Generation of Alloantigen-Stimulated Anti–Human Immunodeficiency Virus Activity Is Associated with HLA-A*02 Expression

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Stimulation of peripheral blood mononuclear cells (PBMC) with allogeneic PBMC (ALLO) can result in activity that inhibits the replication of human immunodeficiency virus (HIV). The present study demonstrates that strong anti-HIV activity is dependent on expression of HLA-A*02 by the responding PBMC. Anti-HIV activity was equally effective against 2 primary isolates that use different coreceptors. Neither ALLO-stimulated cell proliferation nor cytokine and β-chemokine production was associated with the expression of HLA-A*02. ALLO-stimulated production of strong anti-HIV activity required intact PBMC and was not inhibited by monoclonal antibodies directed against nonpolymorphic regions of human leukocyte antigens (HLAs). Anti-HIV activity was generated by ALLO-stimulated CD4+ cells, CD8+ T lymphocytes, and monocytes from HLA-A*02–positive patients. These findings provide the first evidence that the production of an HIV inhibitory factor or factors is associated with certain HLA genes and raise new possibilities concerning the role of the major histocompatibility complex in controlling viral infections via alloantigen stimulation.

Several studies have addressed the issue of whether susceptibility to human immunodeficiency virus (HIV) infection, disease progression, or both is associated with human leukocyte antigens (HLAs). Resistance to infection in a cohort of repeatedly exposed female sex workers recently was reported to be associated with HLA-DRB1*01 [1]. Other HLA class II alleles have been associated with rapid HIV disease progression [2–5]. HLA class I genes also have been reported to exert a protective effect (reviewed in Westby et al. [6]). The report about sex workers also indicated that protection from HIV infection was strongly associated with a cluster of related HLA alleles belonging to the A2/6802 supertype [1]. The same study reported that HLA-A*2301 was associated with increased risk of HIV infection. HLA-B*35 and HLA-Cw*4 were also observed to be associated with rapid disease progression [7, 8].

Heterozygosity at HLA class I was found to provide a selective advantage over homozygosity in HIV-infected patients for delaying the onset of HIV disease [7]. This finding is consistent with the overdominant selection hypothesis, in which HLA heterozygous people have a broader spectrum of class I HLA determinants for the presentation of a greater combination of antigenic peptides of infectious agents than would people who were HLA homozygous [9]. Some of the HLA-A*02 subtypes were suggested to contribute to resistance against HIV infection by presenting the same protective HIV epitopes to HIV-specific cytotoxic T lymphocytes (CTLs) and may provide a protective role for HIV-specific, HLA-A*02–restricted cellular immunity [10, 11].

The possibility that immune reactivity against HLA alloantigens could contribute to protection against HIV infection has been raised [12–14]. This suggestion is based on a number of independent observations, including the following: (1) immunization of macaques with allogeneic or xenogeneic cells can protect against challenge with simian immunodeficiency virus grown in the same immunizing cells (reviewed in Lehner et al. [14]); (2) risk of vertical transmission at birth is decreased with increased mother-infant class I discordance [10]; (3) peripheral blood mononuclear cells (PBMC) from HIV-infected and HIV-uninfected patients generate soluble anti-HIV activity when stimulated in vitro with allogeneic PBMC (ALLO) [15–17]; and (4) PBMC from HIV-uninfected alloimmunized patients produce anti-HIV factors and can resist infection when challenged in vitro with HIV [18].

Soluble factors have been demonstrated to inhibit HIV rep-
lication at different steps in the viral cycle. These factors include the following: (1) the HIV-suppressive β-chemokines macrophage inflammatory protein (MIP)-1α, MIP-1β, and RANTES, which bind to HIV coreceptors and block viral entry [19]; (2) the CD8 antiviral factor [20], which inhibits viral replication at transcription [21]; and (3) the anti-HIV activity generated by ALLO stimulation, which inhibits both early and late reverse transcripts, indicating that ALLO-stimulated anti-HIV activity occurs before reverse transcription [17].

The present study investigates whether HLA genes contribute to the anti-HIV activity resulting from primary in vitro stimulation with allogeneic leukocytes. We specifically tested whether strong ALLO-stimulated antiviral activity is associated with expression of HLA class I alleles, class II alleles, or both.

