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Induction of HIV-1 Replication by Allogeneic Stimulation

Hiroyuki Moriuchi,1,2 Masako Moriuchi,1 and Anthony S. Fauci

Allogeneic stimulation presents an immunologic challenge during pregnancy, blood transfusions, and transplantations, and has been associated with reactivation of latent infected virus such as CMV. Since HIV-1 is transmitted vertically, sexually, or via contaminated blood, we have tested the effects of allogeneic stimulation on HIV-1 infection. 1) We show that allogeneically stimulated lymphocytes are highly susceptible to acute infection with T cell-tropic or dual-tropic HIV-1. 2) We show that allogeneic stimulation has dichotomous effects on replication of macrophage-tropic HIV-1; it activates HIV expression in already infected cells but inhibits HIV entry by secreting HIV-suppressive CC chemokines. 3) We show that allogeneic stimulation of latently infected, resting CD4+ T cells induced replication of HIV-1 in these cells. These observations suggest that allogeneic stimulation may play a role in the transmission, replication, and phenotypic transition of HIV-1. The Journal of Immunology, 1999, 162: 7543–7548.

Disparity between MHC Ags elicits anti-allo-MHC immune responses. In contrast to conventional antigenic stimulation, which requires priming immunization to elicit potent immune responses, allogeneic responses can be quite robust with no previous exposure (1, 2). Allogeneic stimulation also presents an immunologic challenge during pregnancy, blood transfusion, and transplantation and has been associated with reactivation of latent human CMV (3), a herpesvirus that is transmitted vertically, sexually, or through blood transfusion or transplantation (4). Thus, reactivation of latent virus by allogeneic stimulation may be a useful strategy for viruses including HIV, which is transmitted vertically, sexually, or via contaminated blood.

Previous studies have demonstrated potentially important relationships between MHC and the pathogenesis of HIV disease. First, studies using SIV infection of rhesus macaques as a model for HIV infection showed that serologic responses to class I MHC molecules can confer sterilizing immunity against challenge with SIV in some or all immunized animals (5–9); passive transfer experiments demonstrated that the protection is mediated, at least in part, by anti-allo-MHC Abs (10). Second, it has been shown that HIV virions acquire host cell proteins including class I and class II MHC Ags as they bud from the cell membrane and that the integration of MHC molecules in the viral envelope increases infectivity of HIV virions, while anti-allo-MHC Abs can block the infectivity of the virus (Ref. 11; reviewed in Ref. 12). Furthermore, women who were alloimmunized as a therapy for unexplained recurrent spontaneous abortions developed anti-allo-MHC Abs that were able to neutralize HIV-1 in vitro (13), and women who remained uninfected despite obvious repeated exposure to HIV expressed rare HLA types for their geographic location (14). These studies indicate that alloimmunized individuals may be able to protect themselves from HIV infection by immune responses against alloantigens expressed on HIV-1 virions or HIV-infected cells.

In this study, we investigate the effects of allogeneic stimulation on replication of HIV in vitro. We show that allogeneic stimulation renders PBMC highly susceptible to acute infection with T cell (T)-tropic or dual-tropic HIV-1, while it has dichotomous effect on macrophage (M)-tropic HIV-1 infection; it activates HIV-1 expression in already infected cells but inhibits M-tropic HIV-1 entry by secreting CC chemokines. We also show that in vitro allogeneic stimulation induces replication of HIV in CD4+ T cells from HIV-infected individuals. Furthermore, allogeneic stimulation induced reactivation of HIV in latently infected, resting CD4+ T cells derived from patients who had been treated with highly active antiretroviral therapy (HAART) and in whom plasma viremia was below the level of detectability. Our data, in addition to the previous studies described above, indicate that allogeneic stimulation may play a role in the pathogenesis of HIV disease by influencing viral transmission, replication, and phenotypic transition.

