Molecular Analysis of Decreased Interleukin-12 Production in Persons Infected with Human Immunodeficiency Virus

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Human immunodeficiency virus (HIV) disease is associated with loss of type 1 responses, including interleukin (IL)-12 production. The dramatic drop in p70 production seen at early stages of disease was found not to be associated with a similarly decreased p40 mRNA expression. p35 mRNA expression was more extensively reduced than p40 mRNA expression at these early stages. Monocytes infected in vitro with HIV displayed decreased p35 expression and p70 production, suggesting that such decreased IL-12 expression may contribute to reduced IL-12 production in HIV-positive patients’ cells. In addition, treatment of cells with IL-10 increased IL-10 mRNA expression and decreased p40 expression in both HIV-positive and -negative cells, while neutralization of IL-10 increased p40 mRNA levels. These observations, together with the observed hyperproduction of IL-10 in HIV-positive patients, may explain the dysregulation of IL-12 production seen in HIV disease.

Interleukin (IL)-12 is a powerful inducer of type 1 immune responses and plays a central role in resistance to intracellular pathogens [1]. This cytokine is secreted by monocytes and other antigen-presenting cells, such as dendritic cells [1-3]. Biologically active IL-12 is a heterodimer of 70 kDa (p70) formed by two covalently linked chains of 40 kDa (p40) and 35 kDa (p35) [1]. Expression of p40 is very low or undetectable in unstimulated peripheral blood cells but is increased after stimulation by bacteria, bacterial products, and intracellular parasites [2, 3]. p35 is constitutively expressed, and its expression is less affected by stimulation than is that of p40, possibly resulting in an excess production of p40 in comparison with the p70 heterodimer [2, 3].

Human immunodeficiency virus (HIV) disease is associated with a progressive loss of type 1 responses and an increase in type 2 responses [4]. Persons whose T helper cell functions are the most severely compromised are also those who produce higher levels of IL-10 [5]. Addition of exogenous IL-12 to peripheral blood mononuclear cells (PBMC) from HIV-positive persons restored type 1 function in vitro [6] and effectively blocked activation-induced programmed cell death [7, 8]. Cells from HIV-positive persons have been reported to produce less IL-12 than cells from HIV-negative controls [9-11]. Therefore, to analyze further the mechanisms involved in decreased IL-12 production, we analyzed IL-12 and IL-10 mRNA and protein levels in PBMC isolated from HIV-negative persons and compared these parameters with those seen in HIV-negative persons. In addition, we analyzed at the mRNA level several potential mechanisms that may be involved in IL-10 decrease, namely viral infection and IL-10 cross-regulatory role.

Materials and Methods

Patient and control blood. Blood samples were obtained from 46 HIV-positive donors (CD4 cell range, 0-1024) from Wilford Hall Medical Center, Lackland Air Force Base, and from Rush Medical College. They were shipped by overnight express to the Bethesda laboratory, where they were processed within 24 h after sampling. Samples from 27 HIV-negative controls were obtained from the Transfusion Department, National Institutes of Health, and held overnight before processing under the same conditions as HIV-positive samples. PBMC were separated on lymphocyte separation medium (Organon Teknika, Rockville, MD) and resuspended at 3 × 10^6 cells/mL in complete medium (RPMI 1640 containing 100 U/mL penicillin, 100 μg/mL streptomycin, 5 mM HEPES, 2 mM glutamine) supplemented with 5% AB+ serum.

IL-12 and IL-10 production. The production of IL-12 and IL-10 by PBMC was determined by culturing 3 × 10^6 PBMC at 37°C in a moist, 7% CO2 atmosphere. Cells were either unstimulated or stimulated with Staphylococcus aureus Cowan strain 1 (SAC) (Pansorbin; Calbiochem-Behring, La Jolla, CA; 0.0075%). Supernatants were harvested after 24 h, filtered through a 0.22-μm filter, and stored frozen at -70°C [2]. IL-12 p70 heterodimer and IL-10 were measured by ELISA (R&D Systems, Minneapolis, and Pharmingen, San Diego, respectively), following manufacturers’ instructions. The detection limits were 4 and 40 pg/mL for IL-12.
and IL-10, respectively. For statistical purposes, all values below detection limits were assigned an arbitrary value of half the detection limit (2 and 20 pg/mL for IL-12 and IL-10, respectively).

