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HIV-1 Envelope gp120 Inhibits the Monocyte Response to Chemokines Through CD4 Signal-Dependent Chemokine Receptor Down-Regulation

Ji Ming Wang,‡ Hirotsugu Ueda,§ O. M. Zack Howard,‡ Michael C. Grimm,§ Oleg Chertov,‡ Xiaoqi Gong,§ Wanghua Gong,‡ James H. Resau,¶ Christopher C. Broder,‖ Gerald Evans,‡ Larry O. Arthur,§ Francis W. Ruscetti,¶ and Joost J. Oppenheim*  

Since HIV-1 infection results in severe immunosuppression, and the envelope protein gp120 has been reported to interact with some of the chemokine receptors on human T lymphocytes, we postulated that gp120 may also affect monocyte activation by a variety of chemokines. This study shows that human peripheral blood monocytes when preincubated with gp120 either purified from laboratory-adapted strains or as recombinant proteins exhibited markedly reduced binding, calcium mobilization, and chemotactic response to chemokines. The gp-120-pretreated monocytes also showed a decreased response to FMLP. This broad inhibition of monocyte activation by chemoattractants required interaction of gp120 with CD4, since the effect of gp120 was only observed in CD4+ monocytes and in HEK 293 cells only if cotransfected with both chemokine receptors and an intact CD4, but not a CD4 lacking its cytoplasmic domain. Anti-CD4 mAbs mimicked the effect of gp120, and both anti-CD4 Ab and gp120 caused internalization of CXCR4 in HEK 293 cells provided they also expressed CD4. Staurosporine blocked the inhibitory effect of gp120 on monocytes, suggesting that cellular signaling was required for gp120 to inhibit the response of CD4+ cells to chemoattractants. Our study demonstrates a broad suppressive effect of gp120 on monocyte activation by chemoattractants through the down-regulation of cell surface receptors. Thus, gp120 may be used by HIV-1 to disarm the monocyte response to inflammatory stimuli.  


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3 Abbreviations used in this paper: CXCR, CXC chemokine receptor; CCR, CC chemokine receptor; SDF-1, stromal cell-derived factor; MIP, macrophage inflammatory protein; MCP, monocyte chemotactic protein.

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gp120 INHIBITS MONOCYTE RESPONSE TO CHEMOATTRACTANTS

Materials and Methods

Reagents and cells

Natural gp120 was isolated from the culture fluid of HIV-1 (MN or RF)-infected H9 cells by immunoadfinity chromatography (22). Recombinant gp120 (strains MN and IIIB) and recombinant soluble CD4 were purchased form Intracel (Cambridge, MA). At the highest concentration tested, the gp120 preparations had <0.2 ng/ml of endotoxin activity. Anti-CD4 mAbs (clone K4) was purchased from Dako (Carpinteria, CA). Recombinant chemokines were obtained from PeproTech (Rocky Hill, NJ). Radioiodinated chemokines were purchased from DuPont-New England Nuclear (Boston, MA). Human PBMC were isolated from buffy coat (National Institutes of Health, Bethesda, MD) enriched for mononuclear cells using iso-osmotic Percoll gradient as previously described (23). Neutrophils were isolated from buffy coat blood with dextran sedimentation as previously described (23). The purity of the cell preparations was examined by morphology and was >90% for monocytes and >98% for neutrophils. The CCR5-transfected HEK 293 cells were a gift from Dr. P. Gray (ICOS, Seattle, WA). CCR1 and CXCR4/fusin cDNA were isolated in this laboratory and were transfected into 293 cells (University of California, San Francisco, CA) and was stably transfected as previously described (24). CCR2b cDNA was supplied by Dr. I. Charo (University of California, San Francisco, CA) and was stably transfected into 293 cells in this laboratory. The viability of monocytes or HEK 293 cells before and after gp120 treatment was examined by trypan blue exclusion and was >95% after up to 18-h treatment at 37°C.

Calcium mobilization

Ca2+ mobilization was performed using FACS analysis through the courtesy of Ms. L. Finch (SAIC Frederick, Frederick, MD). Monocytes (10⁶ cells) loaded with indo-1 were treated with gp120 (MN; 50 nM) or medium (RBPI, 1640, 10 mg/ml BSA) alone for 60 min at 37°C. The loaded cells were washed, resuspended in fresh medium, then stimulated with gp120 (MN; 50 nM), chemokines (10 nM), or FMLP (10 nM). The bound/free ratio of indo-1 was continuously recorded using EPICS 753 (Coulter, Miami, FL). The percentage of responding cells was estimated.