Materials and Methods

Establishment of allogeneically stimulated PBMC cultures and acute HIV infection

PBMCs were isolated from HIV-seronegative healthy donors, as described previously (15). Unfractionated PBMCs were resuspended in RPMI 1640 supplemented with 10% heat-inactivated FBS (Life Technologies, Gaithersburg, MD) at a cell concentration of 2 × 10^6 cells/ml. For allogeneic stimulation cultures, equal numbers of cells from two different donors were mixed at the same cell concentration. MHC disparity between donors was confirmed by HLA typing, which was kindly performed by the HLA laboratory of the Department of Transfusion Medicine (Warren Grant Magnuson Clinical Center, National Institutes of Health). After 4 days of culture at 37°C in 5% CO2, a fraction of the cultures was depleted of CD8+ cells by immunomagnetic beads (Dynal, Lake Success, NY), as described previously (15). Unfractionated or CD8-depleted PBMC cultures were infected with either the T-tropic laboratory strain HIV-1 IIIB, the M-tropic laboratory strain HIV-1 Ba-L, or a dual-tropic primary HIV-1 isolate AU at an approximate multiplicity of infection of 0.05. Viral replication was measured by RT activity in the cell-free culture supernatants.

Single round virus replication assays and fusion assays

Single round virus replication assays and fusion assays were performed as described previously (16). Where indicated, neutralizing Abs to RANTES,

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3 Abbreviations used in this paper: T, T cell; M, macrophage; HAART, highly active antiretroviral therapy; MIP, macrophage inflammatory protein; MMC, mitomycin C; CXCR4, CXC chemokine receptor 4; AMV, amphotropic murine leukemia virus.

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that all subpopulations of PBMCs analyzed, such as CD4+ activated PBMCs (Table I). Double staining of PBMCs demonstrated midine, also confirmed that allogeneic stimulation potently activates CD4+ T cell subpopulations by FACS. The percentages of CD4+, CD19, and CD14+ subsets in the culture were 78, 15, and 4%, respectively. The mean fluorescence intensity (MFI) of CCR5 or CXCR4 staining is shown in parentheses. MFI for isotype control was 12 and 11, respectively. [3 H]Thymidine incorporation by 10^6 PBMCs was also measured. Results are representative of five independent experiments.

Isolation of resting CD4+ T cells from HIV-infected patients and allostimulation

Resting CD4+ T cells were isolated from six HIV-infected patients as described previously (17). Purity of resting CD4+ T cells, as determined by staining with anti-CD4, anti-CD9, and anti-HLA-DR Abs, was >99%. All the patients had been on HAART and had fewer than 500 HIV-1 RNA copies per milliliter of plasma, as determined by branched DNA assays (Chiron, Emeryville, CA). For allogeneic stimulation experiments, unfractionated PBMCs were isolated from HIV-uninfected donors, treated with mitomycin C (MMC) (0.25 mg/ml; Sigma, St. Louis, MO) for 30 min to render them incapable of propagation and infection, and cocultured with equal numbers of resting CD4+ T cells from HIV-infected patients at a cell concentration of 2 x 10^6 cells/ml. Release of HIV was monitored by p24 Ag ELISA using commercially available kits (Coulter, Miami, FL).

Flow cytometric analyses and [3 H]thymidine incorporation

Cell surface expression of CD4, HIV coreceptors (CCR5 and CXCR4), and cellular activation markers (CD69, HLA-DR) was analyzed in unstimulated and allostimulated PBMCs by FACS (FACScan, Becton Dickinson Immunocytometry Systems, San Jose, CA). PE-conjugated mAbs to CD4, CD8, and CD14; FITC-conjugated mAbs to CCR5 and CXCR4 were purchased from PharMingen (San Diego, CA). PE-conjugated mAbs to CD4, CD8, and CD14; FITC-conjugated mAbs to CD4, CD69, CD25, and HLA-DR; and isotype controls were obtained from Becton Dickinson Immunocytometry Systems.

[3 H]Thymidine incorporation was measured in unstimulated and allostimulated PBMCs.

Results

Allogeneically stimulated PBMCs are highly susceptible to acute infection with T- or Dual-Tropic HIV-1

The replication of HIV-1 is closely associated with the state of cellular activation of susceptible cell populations (reviewed in Ref. 18). Allogeneic stimulation has been shown to elicit robust immune responses without priming (1, 2). In this regard, we first examined the expression of cell surface activation markers on unstimulated and allostimulated PBMCs. While unstimulated PBMCs expressed barely measurable CD69, CD25, and HLA-DR on their surfaces, allostimulated PBMCs expressed a variable degree of cellular activation markers (Table I; data not shown). Cellular proliferation, judged by incorporation of DNA precursor [3 H]thymidine, also confirmed that allogeneic stimulation potently activated PBMCs (Table I). Double staining of PBMCs demonstrated that all subpopulations of PBMCs analyzed, such as CD4+ T cells (HIV targets), CD8+ T cells (effectors), and monocytes (HIV targets), were similarly activated (Table I; data not shown). HLA disparity between the donors was confirmed by HLA typing (data not shown). In the experiment shown in Table I, CCR5 expression was moderately down-regulated in allostimulated cells as compared with unstimulated cells; however, this was not a consistent finding in other experiments (data not shown).

