Human anti-human leukocyte antigen (HLA) antibodies were assessed for neutralizing activity against human immunodeficiency virus type 1 (HIV-1) carrying HLA alleles with matching specificity. Multiparous women carrying anti-HLA antibodies were identified. Plasma samples from those women were confirmed as having antibodies that specifically bound to HLA proteins expressed on the peripheral blood mononuclear cells (PBMCs) of their husbands. A primary HIV-1 isolate was cultured in the husband's PBMCs so that the virus carried matching HLA alleles. To determine the HIV-1-neutralizing activity of anti-HLA antibodies, the infectivity of the virus for GHOST cells (which express green fluorescent protein after HIV infection) was investigated in the presence of a plasma sample positive for the respective anti-HLA antibody. A neutralization assay was also performed using purified immunoglobulin G (IgG) from two plasma samples, and two plasma samples were investigated in the presence of complement. The prerequisite for anti-HLA antibody-mediated neutralization is incorporation of HLA proteins by HIV-1. Therefore, the extent of incorporation of HLA proteins by the primary HIV-1 isolate was estimated. The ratios of HLA class I protein to HIV-1 capsid (p24) protein cultured in the PBMCs of two healthy individuals were 0.017 and 0.054. These ratios suggested that the virus strain used in the assay incorporated more HLA proteins than gp160 trimers. Anti-HLA antibody-positive plasma was found to contain antibodies that specifically reacted to HIV-1 carrying cognate HLA alleles. However, incubation of HIV-1 with anti-HLA antibody-positive plasma or purified IgG did not show a reduction in viral infectivity. HIV-1-neutralizing activity was also not detected in the presence of complement. This study shows that HIV-1 primary isolates cultured in PBMCs contain significant amounts of HLA proteins. However, the binding of antibodies to those HLA proteins does not mediate a reduction in viral infectivity.

An accidental finding that macaques immunized with human cells alone exhibited sterilizing immunity against simian immunodeficiency virus (SIV) cultivated in the human cell lines (33, 34) led to a hypothesis that the alloimmune response may have a role in protection against human immunodeficiency virus (HIV) infection. These findings were further substantiated by studies in which macaques were immunized with purified human leukocyte antigen (HLA) class I (8) or class II (2) proteins and subsequently challenged with SIV cultured in human cells. These studies showed a strong correlation between protection against SIV infection and xenogeneic anti-HLA antibody responses (9). The numbers of HLA molecules incorporated into the envelopes of various strains of SIV as well as HIV type 1 (HIV-1) were found to exceed the numbers of viral envelope gp120 trimers (1a). It was hypothesized that the binding of anti-HLA antibodies to HLA proteins in the viral envelope may cause steric hindrance to the interaction of viral gp120 with its receptor on the target cell (9). As with xenogeneic anti-HLA antibodies, few studies suggest a protective role of allogenic anti-major histocompatibility complex antibodies of human origin against HIV-1. Neutralization of HIV-1 virions was observed in the presence of immunoglobulin G (IgG) from women undergoing leukocyte immunotherapy (14) and in the sera of polytransfused patients (30, 31, 40) in vitro.

Several investigators have documented the protective role of an alloimmune response against HIV-1. An alloimmune response at mucosal sites has been found to confer resistance to HIV-1 infection (3, 20). Among several therapeutic HIV-1 vaccine trials, instances of apparent clinical benefit were associated with the usage of vaccine candidates that contained host cell antigens and acted in accordance with the principles of alloimmunization (5, 6). Various soluble factors (CD8 suppressor factor, β-chemokines, anti-CCR5 antibodies) produced during the alloimmune response have been found to mediate protection against HIV-1 (37–39). Alloimmunization has been suggested as a strategy for the development of vaccines against HIV/AIDS (13, 28, 29). Anti-HLA antibody-mediated HIV-1 neutralization may be one of the potential mechanisms of protection induced after alloimmunization (13). In some studies, however, no protective role of anti-HLA antibodies against HIV-1 was observed. Anti-HLA alloantibodies found in children did not correlate with a lack of HIV-1 transmission from the infected mother (16). Similarly, the presence of anti-HLA antibodies did not correlate with resistance to HIV-1 infection by female commercial sex workers in the Nairobi cohort (17). The HIV-1-neutralizing activity demonstrated by exposed but persistently seronegative individuals did not correlate with the presence of anti-HLA antibodies (15). In a study reported by Polynskaya et al. (21), anti-major histocompatibility complex antibody responses to simian B cells did not show protection of macaques against SIVmac infection.
Due to conflicting evidence from different studies, the role of anti-HLA antibodies in protection against HIV-1 has remained inconclusive (18). Potential mechanisms of protection against HIV-1 are still under investigation. We assessed human anti-HLA antibodies for protection against HIV-1 by testing anti-HLA antibody-positive plasma against HIV-1 virions carrying HLA types with matching specificities.