Total p40 production was determined in 24-h SAC-stimulated supernatants by ELISA using antibodies C11.79 and C8.6 (provided by G. Trinchieri, Wistar Institute, Philadelphia). The antibodies recognize both IL-12 p40 and the heterodimer p70, as described [2]. Monoclonal antibody C11.79 was coated overnight at 4°C (10 μg/mL in 0.1 M carbonate buffer, pH 9.6) on flat-bottom microtiter plates (Immulon-4; Dynatech, Chantilly, VA). The plates were washed six times with PBS with 0.025% (vol/vol) Tween 20 and incubated for 2 h at room temperature with 200 μL of PBS containing 10% fetal calf serum (PBS-FCS) as a blocking step. After being washed, 50 μL of supernatants and serial dilutions of recombinant IL-12 (provided by S.F. Wolf, Genetics Institute, Cambridge, MA) were incubated overnight at 4°C. After being washed, 50 μL of biotinylated detection monoclonal antibody C8.6 (dilution 1:1000 in PBS-FCS) was added and plates were incubated at 37°C for 1 h. Plates were washed, incubated at room temperature with 100 μL of avidin-peroxidase (Kirkegaard & Perry, Gaithersburg, MD; 1:1000 in PBS-FCS) for 45 min, washed again, and developed with 100 μL of peroxidase substrate (Kirkegaard & Perry) at room temperature. Plates were read at 414 nm and allowed to develop until the top standard (2500 pg/mL) reached an OD of 1 or the blank reached an OD of 0.1 (usually 45 min to 1 h). The detection limit was 40 pg/mL. For statistical purposes, all values below the detection limit were assigned to an arbitrary value of half the detection limit (20 pg/mL).

Detection of IL-12 and IL-10 mRNA. Expression of hypoxanthine-phosphoribosyl transferase (HPRT), IL-10, and IL-12 p40 and p35 chain mRNA was assessed on PBMC stimulated with SAC for 6 h, using a quantitative reverse transcriptase–polymerase chain reaction (RT-PCR) protocol. The optimal incubation time was 6 h for peak gene induction, determined by preliminary kinetic experiments. The quantitative RT-PCR protocol has been described [5, 12]. Briefly, total RNA was extracted by lysis of the cells with an RNA solvent (RNAstat; Tel Test, Friendswood, TX). Next, 1 μg of RNA was reverse-transcribed using superscript RT (Life Technologies GIBCO BRL, Gaithersburg, MD). A 1:8 dilution of the RT product was amplified to sub saturating levels (28 cycles for p40, IL-10, and HPRT mRNA and 30 cycles for p35). Primer and internal probe sequences for IL-10 and HPRT were as described [13]. Sequences for IL-12 (provided by S.F. Wolf) were as follows: p40 sense, CCAAGAACTTGCAGCTGAAG; antisense, TGGGTCCTATTCGCTGTGTC; probe, GCTCATCTTTGACGGAAATGG; p35 sense, GACACACCAGTCGGCAAG; antisense, GGGCAGCAACCTGGGAAAG; probe, CTCAGAGGCGGCAACACACCA. PCR products were run on a 1% agarose gel and transferred to a nylon membrane (Hybond N+; Amersham, Amersham, UK). Southern blots were hybridized with internal cytotoxic-specific oligonucleotide probes labeled by an enhanced chemiluminescence system (Amersham). Intensity of the bands of HPRT expression when necessary.