Given the fact that allogeneic stimulation potently activates both HIV target and effector subpopulations, we next investigated the susceptibility of allostimulated PBMC to acute HIV-1 infection. Both

<table>
<thead>
<tr>
<th>% CD4+/CD69+</th>
<th>% CD4+/HLA-DR+</th>
<th>% CD4+/CCR5+</th>
<th>% CD4+/CXCR4+</th>
<th>[3 H]Thymidine Incorporation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Donor 1</td>
<td>0.70</td>
<td>1.70</td>
<td>8.54 [16]</td>
<td>69.75 [57] 233 cpm</td>
</tr>
<tr>
<td>Donor 2</td>
<td>0.66</td>
<td>2.25</td>
<td>2.54 [14]</td>
<td>76.39 [61] 145</td>
</tr>
<tr>
<td>Donors 1 + 2</td>
<td>8.00</td>
<td>9.37</td>
<td>1.67 [13]</td>
<td>89.07 [64] 7980</td>
</tr>
</tbody>
</table>

* Unfractionated PBMCs from two donors were unstimulated (donor 1 and donor 2) or allostimulated with each other (donors 1 + 2) for 4 days, and expression of cellular activation markers (CD69, HLA-DR) and HIV-1 coreceptors (CCR5, CXCR4) were determined for CD4+ T cell subpopulation by FACS. The percentages of CD4+, CD19+, and CD14+ subsets in the culture were 78, 15, and 4%, respectively. The mean fluorescence intensity (MFI) of CCR5 or CXCR4 staining is shown in parentheses. MFI for isotype control was 12 and 11, respectively. [3 H]Thymidine incorporation by 10^6 PBMCs was also measured. Results are representative of five independent experiments.
PBMCs were infected with replication-incompetent luciferase reporter virus NL4–3-luc-R.

**FIGURE 2.** Allostimulation-induced CC chemokines inhibit M-tropic HIV-1 infection. A, Single round virus replication assays. Either unstimulated (donor 1 and donor 2 in Fig. 1) or allostimulated (donors 1 + 2 in Fig. 1) PBMCs were infected with replication-incompetent luciferase reporter virus NL4–3-luc-R. E– (21), which had been pseudotyped by the indicated Env, and luciferase activity in the infected cell lysates was assayed 3 days after infection. The culture supernatants were either depleted before infection (sup (−)) or maintained at 1:1 ratio throughout infection (sup (+)). Results were representative of seven independent experiments. B, Fusion assays. Either unstimulated (donor 1 and donor 2 in Fig. 1) or allostimulated (donors 1 + 2 in Fig. 1) PBMCs were infected with replication-incompetent luciferase reporter virus NL4–3-luc-R. E– (21), while BSC-1 cells were infected with vCB21R (encoding the lacZ gene under the control of the T7 promoter (22)) as well as a vaccinia vector expressing the indicated Env (22). After overnight incubation, the two sets of cells were mixed, and β-galactosidase activity was assayed for the infected cell lysates 4 h after mixing. Where indicated, PBMCs were incubated with their own conditioned medium 30 min before and during mixed cultures. Fusion index indicates /galactosidase activity of test samples relative to that of control sample in which supernatants were maintained throughout the 3 day period of infection, M-tropic virus infection was significantly suppressed (Fig. 2A).

To further investigate early events of virus infection, fusion assays were employed. While allostimulated cells themselves were more fusogenic with both M- and T-tropic HIV-1 Envs than were unstimulated cells, the supernatants from allostimulated cultures inhibited cell-cell fusion mediated by M-tropic Env (Fig. 2B). These results indicate that allostimulation provides a more favorable environment for T-tropic and dual-tropic HIV-1 strains, which are prevalent in late stages of HIV disease in certain patients (19, 20), than for M-tropic HIV-1.