Chemotaxis assays

Chemotaxis assays were performed using a 48-well chemotaxis chamber (Neuroprobe, Cabin John, MD) as described previously (25). Chemotactants were placed in the lower wells of the chamber, 50 μl of cells (monocytes or neutrophils at 2 × 10⁶/ml) were placed in the upper wells. The upper and lower wells were separated by a polycarbonate filter (5-μm pore size). For 293 cells the filter of 10-μm pore size was precoated with collagen IV as previously described (25). After stimulation (90 min for monocytes, 60 min for neutrophils, 5 h for 293 cells) at 37°C, the filters were removed and stained, and the cells migrated across the filters were counted after coding the samples. Results were calculated as the mean (± SD) number of migrated cells in three high powered light microscopy fields in triplicate samples. The chemotaxis index was calculated as follows: chemotaxis index = number of migrated cells in response to chemotaxis activator/number of randomly migrated cells (in response to medium). In chemotaxis deactivation experiments, monocytes (or neutrophils) and HEK 293 cells transfected with chemokine receptors were preincubated with chemotactants or gp120 for 30 or 60 min at 37°C, then washed three times with PBS. The cell migration in response to various chemotactants was assessed and computed as described above. After subtraction of background migration (in response to medium), the percent deactivation of chemotaxis to a given chemotactant was calculated by the formula: 1− (mobilization of cells preincubated with gp120/ migration of cells preincubated with medium × 100%).

Binding assays

Binding assays were performed by preincubating duplicate samples of monocytes (2 × 10⁶/sample) or chemokine receptor-transfected 293 cells (1 × 10⁶/sample) with different concentrations of gp120 for 60 min at 37°C in a volume of 200 μl/sample of binding medium (RPMI 1640, 1% BSA, 5 mM HEPES, and 0.05% NaN₃). Radiolabeled chemokines (0.12 mM) were then added to each sample. To parallel duplicate samples, different concentrations of gp120 or unlabeled chemokines (as control) were added simultaneously with radiolabeled chemokines. After incubation at room temperature for 40 min, the cells were centrifuged through a 10% sucrose/ PBS cushion, and the cell-associated radioactivity was measured in a gamma counter. The percent reduction of chemokine binding to monocytes by gp120 treatment was calculated by the formula: 1− (counts per minute associated with cells preincubated with gp120[counts per minute associated with cells incubated with medium alone]) × 100.

To determine the change in the number of binding sites and the affinity for a given chemokine, cells were preincubated with or without gp120 (25 nM) for 60 min at 37°C. The duplicate monocyte samples were then incubated with 0.12 nM radiolabeled chemokines in the presence of increasing concentrations of unlabeled chemokines. The cells were pelleted after 40 min at room temperature and measured for radioactivity. The binding data were analyzed and plotted with a Macintosh computer program, LIGAND (Dr. P. Muson, Division of Computer Research and Technology, National Institutes of Health, Bethesda, MD).

Confocal microscopy

HEK 293 cells expressing CXCR4 with and without intact CD4 were pretreated for 3 h at 37°C with SDF-1α, recombinant gp120 (IIIB, MN, or CM; 25 nM) or anti-CD4 mAb (Ab; 5 μg/ml). The cells were centrifuged onto slides and permeabilized. The slides were then stained with an anti-CXCR4/fusin mAb (2G5) followed by incubation with FITC-labeled goat anti-mouse IgG F(ab')₂. Slides were examined using a Zeiss 310 Confocal Laser Scanning Microscope (Carl Zeiss, New York, NY). Nomarski, FITC (488 nm; green), and DAPI (UV 364 nm; blue) images were prepared for each specimen, and colored images were superimposed on Nomarski.

Statistical analysis

All experiments were performed at least three times unless specified, and the results presented are either from a representative experiment or from a pool of experiments. The significance of the difference between experimental and control groups was analyzed with Student’s t test.

Results

Gp120 inhibits monocyte activation by chemokines

Since the chemotactic response of monocytes from AIDS patients has been reported to be defective (19), and soluble gp120 suppresses the expression of receptors for C5a and FMLP on normal monocytes (20), we initially investigated the effect of gp120 on the capacity of monocytes to respond to chemokines as well as to FMLP. We confirmed a previous report (25) that both native and recombinant gp120 were also weakly chemotactic for human monocytes. However, gp120 was not chemotactic for HEK 293 cells transfected with CCR5 or CXCR4, which did show significant migration in response to the chemokines SDF-1α and MIP-1β (data not shown), suggesting that gp120 can only induce CD4+ expressing cells to migrate. The recombinant gp120 of the MN strain at a concentration range of 1 to 100 nM did not induce significant Ca2+ mobilization in monocytes (Fig. 1A and data not shown). This gp120 also did not desensitize the subsequent (within 100 s) monocyte response to chemokines or FMLP (not shown), suggesting that the gp120 used in the assay did not activate the receptors for FMLP or for the chemokines tested. However, preincubation of monocytes with increasing concentrations of gp120 for 1 h at 37°C significantly reduced the monocyte response to a number of chemokines and the bacterial chemotactic peptide FMLP. Figure 1 shows that at 50 nM, gp120 of the MN strain almost completely abolished the Ca2+ mobilization induced by MIP-1α, RANTES, SDF-1α (10 nM), as well as FMLP (10 nM; Fig. 1B). The Ca2+ mobilization induced by MCP-1 (10 nM) was also significantly reduced by preincubation of monocytes with gp120 (Fig. 1B).