Materials and Methods

Study population. Blood samples were obtained from 38 healthy volunteers in the Department of Transfusion Medicine, National Institutes of Health (NIH; Bethesda, MD). The samples were collected by means of a protocol approved by an NIH institutional review board. None of these donors tested positive for anti–HIV type 1 (HIV-1) serum antibodies. HLA typing of donor PBMC was performed by molecular typing using the polymerase chain reaction sequence-specific primers technique [22], by the HLA Laboratory (Department of Transfusion Medicine, NIH).

Cells. PBMC were isolated by density gradient centrifugation from heparinized blood and were washed twice in PBS. PBMC were suspended at 3 × 10^6 cells/mL in RPMI 1640 medium supplemented with 10% heat-inactivated fetal bovine serum (FBS; HyClone), 2 mmol/L-glutamine, penicillin (100 U/mL), and streptomycin (100 μg/mL).

Virus stocks. The primary isolates HIV-1A14, and HIV-1C15 were grown in human phytohemagglutinin (PHA) blasts, as described elsewhere [16]. The HIV-1A14 isolate (215 TCID₅₀/10⁵ cells) uses predominantly CXCR4 and CCR3 coreceptors (G. Blatter and D. I. Cohen, personal communication), and HIV-1C15 isolate (671 TCID₅₀/10⁶ cells) uses CCR5 and CXCR4 (D. I. Cohen, personal communication).

Generation of alloantigen-stimulated culture supernatants. PBMC (3 × 10⁶ cells/mL) from healthy donors (referred to as “responding cells”) were stimulated for 7 days with irradiated (5000 cGy) ALLO (1 × 10⁶ cells/mL) from single donors (referred to as “single ALLO stimulators”) or with a pool of ALLO from 6 HLA-mismatched donors (referred to as “ALLO pool stimulators”). The stimulators were as follows: stimulator 1, HLA-A*02, -A*03, -B*15, and -B*55; stimulator 2, HLA-A*02, -A*03, -B*37, and -B*51; stimulator 3, HLA-A*01 and -B*08; stimulator 4, HLA-A*24, -A*68, -B*13, and -B*44; stimulator 5, HLA-A*02, -A*03, -B*14, and -B*35; and stimulator 6, HLA-A*03, -A*68, -B*33, and -B*44. These mixed lymphocyte cultures were grown in RPMI 1640 medium supplemented with 10% FBS at 37°C in a 7% CO₂ humidified atmosphere.

Cell culture supernatants were collected at the end of 7 days and were frozen at −70°C. The anti-HIV activity contained in the unstimulated (negative control) and ALLO-stimulated culture supernatants was tested on in vitro-infected PHA blasts, as described below. Responder cells (3 × 10⁵ cells/mL) also were stimulated with anti-CD3 (10 μg/mL; Pharmingen) or tetanus toxoid (1:400; Connaught Laboratories) for 7 days. In some experiments, PBMC were cultured in RPMI 1640 medium supplemented with 10% FBS, were stimulated with PHA (2 μg/mL; Sigma) for 3 days at 37°C, and were infected at the indicated concentrations of virus, as reported elsewhere [16]. HIV-1-infected blasts (1 × 10⁶ cells/mL) were co-cultured in triplicate with either ALLO-stimulated or ALLO-unstimulated supernatants in RPMI 1640 medium containing 10% FBS and 10 IU/mL of interleukin (IL)-2 (Boehringer Mannheim) in flat-bottomed, 96-well plates. The final concentration of supernatant used was 50% (vol/vol). Supernatants were collected after 3 days of infection, were inactivated with 100 μL of 2% Triton X-100 (Sigma), and were frozen at −20°C. Supernatants for kinetic studies were collected after 2, 3, and 4 days of infection. The p24 antigen (HIV-1) levels in these supernatants were determined by ELISA (Coulter). The data are expressed as nanograms per milliliter of p24 detected in the infected culture supernatants. In some experiments, data were transformed to the percentage of inhibition by the following formula: [(1 − p24 value from infected cultures/p24 value from infected cultures in the presence of control unstimulated supernatants) × 100].