**MATERIALS AND METHODS**

Study participants, blood sample collection, and processing. HIV-seronegative women who had experienced three or more full-term pregnancies and their husbands were counseled about the study objectives at the postnatal clinic of the Sassoon General Hospital, Pune, India. Informed written consent was obtained from all study participants prior to their enrollment in the study. The study was approved by the ethical committees of the National AIDS Research Institute and B. J. Medical College–Sassoon General Hospital, both in Pune, India. Eight to 10 ml of blood was collected in EDTA Vacutainers from all women and their husbands. Plasma and peripheral blood mononuclear cells (PBMCs) were separated by Ficoll-Hypaque density gradient centrifugation. Plasma and PBMCs were aliquoted and stored at −70°C and −156°C, respectively.

**Screening for anti-HLA antibodies by ELISA.** Plasma from multiparous women was tested for anti-HLA antibodies. Anti-HLA IgG antibodies were detected by commercially available enzyme-linked immunosorbent assay (ELISA) kits (QuikScreen and B-Screen; GTI, Brookfield, WI) according to the manufacturer’s instructions. A pool of plasma samples from 12 healthy, HIV-seronegative individuals was used as a complement source. Antibody W6/32 (a monoclonal antibody of mouse origin that recognizes nonpolymorphic regions of HLA class I and II antigens) was added to separate wells in duplicate, allowing anti-HLA antibodies to bind to immobilized HLA proteins. Anti-HLA antibodies were detected using anti-human IgG conjugated with alkaline phosphatase and p-nitrophenyl phosphate as an enzyme substrate. Plasma samples showing absorbances greater than or equal to twice the mean absorbance for two negative control wells (cutoff) were considered positive for the presence of anti-HLA antibodies, according to the manufacturer’s instructions.

**Binding of anti-HLA antibodies to the lymphocytes of the husband.** Plasma samples from 14 women that contained anti-HLA antibodies were tested against PBMCs from their respective husbands for binding antibodies. The husband’s PBMCs (2 × 10^6) were incubated with the wife’s plasma at room temperature for 45 min, and antibodies bound to lymphocytes were detected by a fluorescein isothiocyanate (FITC)-labeled anti-human IgG antibody (Bangalore Geni, India). Stained cells were fixed in 3% formaldehyde (prepared in phosphate-buffered saline) and analyzed on a flow cytometer (FACSort; Becton Dickinson) using CellQuest software. An electronic gate was formed for lymphocytes based on their forward and side scatter. Ten thousand cells within the gate were acquired and analyzed. A pool of plasma samples from 12 healthy, HIV-seronegative individuals without any known factor for the induction of anti-HLA antibodies such as pregnancy or transplantation was used as the negative control. Specific median fluorescence intensity (SMFI) was calculated as the MFI for the test divided by the MFI for the control. All plasma samples were heat inactivated at 56°C for 30 min before use and were tested at a 1/10 dilution. All plasma samples were also tested against autologous PBMCs.

To confirm that the antibodies that bound to the husband’s PBMCs were HLA specific, heat-inactivated plasma samples (0.3 ml at a 1/10 dilution) from three women, 40W, 43W, and 65W, were each incubated with the respective husband’s PBMCs (3 × 10^6) at 37°C for 1 h. After incubation, the plate was centrifuged, and the supernatant was tested for anti-HLA antibodies and anti-cytomegalovirus (CMV) antibodies (used as an unrelated control) (Plateia CMV IgG kit; Bio-Rad, France). The plasma samples of 40W and 43W were tested at a 1/20 dilution, whereas the plasma of 65W was tested at a 1/2 dilution, for anti-HLA antibodies. All plasma samples were tested at a 1/100 dilution for antibodies against CMV.