We next examined the effect of gp120 on the capacity of monocytes to directionally migrate in response to a variety of chemotactants. As observed for Ca2+ mobilization assays, preincubation of monocytes with gp120, also in a dose-dependent manner inhibited the cell migration occurring in response to a number of chemotactants, including C-C chemokines as well as CXC chemokine SDF-1α, the ligand for CXCR4/fusin (Table I). In Table I, the effect of gp120 (MN) at 10 nM is shown. The inhibition of monocyte migration by gp120 could also be demonstrated for FMLP (Table I), which uses a seven-transmembrane receptor structurally
related to chemokine receptors. Since gp120 had previously been considered to be a ligand for CD4, the contribution of CD4 to these desensitizing effects of gp120 was evaluated. In fact, gp120 failed to inhibit directional migration of chemokine receptor transfected HEK 293 cells lacking CD4 in response to their respective chemokines (data not shown). In addition, as shown in Table I, preincubation of gp120 with soluble CD4 completely abolished the inhibitory effect of gp120 on monocyte migration induced by chemokines and FMLP, suggesting a critical role of cell membrane CD4 molecules in mediating this suppressive activity of gp120. The inhibitory effect of gp120 on monocyte migration was also totally eliminated by preincubation with an anti-gp120 mAb (data not shown). The gp120 did not affect the chemotactic response of neutrophils to FMLP or IL-8 (data not shown), further supporting a requirement for cell surface CD4 for the gp120 effect.

Gp120 inhibits monocyte expression of chemokine binding sites

To test whether gp120 might compete with chemokines for binding sites on monocytes, soluble recombinant or purified natural gp120 (MN) (22) and radiolabeled chemokines were added simultaneously to human peripheral blood monocytes as previously described (23). While all unlabeled chemokines (60–120 nM) showed the expected competition for binding by radiolabeled ligands (Fig. 2, horizontal lines), gp120 showed little or no direct competition with radiolabeled chemokines for binding sites on monocytes or neutrophils (Fig. 2, open circles).

We then examined the possibility that more prolonged incubation with gp120 might down-regulate monocyte expression of chemokine receptors. Preincubation of monocytes with soluble gp120 at 37°C for 60 min resulted in a marked dose-dependent inhibition of the capacity of monocytes to bind C-C chemokines (Fig. 2, A–E).

Table I. Effect of soluble CD4 (sCD4) on the inhibition by rgp120 (MN) of monocyte chemotaxis in response to C-C chemokines, SDF-1 and FMLP

<table>
<thead>
<tr>
<th>Chemotactants</th>
<th>Medium</th>
<th>rgp120</th>
<th>rgp120 + sCD4</th>
</tr>
</thead>
<tbody>
<tr>
<td>MIP-1α</td>
<td>3.51</td>
<td>1.0 (100)b</td>
<td>3.23 (11)</td>
</tr>
<tr>
<td>MIP-1β</td>
<td>2.42</td>
<td>0.96 (100)b</td>
<td>2.50 (0)</td>
</tr>
<tr>
<td>RANTES</td>
<td>3.13</td>
<td>1.0 (100)b</td>
<td>2.70 (20)</td>
</tr>
<tr>
<td>MCP-1</td>
<td>4.60</td>
<td>3.0 (45)b</td>
<td>3.92 (19)</td>
</tr>
<tr>
<td>MCP-2</td>
<td>3.62</td>
<td>2.57 (40)b</td>
<td>3.62 (0)</td>
</tr>
<tr>
<td>FMLP</td>
<td>11.02</td>
<td>4.51 (65)b</td>
<td>11.20 (0)</td>
</tr>
<tr>
<td>SDF-1α</td>
<td>6.43</td>
<td>1.54 (90)b</td>
<td>6.51 (0)</td>
</tr>
</tbody>
</table>

a Recombinant gp120 at 25 nM was preincubated in the presence or absence of sCD4 for 30 min at 37°C, then was further incubated with monocytes for 60 min at 37°C. After washing, the cell migration in response to C-C chemokines (10 nM), SDF-1α (100 nM), or FMLP (10 nM) was determined. The results represent a summary of five experiments performed.

