The Journal of Immunology

Induction of HIV-1 Replication by Allogeneic Stimulation

Hiroyuki Moriuchi, Masako Moriuchi and Anthony S. Fauci

J Immunol 1999; 162:7543-7548; ;
http://www.jimmunol.org/content/162/12/7543

The Journal of Immunology is published twice each month by
The American Association of Immunologists, Inc.,
1451 Rockville Pike, Suite 650, Rockville, MD 20852

Print ISSN: 0022-1767 Online ISSN: 1550-6606.

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 latently infected virus such as CMV. Since HIV-1 is transmitted vertically, sexually, or via contaminated blood, we have tested the effects of allostimulation on HIV-1 infection. 1) We show that allostimulated lymphocytes are highly susceptible to acute infection with T cell-tropic or dual-tropic HIV-1. 2) We show that allostimulation 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 allostimulation 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 serological 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)[3]-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 allostimulation 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 allostimulation 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% CO_2, 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,

Laboratory of Immunoregulation, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892

Received for publication December 16, 1998. Accepted for publication March 26, 1999.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] H. M. and M. M. contributed equally to this project.

[2] Address correspondence and reprint requests to Dr. Hiroyuki Moriuchi, Department of Pediatrics, Nagasaki University School of Medicine, 1-7-1 Sakamoto, Nagasaki 852-8501, Japan. E-mail address: hiromori@net.nagasaki-u.ac.jp

[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.

0022-1767/99/$02.00

Table I. *Cellular activation and proliferation of allostimulated PBMCs*[a]

	% $CD4^+/CD69^+$	% $CD4^+/HLA\text{-}DR^+$	% $CD4^+/CCR5^+$	% $CD4^+/CXCR4^+$	[^{3}H]Thymidine Incorporation
Donor 1	0.70	1.70	8.54 [16]	69.75 [57]	233 cpm
Donor 2	0.66	2.25	2.54 [14]	76.39 [61]	145
Donors 1 + 2	8.00	9.37	1.67 [13]	89.07 [64]	7980

[a] 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 $CD3^+$, $CD19^+$, 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 10^4 PBMCs was also measured. Results are representative of five independent experiments.

macrophage inflammatory protein (MIP)-1α and MIP-1β (R&D Systems, Cambridge, MA), or control Ig were added to the cultures.

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-CD69, 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 2×10^6 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, CD25, and HLA-DR) was analyzed in unstimulated and allostimulated PBMCs by FACS (FACScan, Becton Dickinson Immunocytometry Systems, San Jose, CA). PE-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 allostimulation potently activates both HIV target and effector subpopulations, we next investigated the susceptibility of allostimulated PBMC to acute HIV-1 infection. Both

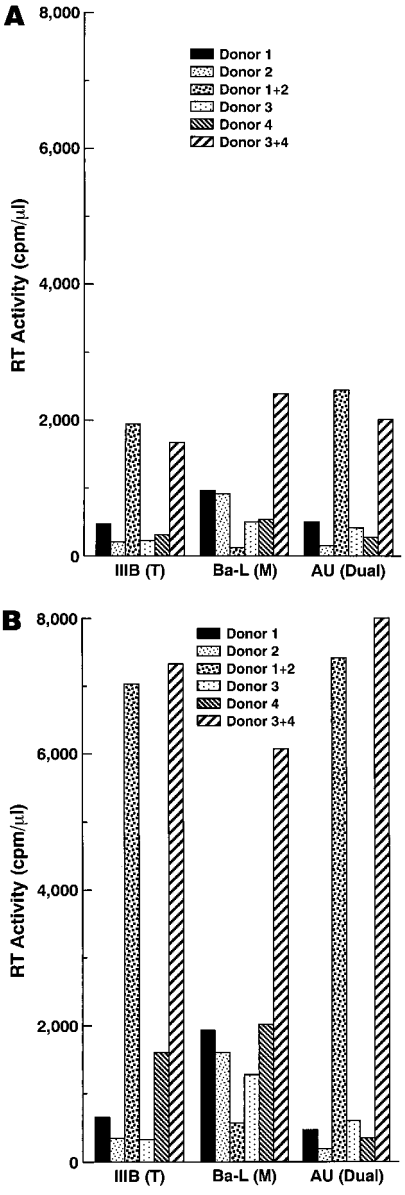

FIGURE 1. Allostimulation has dichotomous effects on acute HIV-1 infection, depending on viral phenotypes. Unfractionated PBMCs from 2 pairs of healthy donors (donors 1 through 4 in Table II) were incubated separately or allostimulated with each other for 4 days. A fraction of PBMCs was depleted of $CD8^+$ cells. Either unfractionated PBMCs (*A*) or CD8-depleted PBMCs (*B*) were infected with the indicated strains of HIV-1. Peak RT titers on day 8 postinfection are shown. Results are representative of eight independent experiments.

