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Induction of Interleukin-12 (IL-12) by Recombinant Glycoprotein gp120 of Human Immunodeficiency Virus Type 1 in Human Monocytes/Macrophages: Requirement of Gamma Interferon for IL-12 Secretion

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We studied the effects of the gp120 glycoprotein of human immunodeficiency virus type 1 on the expression of interleukin-12 (IL-12) in human monocytes and in monocyte-derived macrophages. Induction of the mRNA for both the p35 and p40 subunits of IL-12 was observed in both cell types after gp120 treatment. We then evaluated cytokine secretion by using an enzyme-linked immunosorbent assay which recognizes only the IL-12 heterodimer. No IL-12 was detected in monocytes/macrophages treated with gp120 alone. A consistent IL-12 secretion was found in macrophages primed with gamma interferon (IFN-γ) and subsequently treated with gp120. Low levels of IL-12 were occasionally observed in IFN-γ-primed monocytes stimulated with gp120. The greater response of macrophages than of monocytes to the priming effect of IFN-γ was consistent with the finding that IFN-γ induced a much stronger antiviral state to vesicular stomatitis virus in macrophages than in monocytes. These data indicate that gp120 is an inducer of IL-12 expression in monocytes/macrophages and that IFN-γ is an essential cofactor for IL-12 secretion, especially in differentiated macrophages.

Unbalanced cytokine production is considered to play an important role in the generation of the progressive immunodeficiency observed in the course of human immunodeficiency virus type 1 (HIV-1) infection (7, 16, 18). A decrease in the production of immunoregulatory cytokines (such as interleukin-2 [IL-2] and gamma interferon [IFN-γ]), as well as an increase in proinflammatory cytokines (especially tumor necrosis factor alpha and IL-6), has generally been associated with disease progression in HIV-infected individuals (7, 16, 18). Recently, much attention has been given to IL-12 and to its role in the pathogenesis of HIV-1 infection (2, 4–6).

IL-12, also known as the natural killer cell stimulatory factor, is a heterodimeric cytokine produced by monocytes/macrophages, B cells, and other accessory cells in response to bacteria, bacterial products, or parasites (21). IL-12 has pleiotropic effects on different cell functions both in vitro and in vivo (reviewed in reference 21). These effects include stimulatory effects on natural killer cells and T cells (14), as well as induction of cytokine production and activity (9, 19). The current data on IL-12 indicate that IL-12 is a powerful and probably essential factor for Th1 cell generation and proliferation (20). The mechanisms involved in the regulation of IL-12 production are still poorly understood. Kubin and coworkers reported that IFN-γ priming was important for IL-12 secretion in unseparated peripheral blood mononuclear cells (PBMC) treated with lipopolysaccharide (LPS) (15). Very recently, two studies have shown that pretreatment with IFN-γ is of crucial importance for the secretion of IL-12 by mouse macrophages (8) and human monocytes (12).

IL-12 production is generally impaired in the course of HIV infection (2, 5, 6). The relevance of this IL-12 deficiency in the pathogenesis of HIV-1 infection was recently suggested by the finding that exogenous IL-12 can restore immune responsiveness of peripheral leukocytes from AIDS patients (4). However, in spite of the great interest in this cytokine, no information on the effects of HIV-1 or its gp120 protein on IL-12 expression by macrophages, which represent the major source for IL-12 production, is available.

Macrophages are cellular targets of HIV-1 infection and play important roles in the pathogenesis of AIDS (for a review, see reference 17). In previous studies, we showed that freshly isolated human monocytes spontaneously differentiated into macrophages after 3 to 7 days in culture and acquired an enhanced capability of producing certain cytokines (i.e., tumor necrosis factor alpha, IL-6, and IFN-β) in response to LPS (11). We also found that treatment of monocytes/macrophages with HIV-1 recombinant gp120 (rgp120) induced the expression of IFN-β and IL-10, which were capable of down-modulating HIV replication, especially in differentiated macrophages (1, 10). In this study, we evaluated whether the HIV-1 gp120 protein could induce IL-12 expression in monocytes/macrophages and whether IFN-γ affects IL-12 production differently in monocyte-derived macrophages and freshly isolated monocytes.