b p < 0.05 (Student’s t test) compared to the migration of monocytes preincubated with medium alone.
The IC₅₀ for gp120 inhibition of monocyte binding of chemokines (0.1, 1.0, and 5.0 nM for MIP-1α, MIP-1β, and RANTES, respectively) was similar to the IC₅₀ obtained by the direct competitive effect of the native ligands (0.3, 0.6, and 1.0 nM for MIP-1α, MIP-1β, and RANTES, respectively). Consequently, preincubation of monocytes with gp120 not only inhibited their capacity to bind MIP-1β, which uses exclusively CCR5 (26, 27), but also inhibited their binding of MIP-1α and RANTES, which, in addition, use CCR1 (28, 29) and other C-C chemokine receptors (3, 26). Furthermore, the capacity of monocytes to bind [¹²⁵I]MCP-1 and [¹²⁵I]MCP-2 was inhibited up to 50% by preincubation of the cells with gp120 from MN, IIIB, or CM strains (3, 26) and/or CCR1 (30), respectively. Preincubation with gp120 also significantly inhibited the monocyte expression of the binding sites for SDF-1α, the only known ligand for the T tropic HIV-1 fusion cofactor CXCR4 (Fig. 2F). The suppressive effect on monocyte expression of chemokine binding sites was not limited to purified gp120 of the MN strain. Preincubation with other native and recombinant gp120 from T cell line-adapted virus, such as IIIB, as well as a recombinant gp120 from a monocyte tropic strain, CM, significantly interfered with the capacity of monocytes to bind chemokines (data not shown). In contrast, gp120 did not inhibit the neutrophil binding of the CXC chemokine, IL-8, a potent chemotactic factor for neutrophils rather than monocytes (data not shown). These results showed that the reduction in chemokine binding to monocytes preincubated with soluble gp120 is more generalized than the reported capacity of chemokines to competitively inhibit HIV-1 entry and envelope-mediated cell fusion (31, 32).

**FIGURE 2.** The gp120 inhibits the capacity of monocytes to bind chemokines. Monocytes were preincubated in duplicate with medium alone or with different concentrations of purified gp120 (MN) for 60 min at 37°C. [¹²⁵I]labeled chemokines were added, and the cultures were continued for 40 min at room temperature. The cells were then centrifuged through a sucrose cushion and measured for radioactivity. Direct competition experiments were performed in parallel by adding different concentrations of gp120 simultaneously with [¹²⁵I]labeled chemokines to duplicate monocyte samples (shown as open circles). Unlabeled chemokines were used as control competitors (60 nM for MIP-1α and MIP-1β; 120 nM for RANTES, MCP-1, MCP-2, and SDF-1α). The cells were incubated at room temperature for 40 min and harvested. One of three representative experiments is shown. Shaded bars represent the percent reduction of monocyte binding for chemokines. Hatched bars indicate the levels of direct competition with chemokines for binding by gp120. Horizontal lines depict the maximal level of direct competition of chemokine binding by unlabeled native ligands (60–120 nM). A to E, Reduction of [¹²⁵I]C-C chemokine binding to monocytes pretreated with gp120 (shaded bars) and competition by gp120 of chemokine binding to monocytes (hatched bars). F, Effect of gp120 on monocyte binding of [¹²⁵I]SDF-1α. Experiments performed with recombinant gp120 of the IIIB, MN, or CM strain yielded similar results.

**Down-regulation of chemokine binding sites on monocytes by gp120 requires CD4**

We examined whether the down-regulation by gp120 of chemokine binding sites on monocytes also required the presence of CD4 as manifested in chemotaxis experiments. Preincubation of HEK 293 cells expressing functional CCR5, CCR1, or CXCR4 with gp120 from MN, IIIB, or CM strains did not reduce their ability to bind chemokines (data not shown), indicating that these chemokine receptors did not directly interact with the gp120 tested. On the other hand, the inhibitory effect of gp120 on monocyte binding of C-C chemokines was blocked by pretreatment of gp120 with soluble CD4 (Table II), in correlation with the observation in cell migration (Table I). This suggests that soluble CD4 sequesters gp120 and prevents its binding to cell membrane-anchored CD4 and the consequent down-regulation of chemokine receptors. To examine whether the suppressive effect on monocyte expression of chemokine binding sites was exclusive for gp120 or whether other activators of CD4 have a similar effect, we used anti-CD4 mAbs. Preincubation with anti-CD4 mAbs reproduced the effect of gp120 in inhibiting monocyte binding of MIP-1β, whereas anti-CD14 mAb had no effect (Fig. 3). The anti-CD4 Abs also reduced the capacity of monocytes to bind other chemokines, such as SDF1, MCP-1, and MCP-2 (data not shown).

