, IL-4 at 1 to 50 ng/ml and IFN-γ at 10, 100 and 1000 U/ml.

HIV infection of monocyte/macrophages. Adherent monocytes or macrophages (2 x 10^6) in RPMI medium supplemented with 10% heat-inactivated FCS and 10% human serum were inoculated with cell-free HIV isolates at 100000 c.p.m./ml of reverse transcriptase (RT) activity (10^4 TCID50 on MT4 cells) and allowed to adsorb for 3 h before complete aspiration of medium, washing and addition of fresh medium. Every 3 days 90% of the medium was replaced and the supernatants were stored for quantification of HIV. In this paper we have defined HIV-infected monocytes as those inoculated immediately after adherence and HIV-infected macrophages as those infected 5 days after adherence to plastic.

Detection and quantification of HIV infection. Cell culture supernatants were assayed at 3-day intervals for HIV antigen (HIV antigen assay, Abbott Laboratories) (Goudsmit et al., 1986; Newell et al., 1987). This assay detects the HIV p24 protein. We determined that the HIV p24 protein concentration (pg/ml) was proportional to the optical density (OD) units in the ELISA, and the cutoff (threshold) in OD units corresponded to a limit of detection of HIV p24 of 9 pg/ml. Results were confirmed by RT assays (Hoffman et al., 1985).

Quantification of HIV RNA by hybridization. RNA was extracted from monocytes, macrophages and other cell lines by standard methods (Sambrook et al., 1989), transferred to nylon (Hybond-N +; Amersham) and hybridized with a full-length probe (pNL43, a generous gift from Dr Malcolm Martin, NIH, Bethesda, Md., U.S.A.) by slot blot hybridization. Cellular RNA was also resolved by electrophoresis on a 1.2% agarose gel with 6.8% formaldehyde in 20 mM-MOPS buffer pH 7.8 and examined for HIV RNA by hybridization with the full-length probe or with oligonucleotide probes specific for various HIV sequences. Hybridization signals were quantified by densitometry (Bio-Rad).

In situ hybridization (ISH) for HIV nucleic acid. Monocytes or macrophages grown on microscope slides (Labtek) were fixed in 4% paraformaldehyde in PBS for 10 min at room temperature, rinsed in PBS and stored in 70% ethanol until use. The slides were rehydrated in 5 mM-MgCl2 in PBS for 5 min and prehybridized for 10 min at 70 °C in 70% formamide/2 x SSC. After a wash in 5 x SSC at 55 °C for 10 min they were hybridized at 55 °C for 45 to 60 min with 5 ng of the probe in 5 x SSC/0.5% BSA. The probes were end-labelled with alkaline phosphatase, purified by chromatography on G-50 Sephadex and stored in aliquots at −20 °C. The slides were washed in 1 x SSC at 45 °C for 10 min before addition of the substrate (BCIP and NBT in PBS containing 1 mM-levamisole). The slides were washed in water,
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Results

Effect of IL-4 on morphology of monocytes and macrophages

When IL-4 (10 to 20 ng/ml) was incubated with freshly adherent monocytes, the distribution and morphology of adherent monocytes/macrophages differed from those of control cells or cells incubated with M-CSF or GM-CSF. In IL-4-treated cultures, a significantly increased frequency of monocyte aggregates was observed after 3 to 7 days by phase contrast microscopy [mean of 5 compared with 1/high power field (HPF) respectively]. The number of multinucleated giant cells was also increased on day 12 (mean of 5.5/HPF compared with 0.5/HPF in controls) (Fig. 1a, b and c). IL4 at 50 ng/ml resulted in monocyte detachment from the substrate (data not shown).

Effect of IL-4 and other cytokines on monocyte/macrophage CD4 expression

Neither IL-4 nor IL-1, IL-2, IL-3, TNF-α, M-CSF or GM-CSF consistently and significantly affected the proportion of monocytes expressing CD4 or their mean CD4 density when cultured for between 1 and 5 days (data not shown).

Effect of IL-4 on extracellular EC HIV production by monocytes/macrophages

In an initial series of experiments, the effect of IL-4 (1 to 20 ng/ml) on EC HIV concentration (p24 antigen and RT activity) was determined. The IL-4 concentration was maintained by its incorporation in replacement medium. There was marked and significant stimulation of EC HIV concentrations (p24 antigen and RT activity) which usually peaked between 6 and 10 days post-infection (p.i.). Maximum stimulatory concentrations varied between 10 and 20 ng/ml for monocytes and macrophages. IL-4 had similar stimulatory effects on monocytes and macrophages (representative experiment with monocytes from a single donor and one HIV isolate in Fig. 2).