Induction of IL-12 mRNA in monocytes/macrophages treated with rgp120. As shown in Fig. 1, the addition of 1 μg of rgp120 per ml to either 1-day monocytes or 7-day macrophages resulted in a marked induction of mRNA for both the p35 and p40 subunits of IL-12. The extent of accumulation of IL-12 mRNA in response to gp120 was comparable to that observed in cultures treated with 1 μg of LPS per ml (Fig. 1). By using the *Limulus* amoebocyte assay, we found that no detectable levels of LPS were present in the rgp120 preparations used in these experiments. Similar patterns of IL-12 mRNA induction were obtained in four other experiments using gp120-stimulated monocytes/macrophages from different donors (data not

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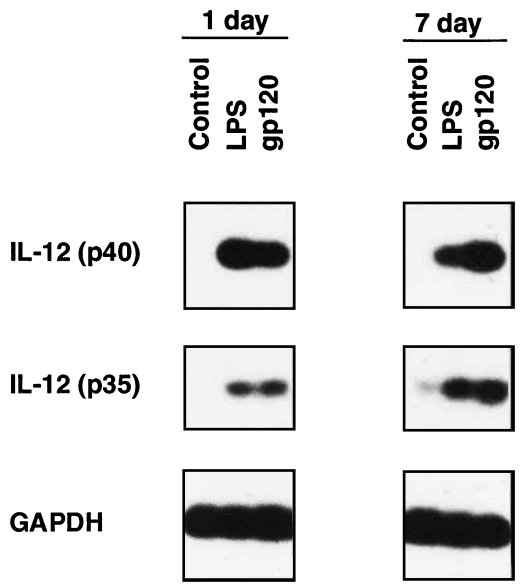

FIG. 1. Induction of IL-12 mRNA in 1-day monocytes and 7-day macrophages treated with either HIV-1 gp120 or LPS. Human monocytes/macrophages were isolated by Ficoll-Hypaque density gradient centrifugation from the peripheral blood of healthy donors and separated from lymphocytes by adherence to plastic dishes (11). Monocytes were seeded in 6-well plates at a density of 1.5×10^5 cells/cm^2 in Iscove's medium supplemented with 15% fetal calf serum as reported elsewhere (10, 11). Cytochemical (i.e., sodium fluoride-inhibited esterase activity) and surface marker (i.e., CD14 antigen) analyses revealed that the adherent cell population consisted of >95% monocytes. At 1 or 7 days of culture, cells were treated for 4 h with 1 μg of either recombinant gp120 (produced in a baculovirus expression system; purchased from Neosystem, Strasbourg, France) or LPS (Sigma) per ml. RNA was extracted by the method of Chirgwin et al. (3). Reverse transcriptase PCR was performed with 1 μg of RNA as previously described (11, 13). The sequences for IL-12 primers were as follows: p40 sense primer, 5′ CATTCGCTCCTGCTGCTTCAC; p40 antisense primer, 5′ TACTCCTTGTTGTCCCCTCTG; p40 probe, ATTCTGGAAAG ATTCTACGCTCCGG; p35 sense primer, 5′ CTTCACCACTCCCAAAACC TG; p35 antisense primer, 5′ AGCTCATCACTCTATCAATAG; p35 probe, 5′ TTACCCTCAACGGACCGGAG. The sequence for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) primers was described previously (11, 13). The signal corresponding to the p35 mRNA was obtained after a 5-day exposure of the film, while that of the p40 mRNA was detected after only an 18-h exposure.

shown). We concluded that gp120 was an efficient inducer of IL-12 mRNA in monocytes/macrophages.

Secretion of IL-12 by monocytes/macrophages primed with IFN-γ and treated with rgp120 protein. We assessed whether treatment of monocytes/macrophages with rgp120 results in any IL-12 secretion, as evaluated by measuring cytokine levels in the culture medium by using a specific enzyme-linked immunosorbent assay (ELISA) which recognizes only the IL-12 p35/p40 heterodimer. In a first set of experiments, we noticed that no detectable levels of IL-12 were generally found in the cell supernatants of gp120-treated cultures of monocytes/macrophages. We then performed a total of six experiments (with monocytes/macrophages from different donors) in which rgp120 was added to cell cultures primed for 3 h with IFN-γ. In all the experiments, remarkable levels of IL-12 were detected in the cell supernatants of IFN-γ-primed 7-day macrophages treated with gp120. Table 1 shows the results of three representative experiments. In 1-day monocytes primed with IFN-γ and treated with gp120, IL-12 secretion was observed in only 2 out of 6 experiments. The results of one of the two experiments are shown in Table 1 (donor 3). In both the experiments in which some IL-12 secretion was also observed in 1-day monocytes after IFN-γ priming and gp120 treatment, the cytokine levels were 10- to 20-fold lower than those detected in the cell supernatants of the corresponding cultures of 7-day macrophages. The IL-12 secreted by IFN-γ-primed gp120-treated macrophages was biologically active, as demonstrated by its capacity of inducing IFN-γ secretion in PBMC activated by phytohemagglutinin and IL-2 (data not shown). IFN-γ-primed monocytes/macrophages did not release IL-12 in response to other stimuli such as HIV-1 p24 protein or monoclonal antibodies to human CD4 antigen (Table 1, donors 1 and 3). Notably, when a typical IL-12 inducer, such as LPS, was added to IFN-γ-primed monocytes/macrophages, the extent of cytokine secretion was consistently higher in 7-day macrophages than in 1-day monocytes (Table 1, donors 1 and 2).