These observations led us to use confocal microscopy to visualize the effect of gp120 as well as anti-CD4 Ab on chemokine binding.
Anti-CD4 mAbs inhibit monocyte binding for MIP-1

**FIGURE 3.**

receptor localization. Confocal microscopy indicated that preincubation with gp120 or anti-CD4 mAb caused marked time-dependent internalization of CXCR4/fusin (Fig. 4) in HEK 293 cells transfected to express both CD4 and CXCR4/fusin, but not in cells expressing CXCR4/fusin alone. The apparent requirement for initial interaction of gp120 with CD4 is in agreement with the recent report that preincubation of CD4+/fusin (CXCR4)+ T cells with gp120 resulted in the formation of a gp120/CD4/fusin complex that could be coprecipitated with Abs against any one of these three molecules (33). Our results also indicate that ligation of CD4 with anti-CD4 is sufficient to internalize chemokine receptors with the resultant inhibition of the ability of CD4+ cells to bind chemokines. Although in Figure 4, only the results obtained with gp120 IIIB and CXCR4/C4D4/293 cells after 3-h treatment are presented, the internalization was already significant at 1-h incubation, and similar internalizing effects were obtained with gp120 of the MN and CM strains. In addition, CCR5 was also markedly internalized by gp120 in 293 cells provided they were also transfected to express CD4 (data not shown).

**Signaling is involved in the inhibition of chemokine receptors on monocytes by gp120**

The interaction of HIV-1 with CD4 and the coreceptors and the resultant HIV-1-cell fusion were reported to be resistant to pertussis toxin, suggesting that the virus competes for chemokine receptor occupancy, resulting in fusion without requiring G protein signaling in target cells (32, 34). However, interaction of gp120 with CD4 and coreceptors has been reported to induce G protein activation (18) and tyrosine kinase signaling events in T cells or transfected cells (35). In our study, the need to preincubate monocytes at 37°C for 1 h with gp120 to down-regulate chemokine binding sites suggested that binding of CD4 by gp120 may transduce signals. It has been observed previously that gp120 triggers intracellular signals, such as translocation of protein kinase C and the production of metabolites of arachidonic acid in monocytes (36, 37). It has also been reported that PMA, a protein kinase C activator, down-regulated an HIV-1 fusion cofactor, later identified as CXCR4/fusin (33), and inhibited T cell viral entry (38). We therefore investigated whether the suppressive effect of gp120 on chemotaxtractant receptors requires CD4-dependent protein kinase activation. We confirmed that gp120 as well as anti-CD4 Ab enhanced the PKC activation in monocytes (G. Evans and H. Ueda, unpublished observation) and pretreatment of monocytes with the protein kinase inhibitor, staurosporine, prevented the down-regulation of MIP-1β binding sites on monocytes by gp120 (Fig. 5). The ability of monocytes to bind other CC chemokines and SDF-1α could also be protected by pretreatment with staurosporine before the addition of gp120 (not shown). A more specific PKC inhibitor, calphostin C, could mimic the protective effect of staurosporine (data not shown). These observations suggest that binding of gp120 to CD4 on monocytes activates intracellular protein kinase activity, which, in turn, down-regulates chemotaxtractant receptor expression and function. This was further investigated by comparing the response to gp120 of HEK 293 cells transfected with tail-less or intact CD4. The ability of HEK 293 cells to bind MIP-1β could not be inhibited by preincubation of the cells with gp120 or anti-CD4 Ab if the cells expressed CCR5 and