**HIV-1 culture in the husband’s PBMCs.** An HIV-1 subtype C,CCR5-tropic, neutralization-sensitive (12) primary isolate (VB49) obtained from the virus repository of the National AIDS Research Institute, Pune, India, was propagated in PBMCs from the husbands of all women carrying anti-HLA antibodies. The virus was also cultured in PBMCs from two women (28W and 49W). PBMCs were stimulated with phytohemagglutinin (5 μg/ml) for 48 h. Stimulated cells (8 × 10^6 to 10 × 10^6) were infected with 200 50% tissue culture infective doses of the virus. After overnight adsorption, cells were washed to remove cell-free virus and were cultured in an interleukin-2-containing (20 U/ml) medium. The culture supernatant (0.2 ml) was collected immediately after resuspension of the cells in interleukin-2-containing medium (day zero) and at 7 days of incubation at 37°C under a 5% CO2 atmosphere. Culture supernatants collected on day zero and day 7 were tested for HIV-1 p24 protein with an ELISA kit (Vironostika HIV-1 antigen micro-ELISA system; Biomerieux, The Netherlands). The culture supernatant was harvested on day 7, when a high concentration of p24 antigen (absorbance, >2 at a 1/200 dilution) was detected. The supernatant was centrifuged, aliquoted, and stored at −70°C. The amounts of these virus stocks ranged from 2.6 × 10^3 to 257 × 10^5 50% tissue culture infective doses/ml (determined as described in the NIAID ACTG manual [1]).

**Determination of HIV-1-neutralizing activity of anti-HLA antibodies.** The ability of anti-HLA antibody-positive plasma from multiparous women to neutralize an HIV-1 strain carrying HLA proteins with matching specificities was investigated. HIV-1-neutralizing activity was detected by using CCR5+ GHOST cells, which express green fluorescent protein under the control of HIV-2 Tat, as target cells (35). Anti-HLA antibody-positive plasma was incubated with HIV-1 (at a predetermined dilution that produces 1,000 fluorescent cells at the end of the assay) for 1 h at 37°C under a 5% CO2 atmosphere. The virus-antibody mixture was used to infect the GHOST cells in the presence of DEAE-dextran (8 μg/ml). The plate was incubated at 37°C under a 5% CO2 humidified atmosphere for 4 days. After incubation, cells were harvested, fixed in 2% formaldehyde, and analyzed on a flow cytometer (FACSort; Becton Dickinson). An electronic gate was formed using forward and side scatter, and within the gate 15,000 cells were acquired. The number of fluorescent cells was determined after quadrants were set based on cells from uninfected wells. The number of fluorescent cells in a well infected with virus alone was designated 100% infection. The percentage reduction of fluorescence (percentage neutralization) was determined for each plasma sample as described by Tokola et al. (35). All plasma samples were heat inactivated at 56°C for 30 min before use.

All plasma samples were tested for neutralization activity at a 1/10 dilution. Since HIV-1 inhibition due to undefined plasma components may be seen if a lesser dilution of plasma is used, IgG antibodies from plasma samples from two women (40W and 43W) were purified and tested against the respective virus. IgG was purified from 0.4 ml of plasma using a mean absorbance for two negative control wells (cutoff) of 0.1. Plasma samples were stored and tested as a negative control in all experiments to determine the background response. IgG purified from the negative control was tested along with the IgG from the test plasma. Complement was added to the negative control as well as to the test plasma when the neutralization assay was carried out in the presence of complement.

**Determination of the extent of HLA proteins incorporated by HIV-1 and detection of the reactivity of anti-HLA antibody-positive plasma.** The primary HIV-1 isolate (VB49) was cultured in PBMCs (12 × 10^6) derived from two healthy individuals (donors 1 and 2) as described above. Uninfected PBMCs (12 × 10^6) from both donors were also cultured as a control. The culture supernatants obtained from infected cells (test) or uninfected cells (control) were passed to the test plasma stocks ranged from 2.6 × 10^3 to 257 × 10^5 50% tissue culture infective doses/ml (determined as described in the NIAID ACTG manual [1]).