To confirm that the observed increase in EC HIV antigen reflected the concentration of infectious virus, RT assays were performed on the EC fluid from infected monocytes and found to increase in two separate experiments from 5320 ± 2123 c.p.m./ml (mean ± s.d.) to 10065 ± 1670 and from 9278 ± 429 to 28891 ± 3154 after 7 days incubation with 20 ng/ml IL-4.

These initial experiments were extended to 27 different combinations of donor monocytes (27 donors) and freshly isolated virus strains (10 isolates). All virus strains were used immediately after isolation or after one passage in mitogen-stimulated blood mononuclear cells.

Fig. 1. IL-4 increases aggregate formation in blood-derived monocyte/macrophage cultures. Human blood-derived monocytes were isolated and cultured in plastic cluster well plates as described in Methods. IL-4 or control medium was added to monocytes 3 h after initial adherence and maintained during the 12 day observation period. IL-4-stimulated cultures on days 12(a) and 5(b); control cultures (c). Phase contrast microscopy. Bar marker represents 10 μm.

dehydrated in alcohol, cleared in Histolene and mounted in Eukitt (Zeiss -FRG). The sequences of the probes were 5’TAAATAGTCTTACAATCGGGTT 3’ (gag nucleotide 1765), 5’CCCATATTGGTCTACAGCCTTCTG 3’ (gag nucleotide 947) and 5’GTTGGGTCTTGTAACATTAAT 3’ (env nucleotide 7134).
The magnitude of the stimulatory effect varied according to the donor monocyte and virus strain pairing (ratio of IL-4-stimulated to unstimulated EC HIV antigen of 0.9 to 3.7, Fig. 3). In 24 of 27 donors, the concentration of HIV antigen in EC fluid was enhanced by IL-4 at optimal concentrations (10 to 20 ng/ml) and this was statistically significant in 20 (P < 0.05, Student's t-test). A small decrease was observed in only one experiment. There was no correlation between the magnitude of initial replication of isolates in monocytes (as determined by EC HIV antigen concentration) and the stimulatory effects of IL-4 (Spearman's rank correlation, non-significant). Similar results were obtained with 13 combinations of donor macrophages (13 donors) and HIV strains (six isolates). Stimulatory effects were observed in 11 of 13 experiments and 10 of these were significant (P < 0.05). In one representative experiment, serial dilutions of supernatant aspirated at day 7 from HIV-infected monocytes treated with control medium or IL-4 showed a mean infectious titre of 10^{1.3} and 10^{2.9} TCID_{50}/ml respectively.

To confirm that the stimulatory effects noted were specific for IL-4, HIV-infected monocytes were incubated with recombinant IL-4 alone or with a polyclonal rabbit anti-IL-4 antibody, produced by immunization with recombinant IL4 (Endogen, British Bio-technology). As shown in Fig. 4, the marked increase in EC HIV antigen with recombinant IL-4 was reversed by preincubation with the antibody, demonstrating the specificity of the stimulatory effect. The anti-IL4 MAb did not inhibit stimulation of EC HIV production by IL-2 and GM-CSF (data not shown).

**Comparison of IL-4 with other cytokines**

The effect of IL-4 on EC HIV production by infected monocytes was compared to that of IL-2, GM-CSF and IFN-γ added after HIV inoculation. In six experiments the ratio of mean peak EC HIV antigen in IL-2-stimulated monocytes to that of unstimulated controls ranged from 1.05 to 3.45, and stimulation was significant

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**Fig. 2.** Comparison of the effect of varying concentration of IL-4 on EC HIV production by human blood-derived monocytes and macrophages. (a) Human blood-derived monocytes from a single donor; (b) human blood-derived macrophages from the same donor. □, Control (uninfected monocytes); □, HIV-infected monocytes; HIV plus (□) 1 pg/ml, (□) 10 pg/ml and (□) 20 pg/ml IL-4. Each column represents the mean (± S.D.) of quadruplicate wells.) P < 0.05 as measured by Student's t-test.