**Table II. CC chemokine production by allostimulated PBMCs**

<table>
<thead>
<tr>
<th></th>
<th>RANTES</th>
<th>MIP-1α</th>
<th>MIP-1β</th>
</tr>
</thead>
<tbody>
<tr>
<td>Donor 1</td>
<td>280</td>
<td>990</td>
<td>1240</td>
</tr>
<tr>
<td>Donor 2</td>
<td>31.3</td>
<td>42.9</td>
<td>&lt;31.3</td>
</tr>
<tr>
<td>Donors 1 + 2</td>
<td>31.3</td>
<td>31.3</td>
<td>31.3</td>
</tr>
<tr>
<td>Donor 3</td>
<td>31.3</td>
<td>31.3</td>
<td>31.3</td>
</tr>
<tr>
<td>Donor 4</td>
<td>31.3</td>
<td>31.3</td>
<td>31.3</td>
</tr>
<tr>
<td>Donors 1 + 2</td>
<td>280</td>
<td>990</td>
<td>1240</td>
</tr>
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</table>

Unfractionated PBSs from two pairs of healthy donors were unstimulated (donors 1, 2, 3, and 4) or allostimulated with each other (donors 1 + 2 and 3 + 4) for 4 days, and the concentration of the indicated chemokines in the culture supernatants was determined by ELISAs. Results are representative of three independent experiments.

unfractionated and CD8-depleted PBMCs were infected with either T-tropic (IIIB), M-tropic (Ba-L), or dual-tropic (AU) HIV-1, and viral replication was monitored by RT activity in the infected culture supernatants. Allostimulated PBMCs became highly susceptible to T-tropic and dual-tropic HIV-1 (Fig. 1A). Effects on M-tropic HIV-1 were variable: replication of M-tropic HIV-1 was suppressed in some donor pairs, but enhanced in other donor pairs (Fig. 1A; data not shown). Depletion of CD8-positive cells modestly increased susceptibility of PBMCs to infection with all HIV-1 strains tested (Fig. 1B). These results indicate that allostimulation provides a more favorable environment for T-tropic and dual-tropic HIV-1 strains, which are prevalent in late stages of HIV disease in certain patients (19, 20), than for M-tropic HIV-1.

**Allostimulation induces expression of CC chemokines, which suppress M-tropic HIV-1 infection**

To delineate the mechanisms whereby allostimulation modulates HIV-1 infection, single round virus replication assays were performed. Infectability of allostimulated PBMCs was enhanced for all viruses tested when the allogeneic culture supernatants were removed before infection; however, when the allogeneic culture supernatants were maintained throughout the 3 day period of infection, M-tropic virus infection was significantly suppressed (Fig. 2A).

To further investigate early events of virus infection, fusion assays were employed. While allostimulated cells themselves were more fusogenic with both M- and T-tropic HIV-1 Envs than were unstimulated cells, the supernatants from allostimulated cultures inhibited cell-cell fusion mediated by M-tropic Env (Fig. 2B). These results indicate that allostimulated PBMCs secrete a soluble factor(s) that inhibits M-tropic HIV-1 infection at the level of fusion/entry.

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Peak p24 titers are shown. Cultures with resting CD4<sup>+</sup> T cells expressed low levels of luciferase activity; however, when cultured with CD4<sup>+</sup> T cells from a different donor, luciferase expression from the HIV-1 long terminal repeat was markedly increased (Fig. 3A). These results indicate that allostimulation is able to induce HIV expression in already infected cells.

To confirm that allogeneic stimulation can induce replication of HIV-1 in endogenously infected CD4<sup>+</sup> T cells in patients, we isolated resting CD4<sup>+</sup> T cells from HIV-infected individuals who had been treated with HAART and in whom plasma viremia was undetectable using branched DNA assays, and cocultured these cells with MMC-treated PBMC obtained from HIV-uninfected individuals. Allostimulated cultures released HIV-1 as determined by p24 Ag in 4 of 6 patients, while p24 Ag was undetectable in control cultures with resting CD4<sup>+</sup> T cells from all HIV-infected patients tested (Fig. 3B). Recovered virus was replication competent when passaged into PHA blasts (data not shown). Since MMC treatment renders the cells incapable of supporting HIV-1 infection (Ref. 23; data not shown), the detected virus most probably represents ex vivo reactivation of latent HIV-1 in vivo-infected cells, not spread of infection to allogeneic cells in vitro. Thus, allogeneic stimulation appears to be a potent inducer of HIV-1 replication.

