Preparation and Characterization of an Intravenous Solution of IgG From Human Immunodeficiency Virus-Seropositive Donors

By Larry M. Cummins, Kent J. Weinhold, Thomas J. Matthews, Alphonse J. Langlois, Carlo F. Perno, Richard M. Condie, and Jean-Pierre Allain

An intravenous solution of 99% pure globulin (hyperimmune IgG, HIVIG) was obtained from pooled plasma of selected human immunodeficiency virus (HIV-1)-seropositive asymptomatic donors with >400 CD4+/μL cells per microliter and a high titer of antibody to HIV-1 p24 protein. HIVIG had high titers of antibody to p24, glycoprotein 41 (gp41), and gp120, group-specific neutralizing activity, and binding to the gp120 hypervariable loop region. It inhibited syncytia formation. At low concentration, it enhanced viral production of HIV-1 in infected peripheral blood monocytes but was inhibitory at higher concentration. HIVIG directed group-specific antibody-dependent cellular cytotoxicity against HIV-infected targets. For a period of 6 to 28 months, plasma donors kept stable antibody titers and had a 1.0% decrease in CD4+ cells per month. One gram per kilogram HIVIG injected in two juvenile chimpanzees was well tolerated and did not transmit HIV, as measured by negative cell culture, IgM immune response to HIV proteins, and polymerase chain reaction. The mean half-life of HIV-1 p24 antibody was 15 days. These preliminary data suggest that HIVIG is a safe product suitable for clinical trial in HIV-1-infected individuals.

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Materials and Methods

Plasma donors. Plasma was obtained by plasmapheresis from HIV-seropositive donors. Clinically healthy asymptomatic donors were initially screened for the presence of anti-p24 antibody, and those with titers >1:128 were further evaluated. CD4 lymphocyte count of not less than 400 cells/μL and the absence of detectable HIV Ag and HBsAg by enzyme immunoassays (EIA) were criteria for entering the plasmapheresis program. Plasmaphereses were performed in accordance with the Code of Federal Regulations for collection of source plasma. The health of donors was monitored by measuring the CD4 lymphocyte count and p24 antibody titer at 3-month intervals. Donors were excluded if the CD4 cell counts decreased to <400 cells/μL and/or anti-p24 titers were <1:128 or if the titer decreased twofold from baseline antibody level.

Inactivation of plasma pools. Before fractionation, plasma pools were inactivated by treatment with 1% Tri-N-butyl phosphate and 1% Tween-80 and heated at 30°C for 4 hours. This treatment has been shown to inactivate HIV-1, hepatitis-B, and non-A, non-B hepatitis viruses and provides safety for fractionation personnel. Additional inactivation occurred during fractionation with the cold-ethanol procedure of Cohn-Onley. The crude IgG (fraction II precipitate) paste was further purified using QAE-50 Sephadex, an ion-exchange resin that yielded pure monomeric unfragmented and undenatured human IgG. The final product was formulated as a 5% protein solution in normal saline as a sterile, nonpyrogenic solution.

Neutralization activity. In vitro neutralization activity was evaluated at Duke University Medical Center against HIV strains III-B and MN according to modification of a previously described method. Serial dilutions (100 μL) from 1:10 through 1:2,560 of HIVIG were incubated at 37°C for 30 minutes with 100 μL virus stock in six-well plates (~100 infectious units per milliliter). After 30 minutes of incubation at 37°C, 100 μL AA-5 target cells (106 cells/mL) in complete medium was added and incubated at 37°C in 5% CO2 for 24 hours. On day 1 of culture, 500 μL media (RPMI 1640 containing L-glutamine, 100 U/mL penicillin, 100 μg/mL streptomycin, and 20% fetal calf serum, FCS) was added to all dilutions and incubated for 24 hours. On day 2, 2 mL media was added to all dilutions and incubated. On day 7, the content of each well was collected and cells were pelleted by centrifugation. Cell-free supernatants were recovered and assayed for reverse transcriptase (RT) activity after precipitation with polyethylene glycol.