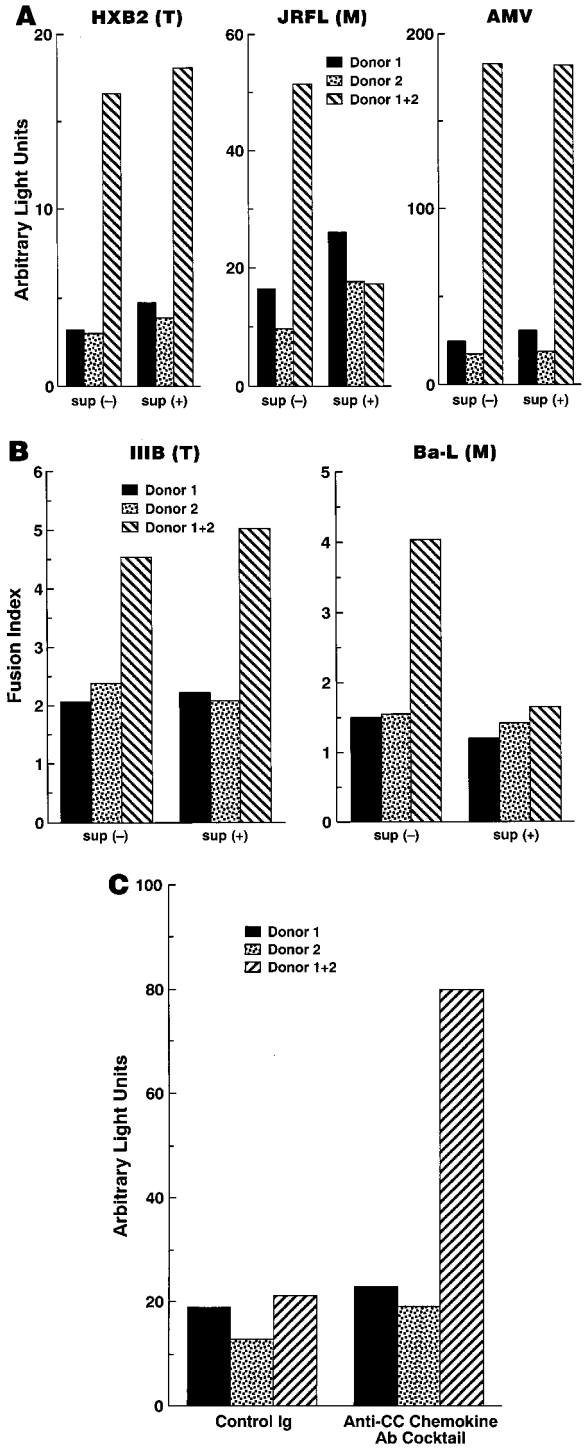

FIGURE 2. Allostimulation-induced CC chemokines inhibit M-tropic HIV-1 infection. *A*, Single round virus replication assays. Either unstimulated (donor 1 and donor 2 in Fig. 1) or allostimulated (donors 1 + 2 in Fig. 1) PBMCs were infected with replication-incompetent luciferase reporter virus NL4–3-luc-R$^-$E$^-$ (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 vTF7–3 (expressing the T7 polymerase (22)), 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 β-galactosidase activity of test samples relative to that of control sample in which wild-type vaccinia vector in place of Env-expressing vector was infected. Results are representative of four independent experiments. *C*, Effects of neutralizing Abs to CC chemokines. The culture supernatants were incubated with either a mixture of neutralizing Abs to CC chemokines (10 μg monoclonal anti-RANTES Ab, 20 μg polyclonal anti-MIP-1α Ab, and 20 μg monoclonal anti-MIP-1β Ab per milliliter of supernatants) or control Igs. Unfractionated or CD8-depleted PBMCs were preincubated with the above supernatants for 2 h and infected with NL4–3-luc-R$^-$E$^-$ that had been pseudotyped by M-tropic HIV-1 JRFL Env. Results are representative of three independent experiments using unfractionated PBMCs. Similar results were obtained for CD8-depleted PBMCs (data not shown).