Cytokine production. The production of the following cytokines after in vitro ALLO stimulation was determined by ELISA: interferon (IFN)-γ (Biosource International), tumor necrosis factor (TNF)-α (Pharmingen), IL-2 (Genzyme DuoSet), IL-4 and IL-10 (Pharmingen), and MIP-1α, MIP-1β, and RANTES (R&D Systems), according to the manufacturer’s instructions.

Blocking with monoclonal antibodies (MAbs). Antibody blocking experiments were performed by incubating PBMC for 7 days in the presence of an anti–class I MAb (W6/32 at 25 μg/mL) [23] and an anti–HLA-DR MAb (L243 at 25 μg/mL) [24] (kindly provided by W. E. Biddison, NIH), both of which recognize nonpolymorphic regions of HLA molecules. The conditions we used were demonstrated to inhibit ALLO-stimulated T cell proliferation. Antibody blocking was also attempted by the anti–HLA-A2 MAb B77.2, which has been shown to inhibit HLA-A2–restricted CTL response [25]. All 3 of the HLA MAbs were shown to interfere with T cell response via the T cell receptor [23–25].

Statistical analysis. The association between the presence or the absence of a given allele and the strength of the viral replication for each of the 2 isolates (HIV-1A14, and HIV-1C15) was determined in 2 ways. Initially, the strength for both isolates was arbitrarily dichotomized at 50%, and the association with every allele that was present in >4 samples was determined as the result of a series of 2 × 2 tables by Fisher’s exact test. This investigation revealed that the HLA-A*02 allele had the greatest potential for association with viral replication. Logistic regression analysis was used to de-
termine the value of the strength of viral replication that would best discriminate among samples that were more likely to be HLA-A*02 negative versus HLA-A*02 positive by using overall percentage of correct classification as the criterion [26].

Once a logistic regression analysis threshold was determined for each of the 2 isolates, the alleles again were examined with respect to the newly dichotomized viral strength categories, by Fisher’s exact test. Odds ratios were constructed for the associations, and confidence intervals for the odds ratios were determined by an exact method (StatXact 4; Cytel Software). The analysis is exploratory, and no formal correction for the number of evaluations performed was made. However, in view of the large number of tests performed, only associations with \( P < .01 \) were considered to be statistically significant.

Results

Donor-dependent inhibition of HIV-1 replication by primary ALLO stimulation. Cell culture supernatants were generated by culturing responding cells with the ALLO pool stimulators. These supernatants were tested for their ability to inhibit replication of the HIV-1BZ167 and HIV-1GT dual tropic isolates in PHA blasts. The kinetics of p24 production for both isolates are shown in figure 1. The experiment compares the viral inhibitory effect of supernatants generated by ALLO pool stimulators from 4 healthy, randomly selected volunteers. Replication of both virus isolates was dramatically inhibited by supernatants from the responding cells of donors 1 and 4. Weaker inhibitory activity against both virus isolates was observed by supernatants from the responding cells of donors 2 and 3.

HLA-A*02-associated ALLO-stimulated inhibition of HIV-1 replication. HLA class I and class II typing were performed on these 4 donors, to determine whether HLA differences could account for the differences observed in ALLO-stimulated anti-HIV activity (see figure 1). HLA-A*02 was the only shared HLA locus between the 2 responding cell donors exhibiting strong anti-HIV activity and was not shared with the 2 responding cell donors exhibiting weak anti-HIV activity. No association between class II and strong antiviral activity was observed.

Because of the possible HLA-A*02 association with ALLO-pool-stimulated anti-HIV activity, donors 1–4 were retested for HLA and anti-HIV activity in a 3-day antiviral assay, as were 30 additional randomly chosen donors. The percentage of HIV inhibition for both virus isolates is shown in figure 2, with a median percentage of inhibition for the HLA-A*02–positive group of 71.5% for HIV-1BZ167 (range, 0%–88%) and 80% for HIV-1GT (range, 0%–90%). The median percentage of inhibition for the members of the HLA-A*02–negative group was 25.5% for HIV-1BZ167 (range, 15%–89%) and 29.5% for HIV-1GT (range, 4%–69%).