To overcome the variability within different PCR experiments and to compare mRNA expression among different persons, an external standard that was assigned an arbitrary 100% value was included in all PCR runs. After normalization with HPRT mRNA levels, results from all donors, both HIV-positive and -negative, were expressed as the percentage of expression compared with this standard. The standard used in these studies was an RT product obtained from an HIV-negative donor whose PBMC responded well to SAC stimulation (producing 122 pg/mL p70 and 1673 pg/mL p40). Quantification by this method was also tested with the same RT product and proved linear in the ranges of cDNA used (results not shown).

In some cases, human recombinant IL-10 (final concentration, 5 ng/mL; DNAX Research Institute, Palo Alto, CA) or recombinant IL-12 (20 U/mL; Genetics Institute) were added to the PBMC cultures alone or at the time of SAC stimulation. Alternatively, cells were preincubated overnight with neutralizing anti–IL-10 antibodies (clone JES 319 F11, 5 or 10 μg/mL; DNAX) and then stimulated with SAC plus anti–IL-10 for an additional 6 h.

Viral infection and cell culture of monocyte/macrophages (M/M). M/M were obtained and infected as described [14]. Briefly, M/M from normal volunteers were purified by counterflow centrifugal elutriation as described [15]. M/M were enriched to >90%, as determined by flow cytometry, and were not activated during the procedure. After purification, they were cultured for 3 days in 48-well flat-bottom plates (Costar, Cambridge, MA) at 2.5 x 10⁴ cells/mL in complete medium consisting of RPMI 1640 containing 100 U/mL penicillin, 100 μg/mL streptomycin, 5 mM HEPES, and 2 mM glutamine and supplemented with 20% heat-inactivated FCS (Hyclone Laboratories, Logan, UT) and 100 U/mL recombinant human granulocyte-macrophage colony-stimulating factor (GM-CSF; R&D Systems). In most cases, cultures were done in duplicate to determine both mRNA expression and protein levels. After 3 days (referred to as day 0), they were exposed or not exposed to viral suspension in complete medium containing HIV-1 blister fluid (gift of M. Popovic, NCI, NIH) at a concentration of 200 ID₅₀/well. They were then kept for 2 weeks, with 80% medium replaced with fresh complete medium every 5 days. After 2 weeks (referred to as day 14), supernatants were harvested and stored at −20°C until they could be assayed for HIV-1 p24 antigen by radioimmunooassay (DuPont, Wilmington, DE). Then, M/M were washed twice with preheated RPMI 1640 and stimulated as described above for PBMC. For mRNA expression, M/M were stimulated for 6 h and RNA was prepared as described above. For protein determination, M/M were stimulated for 24 h, and cell-free supernatants were harvested, inactivated by addition of 0.5% Triton (Sigma, St. Louis), and stored at −70°C. Before determination of p70 protein, supernatants were concentrated 4 times, using centrifugate concentrators (Centri-Por; molecular weight cutoff, 10,000; Spectrum, Houston), and p70 ELISA was run as described above.

Statistical analysis. Statistical comparisons between groups were made by use of Mann-Whitney U tests. Correlations were made by use of simple regression tests. Results were considered significant at P < .05.

Results

Reduction in IL-12 protein production in PBMC from HIV-positive persons. We used SAC stimulation to study IL-12 and IL-10 production in 24-h culture supernatants in PBMC
from 27 HIV-positive and 13 HIV-negative donors. Unstimulated PBMC from HIV-positive and from -negative donors did not produce any detectable IL-12. The two-step ELISA used was specific for the heterodimer p70 and did not detect free or homodimer p40. After SAC stimulation, PBMC from only 3 of 27 HIV-positive donors produced detectable IL-12 (≥4 pg/mL). In contrast, SAC-stimulated PBMC from 12 of 13 HIV-negative donors produced IL-12. Mean IL-12 production by PBMC from HIV-positive donors was significantly decreased compared with uninfected donors (median, 2 vs. 81.5 pg/mL, \( P < .001 \), U test; table 1). The HIV-positive patients had normal monocyte and B cell counts, and the IL-12 deficiency could not therefore be attributed to a reduced number of antigen-presenting cells.