IFN-γ is more effective in inducing an antiviral state to VSV in 7-day macrophages than in 1-day monocytes. We assumed that the lower response of 1-day monocytes to the IFN-γ priming effect for IL-12 secretion in cultures treated with gp120 (or LPS) could be due to a lower level of sensitivity of these cells than of differentiated macrophages to IFN-γ itself. To provide some support for this assumption, we determined whether equal amounts of IFN-γ could exhibit a different antiviral effect to vesicular stomatitis virus (VSV) when added to either 1-day or 7-day monocyte/macrophage cultures. As shown in Fig. 2, IFN-γ induced a much stronger inhibition of VSV yield in differentiated macrophages than in 1-day monocytes. In particular, at both the IFN-γ concentrations used (i.e., 10 and 100 ng/ml), the extent of inhibition of virus yields in comparison with yields for the corresponding untreated cultures was more than 10-fold higher in macrophages than in monocytes.

To evaluate whether the increased response of differentiated macrophages to the biological effect of IFN-γ could be associated with increased receptor expression, we measured the binding at 4°C of ^{125}I-labelled IFN-γ (specific activity, 1,000 Ci/mmol; Amersham) to 1-day monocytes and to 7-day cultured macrophages. The specific IFN-γ binding (defined as the difference between total binding and nonspecific binding in the presence of a 150-fold excess of unlabelled IFN-γ) was consis-

TABLE 1. Secretion of IL-12 by IFN-γ-primed monocytes/macrophages treated with HIV-1 recombinant gp120 protein[a]

Treatment	IL-12 production (pg/ml)[b]					
	Donor 1		Donor 2		Donor 3	
	1-day monocytes	7-day macrophages	1-day monocytes	7-day macrophages	1-day monocytes	7-day macrophages
None	<7.8	<7.8	<7.8	<7.8	<7.8	<7.8
gp120	<7.8	<7.8	<7.8	<7.8	<7.8	<7.8
IFN-γ	<7.8	<7.8	<7.8	<7.8	<7.8	<7.8
IFN-γ + gp120	<7.8	**422**	<7.8	**24**	**48**	**720**
IFN-γ + p24	<7.8	<7.8	ND	ND	<7.8	<7.8
IFN-γ + anti-CD4	<7.8	<7.8	ND	ND	<7.8	<7.8
LPS	<7.8	<7.8	<7.8	<7.8	ND	ND
IFN-γ + LPS	<7.8	**130**	<7.8	**17**	ND	ND

[a] One-day monocytes and 7-day macrophages were cultured in 48-well plates as described in the legend to Fig. 1. Some cultures were treated with 100 ng of IFN-γ (specific activity, 2.4×10^7 U/mg; Becton Dickinson Labware) per ml for 3 h prior to addition of gp120 (1 μg/ml), LPS (1 μg/ml; Sigma), HIV-1 p24 protein (1 μg/ml; catalog no. 382; obtained from the NIH AIDS Research and Reference Reagent Program), or monoclonal antibodies to human CD4 (Q428; catalog no. 1377; obtained from the NIH AIDS Research and Reference Reagent Program); other cells were left untreated. After 24 h at 37°C, cell supernatants were harvested and tested for the presence of IL-12 by using a specific ELISA (detection limit, 7.8 pg/ml of p70 heterodimer; R & D System).

[b] ND, not determined; boldface indicates the positive values.

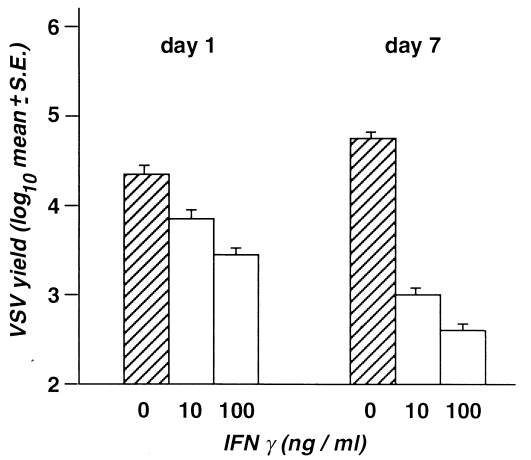

FIG. 2. Inhibition of VSV yield by IFN-γ in 1-day monocytes and 7-day macrophages. One-day monocytes and 7-day macrophages were cultured as indicated in the legend to Fig. 1. Cell monolayers were treated with different concentrations of IFN-γ (100 and 10 ng/ml) for 24 h. Cells were then infected with VSV, and virus was harvested after 18 h and titrated on mouse L929 cells as described elsewhere (11). There were three cell cultures for each experimental point. Error bars indicate standard errors (S.E.).

tently higher in macrophage cultures than in monocytes. In particular, the cell-bound radioactivity observed in cultures treated with increasing ligand concentrations in the range of 0.3 to 2 nM was approximately four times higher in 7-day macrophages than in 1-day monocytes.