Due to conflicting evidence from different studies, the role of anti-HLA antibodies in protection against HIV-1 has remained inconclusive (18). Potential mechanisms of protection against HIV-1 are still under investigation. We assessed human anti-HLA antibodies for protection against HIV-1 by testing anti-HLA antibody-positive plasma against HIV-1 virions carrying HLA types with matching specificities.
of HLA class I A, B, and C alleles; Biodesign International) was added as a capture antibody (2 μg/well). After incubation for 2 h at 37°C, the strips were washed and blocked using 5% bovine serum albumin. The lysate was then added, and HLA proteins bound to anti-HLA antibodies were detected using a biotin-labeled, anti-β2 microglobulin antibody (Fitzgerald International), horseradish peroxidase-conjugated streptavidin, and 3, 3′, 5′, 5′-tetramethylbenzidine-H2O2. The reaction was stopped by 2 N H2SO4, and absorbance was measured at 450 nm after the absorbance value of the blank well was subtracted. Serial dilutions (200, 100, 50, 25, and 12.5 ng/ml) of purified HLA protein (a gift from GTI, Brookfield, WI) were tested along with test samples, and a standard curve was prepared. The amount of HLA proteins in the test sample was determined by using the standard curve.

To determine whether anti-HLA antibody-positive plasma contains antibodies that bind to HLA proteins incorporated by HIV-1, plasma samples from two women (28W and 49W) were incubated with HIV-1 cultured in their respective husbands’ (28H and 49H) PBMCs and also in autologous PBMCs. The virus was also incubated with pooled plasma samples from healthy, anti-HLA antibody-negative individuals (negative control) and with pooled plasma samples from HIV-seropositive individuals (positive control). The virus-plasma mixture was added and incubated in a well coated with rabbit anti-human IgG. The well was washed to remove unbound material after incubation, and virus lysis buffer was added to the well. The presence of bound virus was confirmed by detecting viral particles that bind to HLA proteins incorporated by HIV-1, plasma samples from two healthy, anti-HLA antibody-positive women were tested along with test samples, and a standard curve was prepared. The amount of HLA proteins in the test sample was determined by using the standard curve.

Anti-HLA class I and class II IgG antibodies. Fourteen multiparous women bearing anti-HLA antibodies were identified. Of these 14 women, 6 showed antibodies against both HLA class I and II proteins, 2 showed antibodies against class I only, and the remaining 6 showed antibodies against class II only (Table 1). The signal/cutoff ratios ranged from 5.1 to 17.6 for anti-HLA class I antibodies and from 3.7 to 15.6 for anti-HLA class II antibodies.

The plasma samples of multiparous women showed anti-HLA IgG antibodies that specifically bind to lymphocytes derived from their respective husbands. Binding of a woman’s antibodies to the lymphocytes of her husband was demonstrated by flow cytometry. Plasma samples from 8 of 14 anti-HLA antibody-positive women showed antibodies that bound to lymphocytes derived from their respective husbands. The sMFI ranged between 2.6 to 147.6 (Fig. 1). When the plasma samples were incubated with autologous lymphocytes, no antibody binding was seen (sMFI, <2). The remaining six plasma samples were positive for anti-HLA class II antibodies only (Table 1).

After the incubation of a woman’s plasma (40W, 43W, and 65W) with her husband’s PBMCs, the wife’s plasma showed a >75% reduction in the level of anti-HLA antibodies. However, no reduction was observed in the level of antibodies against CMV (Fig. 2). This indicates that the antibodies that stained the husband’s PBMCs are HLA specific.

Anti-HLA antibodies did not show HIV-1 neutralization. Virus (HIV-1 VB49) stocks, cultured in lymphocytes from the husbands of all participating women, were tested in a neutralization assay against plasma samples from the respective wives. The mean percentages of neutralization (± standard deviations) from two independent experiments are presented in Fig. 3A. The mean percentages of neutralization shown by plasma samples with antibodies against both HLA classes (classes I and II) ranged from −11.4 to 27.9%. The mean percentages of neutralization shown by plasma samples with antibodies against HLA class I only ranged from 10.9 to 15.6%, whereas plasma samples with anti-HLA class II antibodies only showed 0 to 30% neutralization. Thus, none of the test plasma samples showed a level of neutralization activity above the cutoff value.