**Table II. Effect of preincubation with recombinant gp120 on the expression of chemokine binding sites by monocytes**

<table>
<thead>
<tr>
<th>125I-Chemokine</th>
<th>Cells Preincubated with</th>
<th>Medium</th>
<th>gp120 (MN, 10 nM)</th>
<th>gp120 (MN) and sCD4</th>
<th>Kd (nM)</th>
<th>Sites/cell</th>
<th>Kd (nM)</th>
<th>Sites/cell</th>
<th>Kd (nM)</th>
<th>Sites/cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>MIP-1α</td>
<td></td>
<td>0.20</td>
<td>1500</td>
<td>0.30</td>
<td>600</td>
<td>60(60)</td>
<td>0.31</td>
<td>1200</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MIP-1β</td>
<td></td>
<td>0.62</td>
<td>4500</td>
<td>1.33</td>
<td>1300</td>
<td>71(71)</td>
<td>0.84</td>
<td>3900</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RANTES</td>
<td></td>
<td>0.35</td>
<td>4650</td>
<td>1.22</td>
<td>1100</td>
<td>76(76)</td>
<td>0.55</td>
<td>4050</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MCP-1</td>
<td></td>
<td>2.10</td>
<td>6700</td>
<td>3.22</td>
<td>4500</td>
<td>32(32)</td>
<td>2.50</td>
<td>5900</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MCP-2</td>
<td></td>
<td>1.80</td>
<td>8100</td>
<td>2.46</td>
<td>5300</td>
<td>34(34)</td>
<td>2.00</td>
<td>7500</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SDF-1α</td>
<td></td>
<td>2.50</td>
<td>4680</td>
<td>5.32</td>
<td>1240</td>
<td>74(74)</td>
<td>2.43</td>
<td>4440</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Monocytes were preincubated with medium alone or gp120 (MN, 10 nM) with and without sCD4 for 60 min at 37°C, and their capacity to bind C-C chemokines assessed. The binding data were analyzed using the Macintosh program LIGAND, and the results shown are representative of three experiments performed.

**FIGURE 3.** Anti-CD4 mAbs inhibit monocyte binding for MIP-1β. Monocytes were preincubated with recombinant gp120 (MN and IIIB) or different concentrations of anti-CD4 mAbs for 60 min at 37°C. [125I]MIP-1 was added to the cells at room temperature for 40 min. The cells were then pelleted through a sucrose cushion, and the cell-associated radioactivity was determined. Abs from both clones A6 and E9 stained CD4 on infected cells (35). In our study, the need to preincubate monocytes at 37°C for 1 h with gp120 to down-regulate chemokine binding sites suggested that binding of CD4 by gp120 may transduce signals. It has been observed previously that gp120 triggers intracellular signals, such as translocation of protein kinase C and the production of metabolites of arachidonic acid in monocytes (36, 37). It has also been reported that PMA, a protein kinase C activator, down-regulated an HIV-1 fusion cofactor, later identified as CXCR4/fusin (33), and inhibited T cell viral entry (38). We therefore investigated whether the suppressive effect of gp120 on chemotaxtractant receptors requires CD4-dependent protein kinase activation. We confirmed that gp120 as well as anti-CD4 Ab enhanced the PKC activation in monocytes (G. Evans and H. Ueda, unpublished observation) and pretreatment of monocytes with the protein kinase inhibitor, staurosporine, prevented the down-regulation of MIP-1β binding sites on monocytes by gp120 (Fig. 5). The ability of monocytes to bind other CC chemokines and SDF-1α could also be protected by pretreatment with staurosporine before the addition of gp120 (not shown). A more specific PKC inhibitor, calphostin C, could mimic the protective effect of staurosporine (data not shown). These observations suggest that binding of gp120 to CD4 on monocytes activates intracellular protein kinase activity, which, in turn, down-regulates chemotaxtractant receptor expression and function. This was further investigated by comparing the response to gp120 of HEK 293 cells transfected with tail-less or intact CD4. The ability of HEK 293 cells to bind MIP-1β could not be inhibited by preincubation of the cells with gp120 or anti-CD4 Ab if the cells expressed CCR5 and
a CD4 lacking its cytoplasmic tail, whereas the binding capacity of the CCR5/293 cells transfected with intact CD4 was inhibited by gp120 or anti-CD4 Ab (Table III), again suggesting that CD4 is an active rather than passive participant in the down-regulation of chemoattractant receptor expression by gp120.

Discussion

Much attention has been focused on how the gp120 envelope protein may interact with chemokine receptors, and considerable information has been generated concerning the interaction of HIV-1 with CD4 and chemokine coreceptors on T cells and transfected cells. It has been suggested that after binding of gp120 to CD4, a conformational change endows an epitope of gp120 (presumably the V3 loop) with the capacity to bind chemokine receptor cofactors (3, 32). This was supported by the observation that in the presence of cell membrane CD4, gp120 competes with MIP-1β for binding to CCR5 (14, 15). It has also been proposed that a complex of CXCR4 and CD4 may pre-exist, and stimulation with gp120 may further promote the complex formation (33). Studies on T cells and transfectants have shown that interaction of HIV-1 envelope proteins with CD4 and coreceptor CCR5 or CXCR4 activated these receptors, causing G protein-mediated signaling and tyrosine kinase activation (18, 35). Although the chemotactic activity of soluble gp120 for CD4 T lymphocytes and monocytes was documented a number of years ago (17, 25), only recently Weissman et al. (18) reported that mammalian cell-derived recombinant monotropic gp160, a precursor of the gp120, was able to induce Ca²⁺ flux and migration of CD4 T lymphocytes by using CCR5. Since the signaling of gp160 in CD4⁺ T cells could be homologously desensitized by MIP-1β, it was concluded that this gp160 was able to directly activate CCR5. Whether this gp160 was also capable of inducing CD4⁺ monocyte migration or migration of CCR5 transfectants in the presence or the absence of CD4 was not reported. In our study, the recombinant gp120 preparations were only weakly chemotactic for monocytes, but they did not induce significant Ca²⁺ flux. There was no homologous desensitization by gp120 of Ca²⁺ mobilization in monocytes induced by a number of chemokines. Furthermore our gp120 did not induce migration or Ca²⁺ flux in HEK 293 cells transfected with CXCR4 and CCR5 alone or in combination with CD4. Therefore, in our assay systems the gp120 did not show direct activation of CCR5 or CXCR4, but, rather, activation of CD4 was a prerequisite for down-regulation of chemoattractant receptors.