**Fig. 3.** Variation in stimulatory effects of IL-4 on EC HIV production by infected monocytes and macrophages. HIV-infected monocytes (from 27 different donors) or macrophages (13 donors) were incubated with optimum concentrations of IL-4 (10 to 20 ng/ml) or control medium for 7 to 8 days and supernatant medium was assayed for HIV antigen. The results of each experiment are expressed as the ratio of mean EC HIV antigen levels in IL-4-stimulated cultures to the mean HIV antigen levels in unstimulated cultures (EC HIV Ag ratio). Student's t-test corrected for unequal variances was used to calculate the significance of IL-4 stimulation in each individual experiment. Significant stimulation (P < 0.05) was observed in 20 of 27 monocyte and 11 of 13 macrophage experiments.
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Fig. 4. Antibody to IL-4 reverses stimulation of HIV production by infected monocytes. Monocytes were inoculated with HIV (as described in Methods). Various concentrations of anti-IL-4 antibody or control medium were preincubated with IL-4 for 1 h at room temperature and the mixtures were added to HIV-infected cultures. Supernatants were assayed for HIV antigen 6 to 7 days p.i. Both IL-4 stimulation and reversal with anti-IL-4 antibody were highly significant (P < 0.01, Student's t-test) \( \square \), Uninfected monocytes; \( \blacksquare \), HIV-infected monocytes; \( \square \), HIV-infected monocyte plus IL-4; \( \blacksquare \), HIV-infected monocytes plus IL-4 and anti-IL-4 antibody. (P < 0.05, Student's \( t \) test) with four of six virus–donor monocyte pairs. In two experiments, using the same HIV–donor monocyte pairs, IL-2 and IL-4 both produced significant and similar degrees of stimulation (ratios of 1.9 and 2.2 for IL-4 and 2.7 and 1.9 for IL-2 respectively). GM-CSF produced lower levels of stimulation (ratios of 1.00, 1.35 and 1.36, significant in two of three experiments); 10 to 1000 U/ml IFN-\( \gamma \) had an increasing inhibitory effect on EC HIV production (data not shown). Screening experiments with the other cytokines showed statistically significant stimulation of HIV replication only with GM-CSF, M-CSF and TNF-\( \alpha \) (data not shown).

Effect of addition of IL-4 pre- or post-inoculation of monocytes with HIV

As shown in Fig. 5, preincubation of monocytes with IL-4 (10 to 20 ng/ml for 48 h) followed by aspiration and washing of the cytokine from the culture did not enhance EC HIV infection when those cells were immediately inoculated with HIV. However, addition of IL-4 after HIV inoculation (and adsorption) produced significant enhancement of EC HIV production.

Table 1. Effect of IL-4 on EC and IC HIV production by infected monocytes

<table>
<thead>
<tr>
<th>HIV antigen concentration (pg/ml) ( \pm ) s.d.</th>
<th>Expt.*</th>
<th>HIV-infected†</th>
<th>HIV + IL-4‡</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>EC fluid</td>
<td>116 ± 11</td>
<td>335 ± 17</td>
<td>&lt;0.01</td>
<td></td>
</tr>
<tr>
<td>IC fluid</td>
<td>195 ± 13</td>
<td>497 ± 24</td>
<td>&lt;0.01</td>
<td></td>
</tr>
</tbody>
</table>

* EC and IC fluids assayed for HIV antigen 7 days p.i.  † Monocytes inoculated with HIV at \( 10^4 \) TCID\( _{50} \)/ml and incubated with control medium for 7 days.  ‡ Monocytes inoculated with HIV and incubated with IL-4.

Effect of IL-4 on IC HIV production by monocytes and macrophages

As HIV has been shown to accumulate within IC vacuoles in monocytes and macrophages, the effect of IL-4 on IC and EC HIV antigen production was compared. HIV-infected monocytes were studied after 7 days incubation with IL-4 or control medium. EC fluid
was assayed for HIV antigen. The monocytes were carefully washed three times with Hanks' balanced salt solution and the cell pellet was lysed with an equal volume of Triton X-100. After clarification by low-speed centrifugation, the supernatant was assayed for HIV antigen. As shown in Table 1, the HIV antigen concentration was greater in cellular lysates than in supernatants, and increased significantly in cultures treated with IL-4. Similar results were obtained by RT assay (data not shown).

The proportion of monocytes and macrophages infected with HIV with or without stimulation with IL-4

ISH for HIV nucleic acid in monocytes and macrophages was used to quantify the effect of IL-4 on the proportion of monocytes and macrophages infected with HIV, and to quantify IC infection. As shown in Fig. 6 and Table 2, IL-4 increased the proportion of both monocytes and macrophages containing HIV-specific nucleic acids 4 and 7 days after virus inoculation. At 7 days, 92% of monocytes were infected, allowing little scope for an additional effect of IL-4. However, stimulation was more obvious in macrophages, for which the proportion of infected cells increased from 35 to 45% up to 66 to 70% at 7 days. The intensity of infection per cell was more difficult to estimate with immunochemical than with radiometric methods, but there was a marked increase in the proportion of cells displaying more than 20 granules of substrate per cell in cultures stimulated with IL-4. Aggregates of infected cells, which formed intensely infected foci, were observed in IL-4-stimulated HIV-infected monocyte and macrophage cultures, and were most prominent 7 days p.i. In contrast to the live preparations observed by phase contrast microscopy, cellular aggregates were not observed in the ISH preparations of uninfected macrophages stimulated with IL-4 nor in HIV-infected macrophages without cytokine stimulation.