### RESULTS

**Anti-HLA class I and class II IgG antibodies.** Fourteen multiparous women bearing anti-HLA antibodies were identified. Of these 14 women, 6 showed antibodies against both HLA class I and II proteins, 2 showed antibodies against class I only, and the remaining 6 showed antibodies against class II only (Table 1). The signal/cutoff ratios ranged from 5.1 to 17.6 for anti-HLA class I antibodies and from 3.7 to 15.6 for anti-HLA class II antibodies.

The plasma samples of multiparous women showed anti-HLA IgG antibodies that specifically bind to lymphocytes derived from their respective husbands. Binding of a woman’s antibodies to the lymphocytes of her husband was demonstrated by flow cytometry. Plasma samples from 8 of 14 anti-HLA antibody-positive women showed antibodies that bound to lymphocytes derived from their respective husbands. The

### TABLE 1. Detection of anti-HLA antibodies by ELISA

<table>
<thead>
<tr>
<th>Woman ID</th>
<th>Absorbance (signal-to-cutoff ratio)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HLA class I</td>
</tr>
<tr>
<td>40W</td>
<td>17.6</td>
</tr>
<tr>
<td>41W</td>
<td>8.7</td>
</tr>
<tr>
<td>42W</td>
<td>13.7</td>
</tr>
<tr>
<td>43W</td>
<td>12.7</td>
</tr>
<tr>
<td>49W</td>
<td>6.5</td>
</tr>
<tr>
<td>63W</td>
<td>5.1</td>
</tr>
<tr>
<td>65W</td>
<td>13.5</td>
</tr>
<tr>
<td>28W</td>
<td>6.6</td>
</tr>
<tr>
<td>45W</td>
<td>31W</td>
</tr>
<tr>
<td>33W</td>
<td>33W</td>
</tr>
<tr>
<td>44W</td>
<td>&lt;2</td>
</tr>
<tr>
<td>47W</td>
<td>&lt;2</td>
</tr>
<tr>
<td>48W</td>
<td>&lt;2</td>
</tr>
<tr>
<td>56W</td>
<td>&lt;2</td>
</tr>
</tbody>
</table>

*ID, identification number.

**FIG. 1.** Detection of binding of antibodies to lymphocytes by flow cytometry. Results for eight couples are presented. Each woman’s plasma (test) was incubated with autologous PBMCs (W) and her husband’s PBMCs (H) (2 × 10^5) at a 1/20 dilution. PBMCs were also incubated with a pool of plasma obtained from 12 healthy, HIV-seronegative individuals (control). Bound antibodies were detected using FITC-labeled anti-human IgG. The husband’s lymphocytes showed higher fluorescence intensity (x axis) when treated with the wife’s plasma (solid histograms) than when treated with the control (open histograms). The asterisked number in the upper right corner of each panel is the sMFI, calculated as the MFI for the test divided by the MFI for the control.
of 30%. This cutoff value was calculated as the mean plus 3 standard deviations of the percentages of neutralization shown by 20 plasma samples from healthy, HIV-seronegative individuals that were tested against the HIV-1 isolate (VB49) at the dilution reported previously (12).

The purified and concentrated IgG from two plasma samples (40W and 43W) showed higher absorbances than undiluted plasma when tested for anti-HLA antibodies by ELISA. The purified IgG from the plasma samples of 40W and 43W showed 29.3% /H11006 13% and /H11002 15% neutralizing activity,

FIG. 2. Confirmation of the HLA specificity of antibodies that bind to the husband’s PBMCs. IgG antibodies against HLA class I, HLA class II, and CMV in plasma samples from three women—40W (A), 43W (B), and 65W (C)—before and after incubation with their respective husbands’ PBMCs were measured by ELISA. For anti-HLA antibodies, the plasma samples of 40W and 43W were tested at a 1/20 dilution, whereas that of 65W was tested at a 1/2 dilution. All plasma samples were tested at a 1/100 dilution for antibodies against CMV. The absorbance values are shown on the y axis. All plasma samples showed >75% reductions in absorbance values for antibodies against HLA classes I and II after incubation with the respective husbands’ PBMCs, whereas no reduction was observed in the absorbance for antibodies against CMV.