Table II. *CC chemokine production by allostimulated PBMCs*[a]

	RANTES	MIP-1α	MIP-1β
Donor 1	<31.3	42.9	<31.3pg/ml
Donor 2	<31.3	<31.3	<31.3
Donors 1 + 2	660	2320	1980
Donor 3	<31.3	<31.3	<31.3
Donor 4	<31.3	<31.3	<31.3
Donors 3 + 4	280	990	1240

[a] Unfractionated PBMCs 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. 1*A*). 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. 1*A*; data not shown). Depletion of CD8-positive cells modestly increased susceptibility of PBMCs to infection with all HIV-1 strains tested (Fig. 1*B*). 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. 2*A*).

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. 2*B*). These results indicate that allostimulated PBMCs secrete a soluble factor(s) that inhibits M-tropic HIV-1 infection at the level of fusion/entry.

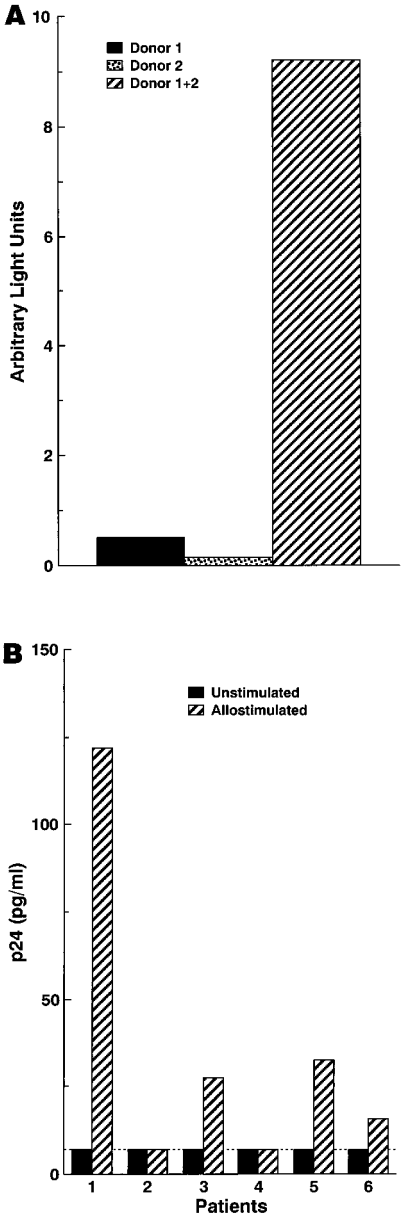

FIGURE 3. Allostimulation induces replication of HIV-1 in latently infected cells. *A*, Freshly isolated, unstimulated PBMCs from two healthy donors were infected with NL4–3-luc-R^-E^- that had been complemented with Env from AMV. A fraction of the infected cells was mixed for allostimulation on day 2 postinfection. Three days later, $CD4^+$ T cells were isolated with immunomagnetic beads (Dynal), and luciferase activity in the infected $CD4^+$ T cell lysates was determined. Results were representative of five independent experiments. *B*, Resting $CD4^+$ T cells were isolated from six HIV-1-infected individuals and either unstimulated or allostimulated by coculturing with unfractionated PBMCs obtained from healthy donors that had been treated with MMC. The culture supernatants were collected for p24 Ag on days 4, 8, 12, and 16 after allostimulation, and peak p24 titers are shown.

Since it has been demonstrated that chemokine ligands for CCR5, a coreceptor for M-tropic HIV-1, inhibit entry of M-tropic HIV-1 into cells and since allostimulated PBMCs secrete a copious amount of CC chemokines (Table II), it is very likely that CC chemokines secreted by these cells suppress M-tropic HIV-1 infection. To test this hypothesis, neutralizing Abs to these CC chemokines were added to the culture supernatants in single round virus replication assays of M-tropic HIV-1 infection. As shown in Fig. 2*C*, the inhibitory effect of the supernatants was abrogated by neutralizing CC chemokines. Taken together, these results indicate that allostimulated PBMCs render themselves relatively refractory to M-tropic HIV-1 by secreting CC chemokines that are suppressive to M-tropic HIV-1.