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Antibody binding to V3 loop peptides. The binding of antibody to the V3 loop peptides of four HIV isolates was tested by enzyme immunoassay. Specific peptides from distinct, well-characterized isolates of HIV-1 (IIB, MN, WMJ, and SC) were used as antigen. Each peptide consisted of 24 amino acids corresponding to a sequence in the V3 domain of glycoprotein (GP)120 for each isolate. A dilution of 1:100 of the IgG preparation was assayed in microtiter plates previously coated with the peptides as described, then incubated at room temperature for 90 minutes, washed and reacted with alkaline phosphatase-conjugated goat anti-human IgG, and visualized by reaction with paranitrophenylphosphate.

Syncyta formation blocking assay. The assay previously described used MOLT-4 cells incubated with HIV-1 isolates IIB and RF-infected CEM cells in the presence of 10-fold serial dilutions of the Igs. Cell fusion mediated by antibody activity was scored by counting the number of giant cells with a diameter greater than five times that of the MOLT or infected CEM cell.

Antibody-dependent cellular cytotoxicity (ADCC). The procedure used to detect ADCC was described previously by Lyerly et al. HIV-infected (IIB RF and MN) CEM.NKR target cells were radiolabeled in suspension at a concentration of 1 x 10^6 cells/mL in RPMI 1640 media with 100 to 200 μCi C^14. After being washed, the labeled target cells were resuspended in media at 1 x 10^6 cells/mL and 10^6 cells were added to triplicate wells of a U-bottomed 96-well plate (Costar, Cambridge MA). Effector cells were freshly prepared peripheral blood mononuclear cells (PBMC) from HIV-seronegative donors. HIVIG and control IVIG were tested at 10-fold serial dilutions with effector/target cells at a ratio of 50:1 and incubated at 37°C in 5% CO2 for 6 hours.

HIVIG inhibition of HIV cytopathic effect in T cells. The ATH8 cell line [an interleukin-2 (IL-2)-dependent, human T lymphotrophic virus (HTLV-I)-transformed T-cell line] was used previously described by Mitsuya and Broder. Cells (2 x 10^6 cells per tube) were exposed to the HTLV-I-III strain of HIV-1 (300 minimum infectious doses per tube) with 10-fold dilutions of either HIVIG (starting from 1:10) or control negative IgG added. Antibody-mediated inhibition of HIV-induced cytopathogenicity was measured by cell counting and trypan blue exclusion 6 to 7 days after viral exposure.

Inhibition of HIV replication in human peripheral blood monocytes/macrophages (M/M). The interaction of HIVIG with HIV-1 replication was evaluated using purified peripheral M/M was evaluated with a method previously described. Purified, normal M/M were obtained from healthy seronegative donors by countercurrent centrifugal elutriation. One lot of HIVIG (lot 2), lot MG1 (an Ig preparation obtained from HIV-seropositive plasma devoid of anti-p24 but containing both anti-gp41 and gp120 antibodies) and a control IVIG were used in these experiments after dilution to 50 mg/mL in saline. Three hundred minimum infectious doses (MID) per well HIV Ba-L strain were mixed with serial 10-fold dilutions of the Ig preparations in 200 μL complete medium and incubated at 4°C for 2 hours. The virus/Ig mixtures were then added to the M/M (10^6 cells per well in 48-well plates, 1 mLwell), and reincubated for 2 days at 37°C. Cells were then washed extensively to remove free virus and Igs and then cultured in 1 mL complete media. Viral production was assessed by enzyme-linked immunoassay (ELISA) specific for HIV-p24 antigen (Dupont, Wilmington, DE). Preliminary experiments showed that HIVIG preparation interferes with the gag-p24 assay. Extensive washing at day 2 after viral exposure substantially reduced such interference that becomes undetectable. Confirmation of data obtained with gag-p24 assay was assessed by RT assay on cell-free supernatants.

HIV-1 culture assay. Donor plasma samples and Ig solutions were assayed at the retrovirus laboratory at the University of Minnesota School of Medicine. The assay previously described involved incubation of the sample with phytohemagglutinin-stimulated PBMC in RPMI 1640 medium, 20% FCS, 2 mmol/L glutamine, 5% interleukin-2 (IL-2), 5 μg/mL polybrene, 200 U/mL penicillin, and 200 μg/mL streptomycin. Cocultures were incubated at 37°C in 5% CO2 for ≤ 35 days with testing and medium exchange at 7-day intervals. Viral replication was determined with the HIV p24 antigen assay described below.