The purified and concentrated IgG from two plasma samples (40W and 43W) showed higher absorbances than undiluted plasma when tested for anti-HLA antibodies by ELISA. The purified IgG from the plasma samples of 40W and 43W showed 29.3% ± 13% and −15% ± 4.9% neutralizing activity,

FIG. 3. Anti-HLA antibodies do not show HIV-1-neutralizing activity. (A) Anti-HLA antibody-positive plasma samples from 14 women were tested for neutralizing activity against HIV-1 cultivated in their respective husbands’ PBMCs by a GHOST cell assay. Neutralizing activity (percent reduction in infection compared to infection with virus alone) is shown on the y axis. The reference positive control (a pool of four plasma samples from HIV-infected individuals that showed HIV-1-neutralizing activity) showed >90% neutralization, whereas the reference negative control (a pool of plasma samples from 12 healthy, HIV-seronegative individuals) showed a background response of <30%. Shown are percentages of neutralization by plasma samples from six participants with antibodies against HLA classes I and II (A1), two participants with antibodies to HLA class I alone (A2), and six participants with antibodies to HLA class II alone (A3). All anti-HLA antibody-positive plasma samples were tested at a 1/10 dilution. (B) Percentages of neutralization by purified IgG from women 40W and 43W, tested at a 1/2 dilution. (C) Percentages of neutralization by plasma from women 40W and 43W tested at a 1/10 dilution in the presence of complement. Data in panels A and B are means and standard deviations from two independent experiments.
respective control.

husband's PBMCs but did not bind to HIV-1 cultured in autologous PBMCs.

ELISA was used for detection of HIV-1 p24 antigen. Each wife's plasma contained antibodies that specifically bound to HIV-1 cultured in her husband's (28H and 49H, respectively) PBMCs or autologous PBMCs. The virus was also incubated with pooled plasma samples from healthy, anti-HLA antibody-negative individuals (negative control) and from HIV-seropositive individuals (positive control).

Donor 1 contained 1,637 and 906 ng of p24 protein/ml and 150 and 224 ng of HIV-1 culture supernatant for donors 1 and 2, respectively, when tested at a minimal (1/2) dilution against the respective virus (Fig. 3B). No HIV-1 neutralization above the cutoff value was observed, even after complement was added to the reaction mixture. Plasma samples from 40W and 43W, when tested in the presence of complement, showed 27% and 29% neutralization, respectively (Fig. 3C). Thus, neither the purified and concentrated IgG nor the plasma tested in the presence of complement showed HIV-1 neutralization. Therefore, there was no evidence for HIV-1-neutralizing activity by anti-HLA antibodies. The reference positive-control plasma showed >90% neutralization in all experiments.

HLA proteins were incorporated by HIV-1, and binding of antibodies to HLA proteins was detected. The HIV-1 lysate derived from in vitro-infected PBMCs from donors 1 and 2 contained 1,637 and 906 ng of p24 protein/ml and 150 and 224 ng of HIV-1 culture supernatant for donors 1 and 2, respectively. The HIV-1 culture contains cell membrane microvesicles that carry host HLA proteins (4). Therefore, the amount of HLA proteins in the control was determined and subtracted from the amount of HLA proteins detected in HIV-1 culture. The molar ratio of HLA class I proteins to HIV-1 p24 was 0.017 for donor 1 and 0.054 for donor 2 (Table 2). Since there are an average of 2,500 capsid molecules per virion (10, 36), our estimate of the HLA/p24 ratio suggests the acquisition of 42 to 135 HLA class I molecules per virion. Since there are ~14 envelope trimers per HIV-1 virion (10), our data suggest that the viromics of the primary HIV-1 isolate may carry more HLA molecules than envelope trimers.

The anti-HLA antibody-positive plasma samples of the women contained antibodies that specifically bound to HIV-1 cultured in their respective husbands’ PBMCs but did not bind to the virus cultured in their autologous PBMCs (Fig. 4).