Allostimulation of latently infected, resting $CD4^+$ T cells induces replication of HIV-1 in these cells

We next examined whether allostimulation can induce HIV expression from already infected cells. First, we tested the effects of allostimulation on HIV expression in an in vitro system in which $CD4^+$ T cells derived from HIV-uninfected individuals were infected with replication-incompetent luciferase reporter virus NL4–3luc-R^-E^- which had been complemented with amphotropic murine leukemia virus (AMV) Env (21). When unstimulated, the infected $CD4^+$ T cells expressed low levels of luciferase activity; however, when cultured with $CD4^+$ T cells from a different donor, luciferase expression from the HIV-1 long terminal repeat was markedly increased (Fig. 3*A*). 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 $CD4^+$ T cells in patients, we isolated resting $CD4^+$ 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 $CD4^+$ T cells from all HIV-infected patients tested (Fig. 3*B*). 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 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.

Alloantigens elicit robust immune responses without a requirement of previous exposure (1, 2). Allogeneic stimulation can occur through sexual contacts, pregnancy, lactation, transfusion, or transplantation. Since several viruses including HIV-1 are transmitted sexually, vertically, or via contaminated blood products or organs, it is possible that allogeneic stimulation may play a role in viral transmission or replication. In this regard, a recent study demonstrated that allogeneic stimulation can induce reactivation of latent human infection of macrophages with CMV (3), a ubiquitous pathogen that is the major cause of morbidity and mortality in transplant patients, as well as a leading cause of congenital birth defects (4).

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 unfractionated 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

We thank the Department of Transfusion Medicine (Warren Grant Magnuson Clinical Center, National Institutes of Health) for blood samples and HLA typing, L. A. Ehler and S. Mizell for recruiting HIV-infected patients, N. Landau and E. A. Berger for reagents, T.-W. Chun for helpful discussions, and John Weddle for graphic work.