Measurement of p24 antigen. HIV p24 antigen was measured with a solid-phase sandwich-type ELISA (Abbott Laboratories, North Chicago, IL).

Measurement of antibodies to p24 and gp41 antigens. The antibodies were measured with competitive ELISAs (Envacore, Abbott Laboratories, Delfkenheim, Germany). The assays use polystyrene beads coated with recombinant-produced p24 and gp41 antigens.

Specific antibodies in the sample compete against homeradish peroxidase-labeled human anti-HIV for the antigenic sites on the coated bead. Titers were determined by assaysing serial dilutions of the test samples, and the dilution corresponding to an A492 of 50% of negative minus positive control was then determined from a linear curve relating dilution and absorbance. In some experiments, a second assay was used to titrate antibody to p24.

Measurement of antibody to gp120. Anti-gp120 antibody titers were determined using an ELISA. The test system has been described previously. A nitrocellulose membrane coated with recombinant gp120 is incubated with sample for 1 hour at 37°C. The gp120 antibody is then detected with a human anti-gp120 alkaline phosphatase-labeled conjugate using para-nitrophenol as a substrate. Titers were determined for Igs from a linear curve relating dilution and reflectance value. The reference was a dilution of 1:100 of the IgG lot 2 with an assigned titer of 1,000.

HIV-1 gene amplification and probe. The in vitro polymerase chain reaction (PCR) was described previously. The PCR product of a 680-base pair (bp) segment of the gag region was hybridized with a single-stranded 32P-labeled DNA probe, then electrophoresed in polyacrylamide gel and detected by autoradiography. The hybridization assay has a sensitivity of 6 x 10^-10 mol target. Free probe was separated from hybrids by gel-exclusion chromatography.

β2-Microglobulin assay. β2-Microglobulin was measured by a commercially available solid-phase competitive radioimmunoassay (Abbott).

Animal safety study. Juvenile chimpanzees (Pan troglodytes) were chosen as an animal model because they are easily infected with HIV-1 virus and because they tolerate human globulin products without heterospecies reactions. The animals had previously been immunized against hepatitis B with a test vaccine and were anti-HBs antibody positive. They had no previous exposure to hepatitis-A or HIV-1. The study used one animal as a control. Weekly, it received two 500-mg/kg doses of intravenous (IV) IgG (Gammimune Cutter Biological, Berkeley, CA). Two animals received a total of 1,000 mg/kg HIVIG (lot 1), one at weekly 500-mg/kg doses and the second at weekly 250-mg/kg doses. Serum and lymphocyte samples were taken weekly for 1 month; samples were then taken at 2-week intervals for 6 months. Serum samples were tested by ELISA assays for p24 antigen and IgM antibodies to HIV-1 p24, gp41, and gp120. Lymphocytes were tested for evidence of HIV-1 by the previously described culture assay. Pharmacokinetics of the administered Igs were determined by measurement of serum antibodies to rubella, hepatitis A, HIV-1 p24, and gp41 with commercial ELISA assays (Abbott).

RESULTS

Donor selection and surveillance. In an 18-month period, 246 asymptomatic HIV-seropositive donors were screened;
were monitored to detect the occurrence of a clinical or laboratory abnormality requiring donor exclusion. Data in Table 1. All donors remained asymptomatic, and none had a CD4 cell count >400/µL. The 35 selected donors had a CD4 cell count >400/µL. The 35 selected donors who gave an average of 48 U (29 L) plasma are shown.