This decreased IL-12 production by PBMC from the HIV-positive patients was not due to differences in the kinetics of production of this cytokine in the different donor cells. Thus, supernatants of cultures from 6 HIV-positive and 4 HIV-negative subjects were harvested at 16, 24, 40, and 48 h after SAC stimulation. IL-12 was not detected in any of the supernatants from cultures HIV-positive patients but was detectable at all time points in supernatants from cultures from HIV-negative donors, with production peaking at ~24 h in these cultures (results not shown).

Monoclonal antibodies specific for p40, both free and in combination with p35 in the p70 heterodimer, were used to detect p40 in the same SAC-stimulated supernatants from PBMC from HIV-positive and -negative donors. PBMC from HIV-positive donors produced significantly less p40 than did those from HIV-negative donors (median, 641 vs. 1460 pg/mL, \( P = .025 \), U test; table 1).

**Correlation between IL-12 and p40 production and clinical stages.** Because IL-12 production and expression of p35 and p40 mRNA were not correlated in HIV-positive donors, we tested whether there was a differential evolution of these markers during disease progression. Impairment of p70 production did not correlate with the drop in CD4 cell counts (regression, \( r^2 = 0.057 \), \( df = 25 \); \( P = .24 \); figure 1). No p70 was detected in the supernatants from 13 of 14 HIV-positive donors with relatively high CD4 cell counts (200–500) nor from 5 of 7 donors with counts >500. In contrast, p40 mRNA expression correlated highly with CD4 cell counts (regression, \( r^2 = 0.897 \), \( df = 24 \); \( P < .001 \)).

**Correlation of IL-12 expression with CD4 cell counts and clinical stages.** Because IL-12 p70 production and expression of p35 and p40 mRNA were not correlated in HIV-positive donors, we tested whether there was a differential evolution of these markers during disease progression. Impairment of p70 production did not correlate with the drop in CD4 cell counts (regression, \( r^2 = 0.057 \), \( df = 25 \); \( P = .24 \); figure 1). No p70 was detected in the supernatants from 13 of 14 HIV-positive donors with relatively high CD4 cell counts (200–500) nor from 5 of 7 donors with counts >500. In contrast, p40 mRNA expression correlated highly with CD4 cell counts (regression, \( r^2 = 0.897 \), \( df = 24 \); \( P < .001 \)).

**Table 1.** IL-12 and IL-10 production in *Staphylococcus aureus* Cowan (SAC)—stimulated peripheral blood mononuclear cells.

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<tr>
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<th>p70 (pg/mL)</th>
<th>p40 (pg/mL)</th>
<th>IL-10 (pg/mL)</th>
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<td></td>
<td>Media</td>
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<td>HIV-positive ((n = 27))</td>
<td>2 (0(^\prime) = 0)</td>
<td>2 (2-19) ((n(^\prime) = 3))</td>
<td>20 (0(^\prime) = 0)</td>
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<tr>
<td>HIV-negative ((n = 13))</td>
<td>2 (0(^\prime) = 0)</td>
<td>81.5 (2-508(^\prime) ((n(^\prime) = 13))</td>
<td>20 (0(^\prime) = 0)</td>
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NOTE. Data are median (range). Values below ELISA detection limits were assigned arbitrary value of half detection limit: 2 pg/mL for IL-12 and 20 pg/mL for p40 and IL-10. \( n' \) represents no. of measurable samples (above detection limit).

* Significant difference \((P < .05\), Mann-Whitney U test\) vs. HIV-positive donors.
the disease (Walter Reed classification, stage I–VI), p40 levels in the supernatants also correlated with disease stages (regression, $r^2 = 0.158$, df $= 26$; $P = .04$), while p70 levels and p35 mRNA expression were decreased even at stages I and II of the disease and did not correlate with the stage of the disease (regression, all $P > .08$).