References

1. Clerici, M., L. DePalma, E. Roilides, R. Baker, and G. M. Shearer. 1993. Analysis of T-helper and antigen-presenting functions in cord blood and peripheral blood leukocytes from healthy children of different ages. *J. Clin. Invest. 91:2829.*
2. Risdaon, G., J. Gaddy, and H. E. Broxmeyer. 1994. Allogeneic responses in human cord blood. *Blood Cells 20:566.*
3. Soderberg-Naucler, C., K. N. Fish, and J. A. Nelson. 1997. Reactivation of latent human cytomegalovirus by allogeneic stimulation of blood cells from healthy donors. *Cell 91:119.*
4. Britt, W. J., and C. A. Alford. 1996. Cytomegalovirus. In *Fields Virology*. B. N. Fields, D. M. Knipe, and P. M. Howley, eds. (Lippincott-Raven Publishers, Philadelphia, pp. 2493–2523.
5. Scott, E. J. 1991. Anti-cell antibody in macaques. *Nature 353:393.*
6. Chan, W. L., A. Rodgers, R. D. Hancock, F. Taffs, P. Kitchin, G. Farrar, and F. Y. Liew. 1992. Protection in simian immunodeficiency virus-vaccinated monkeys correlates with anti-HLA class I antibody responses. *J. Exp. Med. 176:1203.*
7. Langlois, A. J., K. J. Weinhold, T. J. Matthews, M. L. Greenberg, and D. P. Bolognesi. 1992. Detection of anti-human cell antibodies in sera from macaques immunized with whole-inactivated virus. *AIDS Res. Hum. Retroviruses 8:1641.*
8. Arthus, L. O., J. W. Bess, Jr., R. G. Urban, J. L. Strominger, W. R. Morton, D. L. Mann, L. E. Henderson, and R. E. Benveniste. 1995. Macaques immunized with HLA-DR are protected from challenge with simian immunodeficiency virus. *J. Virol. 69:3117.*
9. Chan, W. L., A. Rodgers, C. Grief, N. Almond, S. Ellis, B. Flanagan, P. Silvera, J. Bootman, J. Scott, K. Kent, and R. Bomford. 1995. Immunization with class I human histocompatibility leukocyte antigen can protect macaques against challenge infection with SIVmac-32H. *AIDS 9:223.*
10. Gardner, M., A. Rosenthal, M. Jennings, J. Yee, L. Antipa, and E. Robinson, Jr. 1995. Passive immunization of rhesus macaques against SIV infection and disease. *AIDS Res. Hum. Retroviruses 11:843.*
11. Arthur, L. O., J. W. Bess, Jr., R. C. Sowder, II, R. E. Benveniste, D. L. Mann, J. C. Chermann, and L. E. Henderson. 1992. Cellular proteins bound to immunodeficiency viruses: implications for pathogenesis and vaccines. *Science 258: 1935.*
12. Tremblay, M. J., J.-F. Fortin, and R. Cantin. 1998. The acquisition of host-encoded proteins by nascent HIV-1. *Immunol. Today 19:346.*
13. Kiprov, D. D., H. W. Sheppard, and C. V. Hanson. 1994. Alloimmunization to prevent AIDS. *Science 263:161.*
14. Plummer, F. A., K. Fowke, N. J. D. Nagelkerke, J. N. Simonsen, J. Brayo, and E. Ngugi. 1993. Evidence of resistance to HIV among continuously exposed prostitutes in Nairobi, Kenya [abstract WS-A07–3]. In *Program and Abstracts: IX International Conference on AIDS/HIV STD World Congress (Berlin)*. Welcome Foundation, London.
15. Moriuchi, H., M. Moriuchi, C. Combadiere, P. M. Murphy, and A. S. Fauci. 1996. $CD8^+$ T-cell-derived factor(s), but not β-chemokines, RANTES, MIP-1α, and MIP-1β, suppress HIV-1 replication in monocyte/macrophages. *Proc. Natl. Acad. Sci. USA 93:15341.*
16. Moriuchi, H., M. Moriuchi, J. Arthos, J. Hoxie, and A. S. Fauci. 1997. Promonocytic U937 clones expressing CD4 and CXCR4 are resistant to infection with and cell-to-cell fusion with T-tropic HIV-1. *J. Virol. 71:9664.*
17. Chun, T.-W., D. Engel, M. M. Berrey, T. Shea, L. Corey, and A. S. Fauci. 1998. Early establishment of a pool of latently infected, resting $CD4^+$ T cells during primary HIV-1 infection. *Proc. Natl. Acad. Sci. USA 95:8869.*
18. Pantaleo, G., and A. S. Fauci. 1995. New concepts in the immunopathogenesis of HIV infection. *Annu. Rev. Immunol. 13:487.*
19. Connor, R., K. E. Sheridan, C. Ceradini, S. Choe, and N. R. Landau. 1997. Change in coreceptor use correlates with disease progression. *J. Exp. Med. 185: 621.*
20. Schuitemaker, H., M. Koot, N. A. Kootstra, M. W. Dercksen, R. E. de Goede, R. P. van Steenwijk, J. M. Lange, J. K. Schattenkerk, F. Miedema, and M. Tersmette. 1992. Biological phenotype of human immunodeficiency virus type 1 clones at different stages of infection: progression of disease is associated with a shift from monocytotropic to T-cell-tropic virus populations. *J. Virol. 66:1354.*
21. Deng, H., R. Liu, W. Ellmeier, S. Choe, D. Unutmaz, M. Burkhart, P. Di Marzio, S. Marmon, R. E. Sutton, C. M. Hill, et al. 1996. Identification of a major coreceptor for primary isolates of HIV-1. *Nature 381:661.*
22. Feng, Y., C. C. Broder, P. E. Kennedy, and E. A. Berger. 1996. HIV-1 entry cofactor: functional cDNA cloning of a seven-transmembrane, G protein-coupled receptor. *Science 272:872.*
23. Busch, M. P., T.-H. Lee, and J. Heitman. 1992. Allogeneic leukocytes but not therapeutic blood elements induce reactivation and dissemination of latent human immunodeficiency virus type 1 infection: implications for transfusion support of infected patients. *Blood 80:2128.*
24. Fauci, A. S. 1996. Host factors and the pathogenesis of HIV-induced disease. *Nature 384:529.*
25. Stanley, S. K., M. A. Ostrowski, J. S. Justement, K. Gantt, S. Hedayati, M. Mannix, K. Roche, D. J. Schwartzentruber, C. H. Fox, and A. S. Fauci. 1996. Effect of immunization with a common recall antigen on viral expression in patients infected with human immunodeficiency virus type 1. *N. Engl. J. Med. 334:1222.*
26. Ostrowski, M. A., S. K. Stanley, J. S. Justement, K. Gantt, D. Goletti, and A. S. Fauci. 1997. Increased in vitro tetanus-induced production of HIV type 1