In contrast with T cells, the nature of the interaction of gp120 with CD4 and cofactors on monocytes has not been as well characterized. CD4 on monocytes/macrophages is known to act as a HIV-1 fusion cofactor for monocyte tropic strains. In this study, we demonstrated for the first time that HIV-1 gp120 markedly inhibited monocyte responses to a variety of chemokines in association with down-regulation of the receptor expression on the cell surface via a CD4-dependent pathway. This inhibition was apparently not due to a direct competition with chemokines for binding sites by gp120, but required CD4-mediated signaling events operationally resembling heterologous desensitization. This was demonstrated by the facts that 1) the effect of gp120 required a period of preincubation and was observed only on CD4⁺ monocytes and HEK 293 cells transfected to express intact CD4; 2) anti-CD4, but not anti-CD14, mAbs emulated the inhibitory effect of gp120 on both monocytes and CD4⁺/HEK 293 cells; 3) the effect was observed on HEK 293 cells expressing only intact CD4, which retains the cytoplasmic domain, but not on cells expressing a tail-less CD4; and 4) monocytes pretreated with protein kinase inhibitors such as staurosporine (Fig. 5) and calphostin C (data not shown), but not pertussis toxin, were protected from the inhibitory
results. Plots derived from one donor’s cells are shown. Lowed by gp120. Cells from six donors were evaluated and yielded similar with medium and gp120; C labeled MIP-1 1 h at 37°C. The cells were then measured for their ability to bind radio-
further incubated in the absence or the presence of gp120 (MN; 25 nM) for 1 h at 37°C. The cells were then washed and were cultured for 10 h at 37°C

Effect of staurosporine on gp120-mediated down-regulation
FIGURE 5. The data were analyzed and plotted with the LIGAND

Medium (binding in the absence of cold MIP-1β)

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Medium (binding in the absence of cold MIP-1β)</th>
<th>Mediumβ (binding in the presence of cold MIP-1β)</th>
<th>gp120 (MN, 25 nM)</th>
<th>Anti-CD4 (A6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCR5/293</td>
<td>4435</td>
<td>1101 (75)%</td>
<td>4306 (3)</td>
<td>4634 (0)</td>
</tr>
<tr>
<td>Control vaccinia vector</td>
<td>5927</td>
<td>1372 (74)%</td>
<td>5671 (4)</td>
<td>5452 (8)</td>
</tr>
<tr>
<td>VCB2 (tailless CD4)</td>
<td>4203</td>
<td>1449 (66)%</td>
<td>3882 (8)</td>
<td>3981 (5)</td>
</tr>
<tr>
<td>VCB7 (intact CD4)</td>
<td>4217</td>
<td>1027 (76)%</td>
<td>1796 (57)%</td>
<td>1991 (53)%</td>
</tr>
</tbody>
</table>

a CCR5/293 cells were infected with control vaccinia virus, or recombinant vaccinia virus encoding tailless CD4 (VCB2) or intact CD4 (VCB7) at 37°C for 1 h, at a multiplicity of infection of 5 in DMEM containing 2% FCS and antibiotics. The cells were then gently detached with trypsin/EDTA, resuspended in binding medium, aliquoted, and incubated for 10 h in the presence or absence of gp120 or anti-CD4 mAb. Binding assays utilizing [125I]-MIP-1β were performed at room temperature for 40 min. Both VCB2- and VCB7-infected CCR5/293 cells were more than 90% CD4+ as assessed by FACS analysis (not shown).

b Cells were preincubated with medium and bindings were performed in the presence of 120 nM unlabeled MIP-1β.