**Effect of viral infection on IL-12 production.** M/M from 9 different HIV-negative donors were infected by the monocytoproductive strain Ba-L and stimulated with SAC in order to analyze the effect of infection on IL-12 p70 production and p35 and p40 chain expression. Uninfected cells from the same donors were cultured in parallel using similar conditions. Infection was monitored by measurement of p24 levels in supernatants. All 9 infections induced a high level of p24 antigen (8 were $>13$ ng/mL and 1 was $1.5$ ng/mL), while no p24 was detectable in supernatants from uninfected cultures (results not shown). Culture supernatants from cells of 7 donors were assayed for p70 levels, and infection decreased p70 levels in all cases (median, 12.7-fold decrease; figure 2, left). HIV infection decreased SAC-inducible p35 expression in 6 of 9 donors examined (median, 3.7-fold decrease; figure 2, center). In contrast, p40 expression was not significantly affected by infection in 5 cases, although it was slightly decreased in the other 4 cases (median, 0.9-fold decrease; figure 2, right).

**IL-10 production.** Unstimulated PBMC from 11 of 26 HIV-positive donors produced low constitutive levels of IL-10, whereas no IL-10 was detected in unstimulated PBMC from HIV-negative donors. After SAC stimulation, PBMC from HIV-positive donors produced significantly more IL-10 than did those from HIV-negative donors (median, 856 vs. 179 pg/mL, $P = .007$, U test; table 1). No correlation was observed between IL-10 and p70 or p40 levels in supernatants, nor between IL-10 and p40 mRNA expression (regression, all $P > .05$).

**Cross-regulatory effects of IL-12 and IL-10 at the mRNA level.** Since there was a general imbalance between IL-10 and IL-12 production in the HIV-positive patients, we looked at the cross-regulatory effect of exogenous IL-10 and IL-12 at the mRNA level and compared these parameters with those seen in HIV-negative persons. For that purpose, cells from 17 HIV-positive patients (CD4 cell counts, 5–983) and from 14 HIV-negative donors were stimulated with SAC, IL-10 plus SAC, or IL-12 plus SAC for 6 h. A concentration of IL-10 (5 ng/mL) able to inhibit type 1 responses and a concentration of IL-12 (20 U/mL) able to restore type 1 functions were chosen on the basis of previous studies [6, 7]. In HIV-positive donors,
IL-10 plus SAC-treated cells expressed 59.5% less p40 and 65.5% more IL-10 mRNA than did SAC-stimulated cells (figure 3). The effects of IL-10 on p40 and IL-10 mRNA expression were correlated; that is, the more IL-10 decreased p40, the greater effect it had on increasing IL-10 mRNA (regression, \( r^2 = 0.321, df = 16, P = .017 \)). Expression of p35 mRNA was not affected by IL-10 treatment (results not shown). Incubation with IL-10 alone did not induce expression of p40, p35, or IL-10. The addition of IL-12 to SAC-stimulated cells did not affect p35 or p40 mRNA expression, although IL-10 mRNA expression was reduced by 37.4% in HIV-positive donors (results not shown).

The addition of IL-10 to PBMC from HIV-negative donors gave results similar to those seen with HIV-positive donors (both \( P > .07, U \) test): a 76.1% increase in endogenous IL-10 mRNA and a 29% decrease of p40 mRNA (figure 3). Addition of IL-12 to SAC-stimulated cells induced a 37.4% decrease of IL-10 mRNA, which was similar to the findings observed in HIV-positive donors, and it did not affect p40 or p35 expression (results not shown).

Preincubation of PBMC from 8 HIV-positive donors with neutralizing anti-IL-10 antibodies (5 \( \mu \)g/mL) followed by SAC stimulation resulted in a 66.3% mean increase in p40 mRNA expression compared with SAC alone (figure 4). An increased concentration of anti-IL-10 antibodies (10 \( \mu \)g/mL) did not induce higher expression of p40 (results not shown), and antibodies alone did not induce p40 mRNA. A similar increase was obtained with PBMC from 11 HIV-negative donors (58% mean increase of p40 mRNA).