**Discussion**

Activation of the immune system is critical for the initiation and propagation of HIV-1 replication (reviewed in Ref. 24). Common sources of immune system activation include stimulation with a variety of Ags. Paramount among these Ags are a wide range of pathogenic microbes (24). In addition, a number of studies have demonstrated that immunization with common recall Ags results in a transient increase in plasma viremia in HIV-infected individuals (24–28); furthermore, PBMC isolated from uninfected, recently vaccinated individuals become highly susceptible to acute HIV infection in vitro (26). These studies suggest that induction or enhancement of HIV-1 replication in vivo by immune stimulation from exogenous sources may play an important role in the pathogenesis of HIV disease.

Allogeneic stimulation can induce replication of HIV-1 in endogenously infected CD4<sup>+</sup> T cells in patients, we isolated resting CD4<sup>+</sup> T cells from HIV-infected individuals who had been treated with HAART and in whom plasma viremia was undetectable using branched DNA assays, and cocultured these cells with MMC-treated PBMC obtained from HIV-uninfected individuals. Allostimulated cultures released HIV-1 as determined by p24 Ag in 4 of 6 patients, while p24 Ag was undetectable in control cultures with resting CD4<sup>+</sup> T cells from all HIV-infected patients tested (Fig. 3B). Recovered virus was replication competent when passaged into PHA blasts (data not shown). Since MMC treatment renders the cells incapable of supporting HIV-1 infection (Ref. 23; data not shown), the detected virus most probably represents ex vivo reactivation of latent HIV-1 in vivo-infected cells, not spread of infection to allogeneic cells in vitro. Thus, allogeneic stimulation appears to be a potent inducer of HIV-1 replication.
The effects of allostimulation on HIV-1 infection, however, may be more complex. Previous studies suggested that replication of HIV-1 can be increased after transfusion of blood products (23, 29) or allogeneic bone marrow transplantation (30). A recent study reported that pregnancy increases the risk of disease progression for HIV-infected women (31). These studies suggest that allogeneic stimulation can induce replication of HIV-1.

However, a number of studies suggest that allogeneic stimulation may actually elicit protection from HIV-1/SIV infection (5–9, 13, 14). The mechanism of protection was not precisely elucidated, but cellular or humoral immunity against allo-MHC Ags expressed on HIV-1 virions or on HIV-1-infected cells appears to contribute to protection from HIV-1 infection (10–12). Recently, CD8+ T cell-derived soluble factors, which include but are not limited to CC chemokines, RANTES, MIP-1α, and MIP-1β, have been associated with the prevention of SIV infection in macaques by xenogeneic immunization (32) or with anti-HIV activity induced by alloantigenic stimulation (33). Thus, allostimulation could have dichotomous effects on HIV-1 infection; it may induce replication of HIV-1 through cellular activation, while it may also confer a degree of protection from HIV-1 infection through anti-allo-MHC immune responses or the generation of soluble anti-HIV activity.

In the present study we have demonstrated that cellular activation associated with allogeneic stimulation enhances in vitro replication of HIV-1. Allostimulation also induces secretion of CC chemokines, RANTES, MIP-1α, and MIP-1β, which inhibit entry of M-tropic HIV-1 by competing for the CCR5 coreceptor (reviewed in Ref. 34). We have also shown that, in the allogeneic system, depletion of CD8+ T cells modestly increased replication of all HIV-1 stains tested and that neutralization of HIV suppressive CC chemokines markedly enhanced the replication of M-tropic HIV-1, but not of T- or dual-tropic HIV-1 in both un-fractionated and CD8-depleted PBMCs. These results indicate that allostimulation-induced anti-HIV activity is mediated by both CC chemokines and non-CC chemokine factors, and that the net effect of allostimulation on HIV-1 infection is a balance of positive (cellular activation) and negative (induction of HIV-suppressive factors) effects. Differences in the degree and duration of allostimulation may confer different levels and types of HIV suppressor activity, as demonstrated by Pinto et al., who have shown that multiple rounds of allostimulation induced anti-HIV activity against both M- and T-tropic HIV-1 (33). However, since allostimulation-induced CC chemokines inhibit only M-tropic HIV-1 and can enhance replication of T-tropic HIV-1 (35–38), allostimulation would provide a more favorable environment for T-tropic HIV-1 than for M-tropic HIV-1. This effect could lead to a transition from M- to T-tropic phenotype, which is indicative of disease progression in certain patients (19, 20).

In summary, the present study indicates that allostimulation has variable effects on HIV-1 infection; however, the balance of effects favors replication of HIV-1, particularly T- or dual-tropic viruses. Therefore, allostimulation may play a role in transmission, replication, and phenotypic transition of HIV-1.

Acknowledgments

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References