**DISCUSSION**

HIV-1 viromics acquire host HLA proteins while budding out of the infected cell. The number of HLA proteins incorporated by HIV-1 exceeds the number of viral Env gp160 trimers (1a, 36). Due to the close proximity of HIV-1 proteins and gp160 trimers, the binding of antibodies to HLA proteins may sterically hinder the interaction of viral Env protein with the CD4 receptor on the target cell (40). The hypothesis that steric hindrance for interaction between HIV-1 gp120 and CD4 receptor may render the virus noninfective has recently been supported by experimental evidence (23). A similar mechanism of neutralization has also been described for other viruses. For example, the binding of monoclonal antibody 7-1-9 to rabies virus causes steric hindrance of virus-receptor binding (11). Similarly, the binding of monoclonal antibody 4F2 to reovirus causes inhibition of viral binding to the host cell due to hindrance of the interaction between the virus and sialic acid on the host cell (19).

For any clinical application of anti-HLA antibody-mediated HIV-1 neutralization, the diversity of HLA proteins needs to be taken into account. It has been proposed that by appropriately selecting HLA alleles, it may be feasible to induce an immune response that can overcome HIV-1 diversity in a given population (13). The strategy may be particularly useful for the protection of monogamous partners of HIV-1-seropositive individuals. Induction of anti-HLA antibodies was found safe for more than 2,000 women undergoing leukocyte immunotherapy

**TABLE 2. Incorporation of HLA class I proteins by a primary HIV-1 isolate**

<table>
<thead>
<tr>
<th>Donor</th>
<th>Amt (ng/ml) of the following protein in culture supernatant</th>
<th>HLA class I protein/Gag p24 molar ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Infected supernatant</td>
<td>Control supernatant</td>
</tr>
<tr>
<td>Donor 1</td>
<td>150.2</td>
<td>80.4</td>
</tr>
<tr>
<td>Donor 2</td>
<td>223.9</td>
<td>105.7</td>
</tr>
</tbody>
</table>

* The culture supernatant of HIV-1-infected or control cells was ultracentrifuged (at 40,000 × g for 4 h), and the amounts of HLA class I proteins and HIV-1 Gag (p24) protein (for the culture supernatant of HIV-1-infected cells) in the pellet were quantitated by ELISA. The pellet was lysed with lysis buffer (a 1:1:1 mixture of Triton X-100, dipotassium EDTA, and Tween 20) before ELISA.

* After subtraction of the amount of HLA class I proteins obtained in the respective control.

**FIG. 4.** Binding of anti-HLA antibodies to HIV-1 cultured in the husband’s PBMCs, but not to HIV-1 cultured in autologous PBMCs, after incubation with anti-HLA antibody-positive plasma from the wife. Each wife’s plasma (28W and 49W) was incubated separately with HIV-1 cultured in her husband’s (28H and 49H, respectively) PBMCs or autologous PBMCs. The virus was also incubated with pooled plasma samples from healthy, anti-HLA antibody-negative individuals (negative control) and from HIV-seropositive individuals (positive control). The virus-plasma mixture was added and incubated in a well coated with rabbit anti-human IgG. After a wash, the virus bound to the well was lysed, and ELISA was used for detection of HIV-1 p24 antigen. Each wife’s plasma contained antibodies that specifically bound to HIV-1 cultured in her husband’s PBMCs but did not bind to HIV-1 cultured in autologous PBMCs.
to prevent recurrent spontaneous abortions (22). The major advantage of alloimmunization as a strategy for preventing HIV-1 infection is that it is likely to be independent of HIV-1 subtypes and genetic variations. We investigated the infectivity of HIV-1 in the presence of anti-HLA antibodies that can bind to HLA proteins present in the HIV-1 envelope.

Plasma samples from multiparous women that produced antibodies against the polymorphic regions of HLA alleles expressed by their husbands were obtained. Each woman’s plasma was tested against a primary HIV-1 isolate that was cultured in the PBMCs of her husband so that the test virus carried the husband’s HLA alleles. No reduction in viral infectivity was shown by any of 14 anti-HLA antibody-positive plasma samples (Fig. 3).