**Discussion**

The present study compared the production and expression of IL-12 and IL-10 by SAC-stimulated PBMC from HIV-positive and -negative donors. PBMC from the HIV-positive donors exhibited impaired IL-12 heterodimer production compared with that in HIV-negative donors, a result that is in agreement with several previous reports [9–11]. This defect could not be accounted for simply by changes in the number of monocytes, a major source of IL-12, or by differences in the kinetics of...
production of this cytokine in different donor cells. In contrast, SAC-induced production of IL-10 by PBMC from HIV-positive donors was significantly elevated compared with that in HIV-negative donors, as reported [5, 16, 17]. However, no negative correlation between production of IL-10 and IL-12 was observed. Both p40 and p35 chain mRNA were decreased in SAC-stimulated PBMC from HIV-positive donors compared with HIV-negative donors, but several HIV-positive donors showed an impaired production of IL-12 in culture supernatants with a significant level of p40 mRNA expression. In many donors, p35 mRNA expression was more extensively reduced than p40. Impairment of p70 production did not correlate with reduced CD4 cell counts, although p40 and p35 mRNA expression did.

Previous studies have shown SAC to be the most potent stimulus for IL-12 production in PBMC of HIV-negative donors [2]. Therefore, the results observed after SAC stimulation, especially defects, should be representative of the IL-12 secreting—potential of PBMC after stimulation with other antigens from HIV or opportunistic pathogens. Up-regulation of IL-12 p40 in mice after lipopolysaccharide stimulation has been shown to depend on NF-κB family members [18]. However, little is known about the signaling pathways for IL-12 production by other antigens and in human cells. Thus, stimulation of monocytes by different antigens could differ and all stimulation pathways might not be defective in HIV-infected cells. In addition, production of IL-12 after CD40-CD40 ligand interaction has also been reported for human HIV-negative monocytes [19]. This alternative activation pathway should also be studied in the context of HIV infection.

Our results suggest that multiple mechanisms contribute to IL-12 defective production in PBMC from HIV-positive donors and that their appearance during the course of the disease is sequential. One early mechanism could be defective expression of p35 that would lead to an appreciable reduction of the p70 heterodimer, without affecting p40 mRNA expression or free p40 production. Indeed, p35 expression in cells from HIV-positive donors was more acutely decreased than p40, and this p35 defect appeared earlier in disease progression. To test this hypothesis, we infected in vitro M/M from healthy donors with a monocytotropic strain and studied p35 and p40 mRNA expression as well as p70 production after SAC stimulation. Interestingly, we found that p35 was decreased more than p40.

These results suggest that decreased p35 expression after SAC stimulation of infected M/M likely plays an important role in p70 reduction but may not be the only mechanism involved. It has been reported that infection of M/M induced a significant decrease in p40 production, but the effect of infection on p35 expression and p70 production was not studied [9]. In our study, p40 expression was not decreased. Experimental conditions could account for this apparent discrepancy between the two studies, as we cultured infected and uninfected M/M with GM-CSF before SAC stimulation, and GM-CSF provides a priming stimulus for lipopolysaccharide-induced p40 mRNA but primes poorly for inducible p35 mRNA and IL-12 activity [20]. Thus, in our conditions, GM-CSF may have partially restored p40 expression in infected monocytes while it did not restore p35 expression and p70 production. An alternate mechanism of p70 decrease could involve inhibition of the p70 heterodimer by excess free p40 or 80-kDa homodimers [21]. This mechanism could account for those persons who present with a discrepancy between IL-12 heterodimer production and p40 mRNA expression.

In later stages of the disease, p40 expression starts to decline until its expression and production are completely absent. A progressive shift from a dominant type 1 to type 2 cytokine production during the course of HIV disease has been reported [4], and persons whose type 1 function was more severely compromised were also those whose cells produced the highest levels of IL-10 [5]. In this context, hyperproduction of IL-10 by PBMC, described by this study and others [5, 16, 17], and in lymph nodes of HIV-positive patients [22] could be one of the mechanisms involved in decreased p40 expression and production.