The percentage of ALLO pool–stimulated inhibition of HIV replication shown for responding cells from the 34 donors studied was evaluated by logistic regression analysis, to determine the threshold that would best discriminate between the observed strong inhibition in HLA-A*02–positive donors and the weak inhibition in HLA-A*02–negative donors. This analysis identified 40% as the most effective threshold level for HIV-1BZ167 viral inhibition and 45% for HIV-1GT inhibition. By use of these optimal thresholds, responding cells from 19 of 20 HLA-A*02–positive donors demonstrated strong viral replication inhibition levels for HIV-1BZ167 (>40%), compared with 3 of 11 from HLA-A*02–negative donors (\( P < .0001 \), Fisher’s exact test), with an odds ratio of 69.7 (5.4 to >3000).

Similarly, for HIV-1GT, responding cells from 18 of 20 HLA-A*02–positive donors’ cell supernatants exhibited strong viral replication inhibition levels, compared with 4 of 14 from donors who were HLA-A*02 negative (\( P = .0006 \), Fisher’s exact test), with an odds ratio of 22.5 (range, 2.8–255.0). These threshold levels are specific for the data listed here and would require evaluation with independent samples before application in external data sets. Statistical analysis did not reveal strong association of anti-HIV activity with any other HLA class I or class II loci. Subtyping was performed on 13 of 20 of the HLA-
A*02–positive donors. Of these 13, 12 were A*0201, and 1 was A*0202. Responding cells from another A*0202–positive and 3 additional A*0205–positive donors also generated strong anti-HIV activity when stimulated with ALLO pool stimulators (data not shown and not included in the statistical analysis).

Conditions for stimulating strong anti-HIV activity. To determine whether the generation of antiviral activity also would be associated with HLA-A*02 expression by ALLO stimulators, we analyzed the HIV-suppressive activity of supernatants generated by responding cells cultured with 6 HLA-mismatched single ALLO stimulators or as the ALLO pool stimulators. Figure 3 shows a representative example of anti-HIV activity of supernatants generated by responding cells from 2 donors (1 HLA-A*02–positive donor and 1 HLA-A*02–negative donor). Responding cells from the HLA-A*02–positive donor cultured with the ALLO pool stimulators generated supernatants with strong suppression of HIV-1BZ167 virus replication (>75%), regardless of the HLA class I antigens expressed by the ALLO pool stimulators.

In contrast, the supernatants from the HLA-A*02–negative responding cells cultured with the ALLO pool stimulators only weakly inhibited the HIV-1BZ167 isolate (<30%). Similar data were obtained with the HIV-1GT isolate (data not shown). Each of the 6 single ALLO stimulators was equally effective in stimulating the HLA-A*02–positive responding cells to generate anti-HIV activity, regardless of their HLA types, and they were as effective individually as the ALLO pool stimulators. In addition, expression of the HLA-A*02 allele by 3 of 6 of the single ALLO stimulators did not enhance the HIV suppressive activity of supernatants generated by the HLA-A*02–negative responding cells.

To determine whether a different T cell stimulus would generate supernatants with anti-HIV activity, we activated responding cells from an HLA-A*02–positive donor with an anti-CD3 MAb or tetanus toxoid and tested the antiviral activity on PHA blasts infected with HIV-1BZ167 and HIV-1GT. Although anti-CD3 and tetanus toxoid induced T cell proliferation, the supernatants generated did not appreciably inhibit replication of these 2 virus isolates when compared with the ALLO pool–stimulated super-
natants (p24 in the following: unstimulated, 35 ng/mL; ALLO pool stimulated, 8 ng/mL; anti-CD3, 27 ng/mL; and tetanus toxoid, 25 ng/mL). These results demonstrate that the supernatants generated by stimulation via the T cell receptor, which used responding cells from an HLA-A*02-positive donor, is not necessarily sufficient to generate strong anti-HIV activity.