CD4 has been shown to play many roles that serve to either increase or suppress immune responses. CD4 was originally identified as a phenotypic marker and subsequently as a high affinity receptor for HIV-1 envelope protein gp120 (39). CD4 facilitates T cell function by binding to the nonpolymorphic region of the MHC class II Ags expressed on the surface of APCs. However, an inhibitory role for CD4 in the activation of T lymphocytes has been suggested by reports that anti-CD4 Abs abrogate a variety of T lymphocyte effector functions in vitro, including IL-2R γ-chain expression and IL-2-induced lymphocyte proliferation, and graft rejection (39). CD4 participates in signal transduction by its cytoplasmic domain through association with the src-like protein tyrosine kinase lck in T lymphocytes (39), and such signals may lead to down-regulation of TCR as well as cytokine (IL-2) receptors. Our data suggest that signals emanating from CD4 culminate in down-regulation of chemotactic receptors in monocytes. We have also observed in another study that down-regulation of chemokine receptor CXCR4 by gp120 occurred in resting CD4+ T lymphocytes, which involves active participation of CD4. In CD4+, but not CD8+, T cells, gp120 induced rapid tyrosine phosphorylation of the src-like kinase p56k in T lymphocytes (39), and such signals may lead to down-regulation of CXCR4 expression and function (S. S. Su, G. C. Grimm, J. J. Oppenheim, and J. M. Wang, manuscript in preparation). Pretreatment of the CD4 T cells with tyrosine kinase inhibitor herbimycin A, but not staurosporine, completely restored the ability of CD4 T cells to express functional CXCR4. These
observations suggest that although diverse signaling events are triggered by gp120 through CD4 in T cells and monocytes, each of them results in the down-regulation of chemokine receptors.

In our study, natural and recombinant gp120 from T cell tropic strains MN, IIIB, and RF as well as the recombinant monocyte tropic CM strain all exhibited similar effects on monocytes, which differs from the restricted tropism exhibited during the viral fusion process. This could be due to the fact that the CD4 binding region is conserved in gp120 from primary isolates (40) and T cell line-adapted viruses, thus enabling gp120 from different HIV-1 strains to bind and activate monocyte CD4, with consequent down-regulation of chemokine receptor expression. Further efforts are being made in our laboratory to identify critical epitopes responsible for CD4 binding, and possibly signaling, on both mono- and T tropic viral envelope proteins. A 20-amino acid sequence of gp120 from the MN strain corresponding to the putative CD4 binding epitope has been found to be a potent chemostimulant for monocytes and to down-regulate chemokine receptor expression on the cells. The activity of this peptide was abolished by soluble CD4 (H. Ueda and J. M. Wang, unpublished observation), which provides additional evidence that CD4 on monocytes is capable of activating signaling pathways negatively affecting the cell response to other proinflammatory stimulants such as chemokines.

The availability of gp120 in AIDS patients has been documented in a number of reports. In the course of the HIV-1 infection, some of the gp120 detected in the sera of patients may, in fact, be complexed by anti-gp120. However, it is unlikely that the effects of gp120 are completely blocked by the Abs because there are considerable variations in the specificity, amount, and timing of anti-gp120 Ab production (41–43). In most patients, serum anti-gp120 Abs appear relatively late and were first detected only after p24 Ag and infectious virus titer in the peripheral blood had declined manyfold from their highest values (41). Many of the anti-gp120 Abs do not actually have the capacity to recognize the CD4 binding structure on gp120. This is manifested by the failure of these Abs to compete for gp120 binding to CD4 with a neutralizing anti-gp120 human mAb that does recognize the conformational CD4-binding structure on gp120 (43). Such neutralizing Ab appeared later in the patients’ sera than other anti-gp120 Abs that do not block the CD4 binding structure on gp120 (42). These observations suggest that there could be an ample opportunity for the virus-associated or “shed” gp120 (21) to interact with CD4 on immune cells. Furthermore, the binding of gp120 to CD4 is of high affinity, which is comparable to or even greater than the Ab affinities (44). In fact, gp120 that is not associated with viral particles can be detected on the surface of explanted CD4 T cells from patients (45). These observations strongly argue that gp120 can have biologic effects on the cells of infected individuals.

Our findings suggest that gp120 can have a number of intriguing effects on the host that require further evaluation. On the one hand, gp120 may recruit and activate monocytes during the course of viral infection. This activation of monocytes by gp120 results in the internalization of a number of chemoa ATTRACTANT receptors, including HIV-1 fusion coreceptors, which may contribute to the reported viral interference following initial infection (40) as well as to the suppression of monocyte-dependent inflammatory reactions. The inhibitory effects of gp120 on chemokine and other chemoa ATTRACTANT receptors can be exploited to develop anti-inflammatory agents that interfere with monocyte recruitment based on the structure and mechanism of gp120 action.

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References