To address this latter issue, we studied the regulatory effects of exogenous IL-10 on IL-12 and IL-10 mRNA expression in both HIV-positive and -negative donor PBMC. Because it was reported that IL-10 was able to decrease p40 at the transcriptional level in the cells from HIV-negative donors [23], we tested the effects of IL-10 on IL-12 at the mRNA level. As expected, addition of exogenous IL-10 decreased p40 expression in cells from HIV-positive and -negative donors. An unexpected result was the increase in IL-10 mRNA expression after addition of IL-10 to SAC stimulation, and this was observed in both HIV-positive and -negative donors. This result contrasts with that reported in monocytes, for which addition of exogenous IL-10 decreased IL-10 mRNA expression [24]. However, in contrast to that report, we used a shorter incubation time (6 h instead of 24 h) and stimulated unseparated PBMC, including other IL-10-producing cells in addition to monocytes. Thus, the regulatory mechanisms involved in our study may have been different than in the report that used isolated monocytes. Interestingly, there was a correlation between the effect of IL-10 on p40 and IL-10 expression, suggesting the existence of an individual variation in susceptibility to IL-10.

Blockade of IL-10 increased p40 expression in cells from HIV-positive and -negative donors. The restoration occurring in HIV-positive donors suggests that IL-10 contributes to the decrease in p40 expression observed in these donors. However, other cytokines, such as transforming growth factor-β, have also been shown to participate in the down-regulation of IL-12 and p40 production [25]. IL-4 and IL-13 are also involved in the regulation of IL-12 production, being either stimulatory or inhibitory, depending on the experimental conditions [25]. Since both IL-4 and transforming growth factor-β could be elevated in HIV-positive patients [12, 26], their role in IL-12 regulation, in addition or synergy with IL-10, should be investigated in HIV-positive patients. Alternatively and nonex-
clusively, other metabolites such as prostaglandins can contribute to the inhibition of IL-12 and total p40 production [27]. Since HIV infection of monocytes increases prostaglandin E2 production [28], these soluble factors could also contribute to the impaired IL-12 production by PBMC from HIV-positive patients [28a]. In addition, several reports have shown that interferon-γ priming enhances both p40 and p35 expression after SAC or lipopolysaccharide stimulation, even though its presence is not an absolute requirement [20, 29]. During the progression of HIV disease, decreased interferon-γ production was described [4, 30, 31], and this could represent another mechanism involved in the progressive decreased expression of p40 and p35.

We also investigated the inverse regulation of IL-12 on IL-10 expression in cells from HIV-positive and -negative donors. In vivo treatment of normal mice with IL-12 resulted in enhanced IL-10 gene expression [32]. In another study, involving mice infected with Schistosoma mansoni, IL-12 induced a different pattern of regulation of IL-10 expression, depending on the tissue analyzed. IL-10 mRNA was increased in vivo by IL-12 but decreased in T cells from draining lymph nodes restimulated in vitro by antigens [33]. Addition of IL-12 to human CD4 T cells stimulated by allergens inhibited IL-10 and IL-4 production by these T cells [34]. In our study, the addition of exogenous IL-12 at the time of SAC stimulation reduced IL-10 mRNA expression in PBMC from HIV-positive and -negative donors. Our results suggest that IL-12 also has an inhibitory effect on IL-10 expression in cells that are responding to SAC, such as monocytes or B cells. It would be of interest to determine the cell types that are susceptible to IL-12.

In summary, we have reported new observations concerning the molecular analysis of decreased IL-12 production in HIV-positive persons and revealed several potential mechanisms involved in the down-regulation of this important immunoregulatory cytokine. Viral infection appears mainly to affect p35 mRNA expression, while IL-10 reduces p40 expression and simultaneously enhances its own expression. Such detailed studies investigating both mRNA expression and protein secretion are necessary to understand the basis of IL-12 dysregulation occurring during HIV infection and to provide a rational basis for potential immune interventions aiming at restoring normal immune functions.

References


10. Denis, M, Ghadirian E. Dysregulation of interleukin-8, interleukin-10, and IL-4 production by these T cells [34]. In our study, the reference provides a rational basis for potential immune interventions aiming at restoring normal immune functions.

Acknowledgments

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