The data reported here represent the first evidence of the induction of IL-12 gene expression by HIV-1 gp120 in monocytes/macrophages. In our experiments, in spite of the clear-cut accumulation of the mRNA for both the p35 and p40 subunits observed in gp120-stimulated monocytes/macrophages, no apparent secretion of the IL-12 heterodimer was detected, unless the cells were also treated with IFN-γ before stimulation. In this regard, it is worth mentioning that the importance of IFN-γ in IL-12 secretion has already been suggested in some recently published reports (8, 12, 15). Kubin et al. showed that IFN-γ markedly increased the secretion of IL-12 in response to LPS or to *Staphylococcus aureus* in unseparated human PBMC cultures (15). Likewise, Flesch and coworkers reported that IFN-γ was an essential cofactor for the production of IL-12 by murine bone marrow-derived macrophages infected with *Mycobacterium bovis* BCG or treated with LPS (8). Lastly, Hayes and colleagues recently found that optimal expression of IL-12 mRNA and cytokine bioactivity required a specific priming of human monocytes by IFN-γ (12). Thus, our finding that IFN-γ is an essential cofactor for IL-12 secretion in gp120-stimulated monocytes/macrophages further emphasizes the key role of IFN-γ in the regulation of IL-12 production. Further studies are needed to clarify the mechanisms by which IFN-γ induces IL-12 secretion in gp120-stimulated monocytes/macrophages. However, the description of the IL-12 production by monocytes/macrophages in response to IFN-γ–gp120 treatment in itself can be of importance for understanding some of the mechanisms involved in the generation of cytokine dysfunctions observed in HIV-1-infected individuals, especially if this knowledge is integrated with the knowledge of the role of other cytokines (especially IL-10) which may be negative regulators for IL-12 production. In fact, we recently reported that gp120 can induce the expression of considerable levels of IL-10 in human monocytes/macrophages (1). Other authors have shown that IL-10 production is enhanced in HIV-1 infection and is apparently associated with disease progression in patients with AIDS (5, 6). On the basis of the capacity of IL-10 to down-regulate IL-12 production (15), we envisage the following possible scenario occurring in HIV-1-infected individuals. Monocytes/macrophages might produce the amount of IL-12 (in response to HIV-1 gp120 as well as to other stimuli) necessary for the generation of a protective Th1 type of immune response. However, IL-12 expression might be markedly influenced by the local levels of IL-10 and IFN-γ, acting as negative or positive regulators, respectively, for IL-12 secretion. Thus, the decreased production of IFN-γ in response to certain stimuli (18) and the concomitant enhancement of IL-10 expression occurring in the course of HIV-1 infection (5, 6) might result in a general impairment of IL-12 production, subsequently leading to AIDS immune dysfunctions.

In the present study, we also found that consistently higher levels of IL-12 secretion occurred in 7-day cultured monocyte-derived macrophages (primed with IFN-γ and treated with gp120) than in the corresponding cultures of 1-day monocytes, in which IL-12 production was only occasionally detected. This finding was consistent with the higher level of responsiveness to the IFN-γ-mediated antiviral effect to VSV observed in macrophages than in monocytes. The higher degree of sensitivity of 7-day cultured macrophages to the biological effects of IFN-γ can be explained, at least in part, by an increased expression of IFN-γ receptors during the course of monocyte/macrophage differentiation. We previously reported that 7-day macrophages differed from 1-day monocytes in a number of phenotypic and functional characteristics, including their increased cytokine production in response to LPS (11) and their enhanced sensitivity to the antiviral state induced by both IFN-α and -β (10). Thus, we can assume that the enhanced response of macrophages in comparison with that of monocytes to the IFN-γ-induced effects (including the priming for IL-12 production) may represent an additional example of the importance of macrophage differentiation for the acquisition of a suitable repertoire of responses to certain stimuli. Such acquisition may be important for maintaining antiviral activity induced by low levels of endogenous IFNs and may represent a prerequisite for prompt activation of macrophages in response to other signals.

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