following in vivo immunization of HIV type 1-infected individuals with tetanus toxoid. *AIDS Res. Hum. Retroviruses 13:473.*
27. O'Brien, W. A., K. Grovit-Ferbas, A. Namazi, J. Q. Zhao, A. Diagne, K. Idler, J. A. Zack, and I. S. Y. Chen. 1995. Human immunodeficiency virus type 1 replication can be increased in peripheral blood of seropositive patients after influenza vaccination. *Blood 86:1082.*
28. Stapran, S. I., B. L. Hamilton, S. E. Follansbee, T. Elbeik, P. M. Barbosa, R. M. Grant, and M. B. Feinberg. 1995. Activation of virus replication after vaccination of HIV-1-infected individuals. *J. Exp. Med. 182:1727.*
29. Mudido, P. M., D. Georges, D. Dorazio, B. Yen-Lieberman, S. Bae, W. A. O'Brien, J. Spritzler, and M. M. Lederman. 1996. Human immunodeficiency virus type 1 activation after blood transfusion. *Transfusion 36:860.*
30. Bowden, R. A., R. W. Coombs, B. H. Nikora, C. Bigelow, G. E. Sale, E. D. Thomas, J. D. Meyers, and L. Corey. 1990. Progression of human immunodeficiency virus type-1 infection after allogeneic marrow transplantation. *Am. J. Med. 88:49N.*
31. French, R, and P. Brocklehurst. 1998. The effect of pregnancy on survival in women infected with HIV: a systematic review of the literature and meta-analysis. *Br. J. Obstet. Gynaecol. 105:827.*
32. Wang, Y., L. Tao, E. Mitchell, W. M. J. M. Bogers, C. Doyle, C. A. Bravery, L. A. Bergmeier, C. G. Kelly, J. L. Heeney, and T. Lehner. 1998. Generation of CD8 suppressor factor and β chemokines, induced by xenogeneic immunization, in the prevention of simian immunodeficiency virus infection in macaques. *Proc. Natl. Acad. Sci. USA 95:5223.*
33. Pinto, L. A., S. Sharpe, D. I. Cohen, and G. M. Shearer. 1998. Alloantigen-stimulated anti-HIV activity. *Blood 92:3346.*
34. Doms, R. W., and S. Peiper. 1997. Unwelcome guests with master keys: how HIV uses chemokine receptors for cellular entry. *Virology 235:179.*
35. Moriuchi, H., M. Moriuchi, and A. S. Fauci. 1998. Factors secreted by human T lymphotropic virus type I (HTLV-I)-infected cells can enhance or inhibit replication of HIV-1 in HTLV-I-uninfected cells: implications for in vivo coinfection with HTLV-I and HIV-1. *J. Exp. Med. 187:1689.*
36. Kinter, A., A. Catanzaro, J. Monaco, M. Ruiz, J. Justement, S. Moir, J. Arthos, A. Oliva, L. Ehler, S. Mizell, et al. 1998. CC-chemokines enhance the replication of T-tropic strains of HIV-1 in $CD4^+$ T cells: role of signal transduction. *Proc. Natl. Acad. Sci. USA 95:11880.*
37. Dolei, A., A. Biolchini, C. Serra, S. Curreli, E. Gomes, and F. Dianzani. 1998. Increased replication of T-cell-tropic HIV strains and CXC-chemokine receptor-4 induction in T cells treated with macrophage inflammatory protein (MIP)-1α, MIP-1β and RANTES β-chemokines. *AIDS 12:183.*
38. Moriuchi, M., H. Moriuchi, W. Turner, and A. S. Fauci. 1998. Exposure to bacterial products renders macrophages highly susceptible to T-tropic HIV-1. *J. Clin. Invest. 102:1540.*