To test whether intact PBMC were required for stimulating strong anti-HIV activity, the ALLO pool stimulators were lysed by hypotonic shock before coculture with HLA-A*02-positive responding cells. Cell membranes from the lysed ALLO pool stimulators were only marginally effective in stimulating responder cells to generate factor or factors capable of inhibiting replication of the HIV-1BZ167 isolate (p24 in the following: unstimulated, 20 ng/mL; intact ALLO pool, 3.5 ng/mL; and ALLO pool lysed, 16 ng/mL), compared with the anti-HIV activity generated by intact ALLO pool stimulators. Similar results were obtained when these lysed ALLO pool stimulators were tested with the HIV-1GT virus isolate (data not shown).

No association of HLA-A*02-negative dependent, anti-HIV activity with cytokine, chemokine, or proliferative responses. The anti-HIV factor or factors generated by ALLO pool-stimulated T cell lines were reported not to be due to β-chemokines or IFN-γ [16]. We tested whether responding cells from HLA-A*02-positive and HLA-A*02-negative donors that exhibited strong and weak anti-HIV activity, respectively, would generate different amounts of cytokines, chemokines, or T cell proliferative responses on primary in vitro ALLO stimulation. The mean levels of the cytokines IFN-γ, IL-2, IL-4, IL-10, IFN-γ, and TNF-α, as well as the levels of the β-chemokines MIP-1α, MIP-1β, and RANTES in ALLO pool-stimulated supernatants, were not different when generated by HLA-A*02-positive versus HLA-A*02-negative responding cells or were below levels that interfere with HIV-1 replication [19, 27–29]. ALLO pool-stimulated T cell proliferation also was not associated with strong ALLO-stimulated anti-HIV activity, since the HLA-A*02-positive responding cells generated 4459 cpm and strongly inhibited HIV-1BZ167 (79%) and HIV-1GT (80%), whereas the HLA-A*02-negative responding cells gave 5141 cpm but weakly inhibited HIV-1BZ167 (27%) and HIV-1GT (28%) when cultured with ALLO pool stimulators.

Lack of effect of HLA antibodies on blocking anti-HIV activity. To test whether antibodies against HLA would interfere with the generation of ALLO-stimulated anti-HIV activity, responding cells from HLA-A*02-positive donors were incubated with MAbs specific for class I and class II nonpolymorphic determinants. As shown in figure 4A, neither of these antibodies, when incubated separately or together, reduced the ALLO-stimulated anti-HIV activity when tested on the HIV-1BZ167 isolate. Similar results were obtained with the HIV-1GT isolate (data not shown). The W6/32 and L243 antibodies significantly blocked an HLA-A*02-positive donor PBMC proliferative response to ALLO pool when these antibodies were added individually, and they inhibited proliferation by >10-fold when used together (from 18,660 to 1700 cpm; 91% inhibition). The HLA-A2-specific MAb (BB7.2) also did not inhibit the generation of anti-HIV activity under conditions that block HLA-A2-restricted CTL activity [25].

Generation of anti-HIV activity by different leukocyte subpopulations. To determine whether different leukocyte subsets can serve as responding cells to generate ALLO-stimulated anti-HIV activity, PBMC from HLA-A*02-positive donors were depleted of or enriched with CD4+ and CD8+ T cells. In addition, monocytes were elutriated and were further enriched by depleting CD3+ and CD19+ cells. All these subsets were stimu-
lated with the irradiated ALLO pool stimulators for 7 days, and the culture supernatants were tested for anti-HIV activity. As shown in figure 4B, each of these 3 leukocyte subsets was able to generate strong anti-HIV activity.

Association of HLA-A*02 with ALLO-stimulated anti-HIV activity: statistical analysis. Analysis of the data shown in figures 1 and 2 indicates that expression of HLA-A*02 by the responding cells is associated with the generation of supernatants that suppress HIV-1 replication after a single in vitro stimulation with allogeneic leukocytes. By subjecting the antitants that suppress HIV-1 replication after a single in vitro responding cells is associated with the generation of supernatants. Figure 1 and 2 indicates that expression of MHC class I antigen expression in healthy people with irradiated ALLO pool stimulators resulted in no association with the generation of strong anti-HIV activity. However, there was no association with either HLA-A*01 or HLA-A*03 and viral replication. Two of 3 of the strong anti-HIV activity responders that were not HLA-A*02 positive were HLA-B*51 positive. However, HLA-B*51 was not statistically associated with production of anti-HIV activity for the HIV-1 isolate (P = .069) or the HIV-1 isolate (P = .069).