Semi-quantitative kinetics of HIV RNA in HIV-infected monocytes incubated with IL-4 or control medium

To assist in determining whether IL-4 has an early effect in stimulating HIV production by individual monocytes or simply promotes spread of HIV between cells, RNA was extracted from IL-4- and mock-stimulated, HIV-infected and uninfected monocytes at 48, 72 and 96 h. An increased hybridization signal for total HIV RNA was observed in the IL-4-stimulated monocytes, in comparison to equal numbers of unstimulated infected cells, using semi-quantitative linear densitometry (Table 3).

Discussion

In this study, recombinant IL-4 in optimal concentrations was shown to stimulate IC and EC HIV production by infected monocytes and macrophages when added to these cells after virus inoculation. Preincubation of IL-4
Table 2. Effect of IL-4 on proportion of HIV-infected monocytes and macrophages determined by ISH

<table>
<thead>
<tr>
<th>Expt.</th>
<th>Treatment</th>
<th>HIV-infected cells (%)</th>
<th>Aggregates (per 10 HPF)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Day 4</td>
<td>Day 7 (&gt; 20 granules/cell)</td>
</tr>
<tr>
<td>Monocytes</td>
<td>HIV</td>
<td>40</td>
<td>92 (5)</td>
</tr>
<tr>
<td>1</td>
<td>HIV + IL-4</td>
<td>51</td>
<td>98 (11)</td>
</tr>
<tr>
<td>2</td>
<td>HIV</td>
<td>44</td>
<td>92 (7)</td>
</tr>
<tr>
<td>2</td>
<td>HIV + IL-4</td>
<td>55</td>
<td>97 (15)</td>
</tr>
<tr>
<td>3</td>
<td>HIV</td>
<td>54</td>
<td>ND</td>
</tr>
<tr>
<td>3</td>
<td>HIV + IL-4</td>
<td>81</td>
<td>ND</td>
</tr>
<tr>
<td>Macrophages</td>
<td>HIV</td>
<td>32</td>
<td>45 (6)</td>
</tr>
<tr>
<td>1</td>
<td>HIV + IL-4</td>
<td>60</td>
<td>70 (15)</td>
</tr>
<tr>
<td>2</td>
<td>HIV</td>
<td>ND</td>
<td>35 (8)</td>
</tr>
<tr>
<td>2</td>
<td>HIV + IL-4</td>
<td>ND</td>
<td>66 (16)</td>
</tr>
<tr>
<td>3</td>
<td>HIV</td>
<td>4</td>
<td>ND</td>
</tr>
<tr>
<td>3</td>
<td>HIV + IL-4</td>
<td>11</td>
<td>ND</td>
</tr>
</tbody>
</table>

* ND, Not done.

Table 3. Quantification of HIV RNA in HIV-infected monocytes treated with IL-4 or control medium

<table>
<thead>
<tr>
<th>Time p.i. (h)</th>
<th>HIV RNA (optical density units)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>48</td>
<td>1.8</td>
</tr>
<tr>
<td>72</td>
<td>2.4</td>
</tr>
</tbody>
</table>

* Determined by densitometry (see Methods).

with polyclonal anti-IL4 antibody prevented the increase in EC HIV antigen concentration in IL-4-treated HIV-infected cell cultures. Thus, the increase was specific for this cytokine and excluded likely extraneous stimulatory factors such as undetectable levels of contaminating endotoxin (Kornbluth et al., 1989; Molina et al., 1990). The marked stimulatory effects of IL-4 observed in many of our experiments and the increased formation of multinucleated giant cells have been observed previously (Novak et al., 1990). However, we observed no stimulation of monocytes preincubated with IL-4 before HIV infection, confirming the observations of Kornbluth et al. (1989).

The stimulatory effect of IL-4 varied with the monocyte donor and the strain of virus used. Variability in the effects of cytokines other than IL-4 on HIV production by infected monocytes/macrophages (and the U937 promonocytoid cell line) is apparent in reports from different laboratories and probably reflects differences in the systems used, including the HIV strain used, its passage history, differences in donor monocytes, stage of monocyte/macrophage differentiation, and different source and concentration of cytokines (Koyanagi et al., 1988; Kornbluth et al., 1989; Kazazi et al., 1989; Valentin et al., 1990a). As an example of the latter, high concentrations of IL-4 (50 pg/ml) may lead to detachment of monocytes and spurious inhibitory effects on HIV production (see Results).