Factors such as inadequate levels of anti-HLA antibodies in the plasma or inadequate incorporation of HLA molecules in the virus envelope may be responsible for the lack of neutralizing activity. In a study reported by Leith et al. (14), anti-HLA antibodies were derived from women who had been immunized with multiple injections of 10^8 allogeneic lymphocytes. We obtained anti-HLA antibodies from women who were sensitized by exposure to semiallogeneic fetal cells that passed through the maternal-fetal barrier. This may influence the titer of anti-HLA antibodies. We therefore purified and concentrated IgG from two plasma samples and tested it at the highest possible concentration. However, no evidence for any HIV-1-neutralizing activity of anti-HLA antibodies was obtained (Fig. 3B).

In a study reported by Spruth et al. (31) and Wilfingseder et al. (40), significant neutralization of HIV-1 IIIB derived from cell lines was observed in the presence of anti-HLA antibodies and complement. The virus inhibition reported in these studies could be due to complement-mediated viral lysis (40). It has been shown that HIV-1 derived from T-cell lines is highly susceptible to complement, due to low incorporation of complement-regulatory proteins (CD55 and CD59) (32). Another mechanism for enhanced neutralization in the presence of complement may be higher steric hindrance of viral entry by the complement-antibody complex than by the antibody alone, as described for respiratory syncytial virus (41). We tested anti-HLA antibodies against an HIV-1 isolate in the presence of complement; however, no evidence of HIV-1 neutralization was observed (Fig. 3C). This finding might be due to the resistance of the primary HIV-1 isolate cultured in PBMCs to complement-mediated lysis. It has been shown that primary HIV-1 isolates incorporate complement-regulatory proteins (25, 26, 32) that can confer resistance to complement-mediated viral lysis.

The prerequisite for anti-HLA antibody-mediated HIV-1 neutralization is the acquisition of HLA molecules by HIV-1. It has been reported that the amount of HLA proteins acquired by HIV-1 may differ depending on the viral strain and the type of host cell (7). In our study, the molar ratio of HLA class I protein to HIV-1 capsid (p24) protein (0.017 and 0.054 for PBMC donors 1 and 2, respectively [Table 2]) suggested that the virus may carry more HLA molecules than gp160 trimers. Similar findings have been reported previously (1a, 36). Despite the incorporation of a significant number of HLA molecules by HIV-1 virions, anti-HLA antibodies were unable to reduce viral infectivity. Although anti-HLA antibodies bound to HIV-1 (Fig. 4), no evidence was obtained for their virus-neutralizing activity (Fig. 3).

The lack of HIV-1-neutralizing activity by anti-HLA antibodies may probably be attributed to the difference in size between HLA and HIV-1 envelope proteins. The HIV-1 gp160 trimer is 483 kDa, whereas HLA class I and II molecules are 57 and 61 kDa, respectively. The binding of IgG antibody (150 kDa) to HLA class I and II molecules can form a complex of ~210 kDa. Since the HIV-1 envelope molecule is relatively large and it forms a spike that bulges out of the membrane, the binding of anti-HLA antibodies may not interfere with the interaction between viral gp160 and the CD4 receptor. Although this could be anticipated without experimental investigation, the complexity of the mechanism of viral entry into the target cell, experimental evidence (8, 9, 14, 30, 31, 40), and the potential advantage of this approach (13) prompted further investigation. This study shows the lack of HIV-1-neutralizing activity by anti-HLA antibodies.

Alloimmune recognition leads to the induction of various responses, such as generation of anti-HLA antibodies, secretion of β-chemokines (3, 37–39), lymphoproliferative responses (20), formation of anti-CCR5 antibodies (3, 39), and secretion of RNases (24). Although no evidence was obtained for a protective role of anti-HLA antibodies against HIV-1, one or more of these responses might be contributing to the evidence of alloimmune-response-mediated protection against HIV-1.

ACKNOWLEDGMENTS

We thank all the study participants. We thank the staff of B. J. Medical College for HIV diagnosis and P. R. Naphade (B. J. Medical College) for help in sample collection. We acknowledge the technical support of S. S. Kulkarni, NARI, Pune, India, with the GHOST cell assay. We thank S. Zolla-Pazner, New York Veterans Affairs Medical Center, New York, NY, for providing the GHOST cell line. The gift of purified HLA proteins from GTI, Brookfield, WI, is acknowledged.

We thank the Indian Council of Medical Research (ICMR), Government of India, for supporting S. K. Lakhashe with a research fellowship.

REFERENCES