Responding cells from 2 of these 3 exceptions that generated strong ALLO pool-stimulated anti-HIV activity were also HLA-Cw*07 positive. However, 4 HLA-Cw*07-positive samples did not generate anti-HIV activity. Because HLA-B*35 is associated with rapid progression to AIDS [7, 30, 31], close attention was paid to this allele. The responder-cell donors that were HLA-B*35 positive appeared to be negatively associated with ALLO-stimulated anti-HIV activity for both isolates (P = .014). Thus, responding cells from 6 HLA-B*35-positive samples did not generate anti-HIV activity, but these 5 were also HLA-A*02 negative. The one HLA-B*35-positive PBMC donor that generated strong anti-HIV activity was also HLA-A*02 positive. None of the 15 class II alleles (DRβ and DQβ1) were found to be associated with ALLO-stimulated anti-HIV activity by stepwise regression, because all P values were >.3.

Discussion

The results of the present study demonstrate that in vitro stimulation of PBMC (responding cells) from 18 of 20 HLA-A*02-positive and from only 3 of 14 HLA-A*02-negative healthy people with irradiated ALLO pool stimulators resulted in the production of highly effective HIV-suppressive culture supernatants. Strong anti-HIV activity was not associated with the 16 other class I or the 15 class II alleles also tested. The 5 exceptions that were observed (2 HLA-A*02-positive samples that did not and 3 HLA-A*02-negative samples that did elicit strong antiviral activity) raise the possibility of association with a gene that is distinct from but closely linked to HLA-A*02. We observed that responding cells from 2 HLA-A*02 and 3 HLA-A*02 alleles also elicited strong anti-HIV activity, suggesting that these 3 HLA-A*02 subtypes (including HLA-A*021) do not distinguish between strong and weak anti-HIV activity. Thus, the amino acid differences of HLA-A*021, -A*02, and -A*02 do not discriminate between strong- and weak-responder status.

Our finding that ALLO-stimulated anti-HIV activity was not reduced by MAbs against nonpolymorphic class I, class II, or both determinants suggests that this ALLO-stimulated anti-HIV activity does not involve the major histocompatibility complex (MHC) recognition mechanisms that are usually encountered between T lymphocytes and antigen-presenting cells. These antibodies are known to block cellular interactions that involve the T cell receptor [23-25] and inhibited the allogeneic mixed lymphocyte reaction by >90% in our experiments. Our finding that the inhibition of anti-HIV activity was not seen by means of an HLA-A2-specific MAb suggests that the interactions involved in generation of anti-HIV activity might not be the ones required for other immune functions. It is possible that the HLA epitopes recognized are different than these for ALLO-stimulated proliferation or CTL. Alternatively, it is possible that the ALLO recognition involved in the generation of anti-HIV activity is different than that used for T cell proliferation and CTL responses.

The observation that single ALLO stimulators were equally effective, regardless of the presence of HLA-A*02, demonstrates the importance of HLA-A*02 expression by the responding, not the stimulating, cells in this experimental design. Furthermore, expression of HLA-A*02 by both responding cells and single ALLO stimulators did not abrogate or detectably reduce anti-HIV activity. This observation indicates that expression of HLA-A*02 on the stimulator side of the mixed lymphocyte reaction does not affect anti-HIV activity in this in vitro setting. The expression of the HLA alloantigens by the single ALLO stimulators probably activated HLA-A*02 responding cells to generate strong anti-HIV activity, which was unaffected by the additional expression of HLA-A*02 by the irradiated stimulators. The present study also demonstrates that ALLO-stimulated anti-HIV activity can be generated by CD4+ and CD8+ T cells, as well as by monocytes. On the basis of the data presented, it is not clear whether T lymphocytes are necessary for the generation of anti-HIV activity, because the monocytic responding cells were stimulated with irradiated, un fractionated PBMC that contained T cells. Our results that made use of lysed allogeneic cells indicate that intact stimulating cells are required for efficient production of anti-HIV activity.