In comparison with other cytokines, the maximum stimulatory effect of IL-4 was similar to that of IL-2 and exceeded that of IL-1, GM-CSF and M-CSF. Conversely, IFN-γ produced a concentration-dependent inhibitory effect. Hence, the observed effects of these other cytokines on HIV production from monocytes or macrophages qualitatively support the observations of others, despite the marked variability in the degree of stimulation observed (Koyangi et al., 1988; Gendelman et al., 1988; Potts et al., 1990; Hammer et al., 1986; Kornbluth et al., 1990). Recently, Justement et al. (1991) have reported that TGF-β may also produce dichotomous effects, producing stimulation if incubated with cells before HIV inoculation and inhibition if added post-inoculation.

The mechanism by which IL-4 stimulates HIV production is interesting. The results shown above suggest two possible mechanisms: (i) a direct effect on HIV replication and (ii) facilitation of HIV transmission through increased intercellular contact and/or the formation of aggregates. TNF-α and IL-1 have been shown to induce activation and binding of nuclear factor (NF) kB to sites in the HIV long terminal repeat (LTR) (Osborn et al., 1989; Lenardo & Baltimore, 1989). The NFkB- or Sp1-binding sites in the LTR are apparently essential for HIV replication (Parrott et al., 1991). NFkB is constitu-
tively expressed in adherent monocytes and macrophages (Griffin et al., 1989), but is expressed in U937 cells only upon infection with HIV (Bacheleire et al., 1991). This difference in NFκB expression may produce differences in the action of cytokines in these cell types. As IL-4 increases steady-state HIV RNA levels, like TNF-α, it may also act via NFκB. However, effects of IL-4 on RNA stability, also shown with IL-6, may contribute and are being investigated (Poli et al., 1990). The semi-quantitative early increase in HIV RNA in IL-4-stimulated monocytes reflects the effect of IL-4 on multiple rounds of HIV infection of monocytes. Studies are under way to measure single-step growth curves using high concentrations of HIV as inoculum to define further the role of IL-4 in HIV replication.

IL-4 is known to affect the monocyte membrane and its proteins markedly, increasing the expression of CD23 and MHC class II but decreasing CD14 expression (Lauener et al., 1990), reducing adherence to EC ground substance (Elliott et al., 1991) and stimulating the formation of giant cells (McInnes & Rennick, 1988). It is likely that the increased aggregates noted with IL-4-stimulated HIV-infected monocytes and macrophages in the ISH experiments (Fig. 6) reflect a combined effect of HIV and IL-4 in promoting cellular adherence. Presumably these aggregates were more stable under the harsh conditions of ISH than those observed in IL-4-treated uninfected monocyte cultures. Monocyte aggregate could facilitate intercellular transmission of HIV and account in part for the increase in the proportion of infected monocytes and macrophages in IL-4-stimulated preparations. IL-4 may also contribute to the formation of HIV-infected giant cells from monocytes and CD4 lymphocytes, perhaps through effects on membrane integrins (Lifson et al., 1986; Valentin et al., 1990b). As IL-4 increased both the proportion of infected cells and the proportion of intensely infected cells (as determined by ISH), it is possible that IL-4 may both stimulate replication and facilitate intercellular transmission.

It is clear from the above studies and other recent reports (Kornbluth et al., 1989; Justement et al., 1991) that assessment of the effects of cytokines on HIV infection of monocytes and macrophages in vitro should employ multiple fresh isolates of HIV at low passage (rather than high passage laboratory strains), multiple host donor cells in primary culture and incubation of the cytokine with cells before and after HIV inoculation, especially as both TGF-β and IL-4 differ in their effects pre- and post-inoculation of cells with HIV.

Results from these experiments add IL-4 to the list of enhancers of HIV replication in monocytes. Interaction between cytokines (TNF-α and IL-6) (Poli et al., 1990) has been described; synergy between IL-4 and other factors in the enhancement of HIV infection in vitro and in vivo is probable. The variability observed in the stimulatory effects of IL-4 is currently being studied further using multiple strains of HIV to infect cells from a single donor and, conversely, incubating each virus strain with monocytes from multiple donors. This may help define virus or host cell effects affecting virus replication. Individual host variations in response to cytokines may contribute to differences in virus load which may ultimately determine the course of disease.

Our findings also have implications for the biological use of cytokines. As with IL-2 and GM-CSF, IL-4 should only be used in HIV-infected patients with care and under controlled conditions because it may exacerbate rather than slow the course of disease.

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