It is unlikely that the anti-HIV activity generated by ALLO-stimulated HLA-A*02-positive responding cells was due to the β-chemokines. The β-chemokines block HIV isolates that use...
the CCR5 but not the CXCR4 coreceptor for binding to CD4 [19]. In contrast, the ALLO-stimulated anti-HIV activity inhibited replication of HIV isolates that used CXCR4, CCR3, and CCR5 coreceptors. Furthermore, the present study demonstrates that there was no difference in the amount of β-chemokines produced by HLA-A*02-positive and HLA-A*02-negative PBMC samples, compared with the difference in the levels of anti-HIV activity generated by HLA-A*02-positive and HLA-A*02-negative leukocytes.

In addition, the levels of β-chemokines generated by ALLO stimulation were below the levels reported to block HIV entry [19, 29]. The lack of association between anti-HIV activity and β-chemokines is consistent with our previous report that strong anti-HIV activity generated by ALLO-stimulated T cell lines was not inhibited by antibodies specific for β-chemokines [16]. It is also unlikely that the ALLO-stimulated anti-HIV activity is solely due to CD8 antiviral factor, because CD8 antiviral factor has been reported to block HIV replication at transcription [21], whereas the ALLO-stimulated anti-HIV activity inhibited viral replication before reverse transcription [17].

Alloimmunization has been suggested as a strategy for immune-based therapy and as prophylactic immunization against HIV [12–14]. It remains to be determined whether the finding of the present study could be used in this context. The recent report that the repeatedly exposed seronegative Kenyan female sex workers exhibit a high frequency of the HLA-A2/6802 supertype may be relevant to this question [1].

Our results may provide mechanistic insight into the report that HLA-A*02-positive newborns show a 9-fold reduced risk of perinatal HIV infection over HLA-A*02-negative newborns [10]. The present data complement the mother-to-infant transmission study in that they provide a potential mechanism by which neonatal leukocytes could recognize maternal allogeneic class I antigens, resulting in the generation of anti-HIV activity. Thus, it may be significant that HLA-A*02 expression is important both for generating potent, ALLO-stimulated, anti-HIV activity (this study) and for providing protection against HIV transmission in the mother-to-infant setting [10]. Our finding that both T cells and monocytes can serve as responder cells in this in vitro model that generates anti-HIV activity raises the possibility that both innate and adaptive immune mechanisms could contribute to the antiviral effect and protect the fetus from maternal infections that might be transmitted in utero or at birth.

From the perspective of vertical transmission of HIV, it is possible that the antiviral activity generated by allorcognition exerted evolutionary pressure that favored MHC class I polymorphism. However, because HIV is a relatively new virus that has only recently emerged, it would not have had time to exert an evolutionary pressure that would favor class I polymorphism. HLA-A*02, with its numerous subtypes, is the most prevalent human allele worldwide [32]. HLA-A*02 has been suggested to provide the opportunity for recognition of combinations of viral peptide and self HLA class I, including, but not limited to, HIV peptides [1]. Single amino acid substitutions in HLA-A2 proteins can result in changes in the peptide-binding properties of HLA-A*02 subtypes, creating distinct antigenic peptide–HLA-A*02 subtype complexes for presentation to T lymphocytes [33]. Because of high frequency of HLA-A*02 expression and our finding that HLA-A*02 is associated with the production of strong ALLO-stimulated anti-HIV activity, the possibility exists that HLA-A*02 could provide a selective protective advantage against other viruses through a similar ALLO-activated mechanism.

Studies are in progress for the following: (1) to identify and to characterize the soluble factors that inhibit HIV replication, as well as the immunologic mechanisms responsible for the HLA-A*02–negative–associated generation of alloantigen-stimulated antiviral activity; (2) to determine whether this antiviral activity can be generated by neonatal leukocytes in response to maternal HLA alloantigens; and (3) to determine whether the ALLO-stimulated anti-HIV activity can be extended to include the inhibition of other viruses.

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References