Table 1. Donor Surveillance

<table>
<thead>
<tr>
<th>Donor</th>
<th>Duration (mo)</th>
<th>Units Donated</th>
<th>Change* (%)</th>
<th>CD4</th>
<th>p24 Titer</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>27</td>
<td>92</td>
<td>+1.5</td>
<td>-2.1</td>
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</tr>
<tr>
<td>2</td>
<td>17</td>
<td>105</td>
<td>-27.0</td>
<td>-3.0</td>
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<tr>
<td>3</td>
<td>18</td>
<td>29</td>
<td>+0.4</td>
<td>+74.2</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>14</td>
<td>19</td>
<td>-11.0</td>
<td>+49.0</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>10</td>
<td>64</td>
<td>-4.8</td>
<td>+32.7</td>
<td></td>
</tr>
<tr>
<td>6†</td>
<td>6</td>
<td>32</td>
<td>-24.1</td>
<td>-26.0</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>12</td>
<td>36</td>
<td>-10.6</td>
<td>-27.3</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>28</td>
<td>54</td>
<td>-17.3</td>
<td>-22.7</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>9</td>
<td>34</td>
<td>-2.9</td>
<td>-3.6</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>8</td>
<td>19</td>
<td>-19.6</td>
<td>+91.1</td>
<td></td>
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<tr>
<td>11†</td>
<td>17</td>
<td>72</td>
<td>-9.1</td>
<td>+13.1</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>16</td>
<td>71</td>
<td>+12.7</td>
<td>+30.1</td>
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<td>12</td>
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<tr>
<td>14</td>
<td>10</td>
<td>22</td>
<td>-17.4</td>
<td>-21.0</td>
<td></td>
</tr>
</tbody>
</table>

Mean ± SD 14.6 ± 7 48 ± 28 -14.6 ± 8.0

*Percentage of change was calculated from values collected at the initial and the most recent visit.
†Donors discontinued (CD4 <400 cells/µL).

939 had p24 antibody titer >1:128, and all but four of these had a CD4 cell count >400/µL. The 35 selected donors were also negative for p24 antigen by EIA. Thus, 14.2% of seropositive donors tested met the criteria established for selection of HIVIG plasma donors. The selected donors were monitored to detect the occurrence of a clinical or laboratory abnormality requiring donor exclusion. Data collected from the 14 donors followed for 6 to 27 months and who gave an average of 48 U (29 L) plasma are shown in Table 1. All donors remained asymptomatic, and none had a p24 antibody titer <1:128, although four had a substantial decrease in titer over time. The p24 antibody titer increased in six donors in an 8- to 18-month period. A progressive decrease in CD4+ cell count was observed at a rate of ~1% per month, and four donors were excluded when their CD4 cell count decreased to <400/µL (Table 1). In addition to the CD4 cellular marker, β2-microglobulin levels were monitored, and no significant change over baseline was detected in any of the 14 donors.

Preparation and quality control. Before pooling, each unit of plasma was HIV antigen negative. No individual plasma pools of each donor were infectious for HIV-susceptible cells in culture. As shown in Table 2, three lots of HIVIG were HIV antigen negative and not infectious for cultured cells, and the PCR did not yield HIV proviral DNA. Two lots of HIVIG have been tested and met the Center for Biologics Evaluation and Research (CBER) specifications for IVIG.

IVIG lots were sterile and nonpyrogenic; electrophoretically, they contained >99% IgG, which appeared to be 100% monomeric because there was no evidence of aggregation or fragments when they were tested by high-pressure liquid chromatography (HPLC). All preparations contained all four subclasses of IgG; however, HIVIG had a lower percentage of IgG2 but a higher content of IgG1 than polyvalent IVIG (Table 2). The purity and integrity of the product in the absence of stabilizing agents was supported by a minimum of 12 months stability (0°C to 8°C storage) with no changes detected in pH, percentage of monomeric IgG, or antibody titer as assessed by HIV anti-p24. After they had been stored for 1 month at 40°C, we observed a slight haze; the preparation remained 100% monomeric by HPLC. Titers of antibody to the core (p24) and envelope (gp41, gp120) of HIV were high and consistent from lot to lot. Because different assay formats were used to quantify each antibody, we can make no titer comparison between antibodies.

Neutralizing antibody. Neutralizing titers for HIVIG lots against two isolates of HIV are shown in Table 3. The results indicated that the preparations contained broad neutralization activities with higher titers directed at the MN isolate. In addition, with a slightly different technique performed in D. Ho’s laboratory,28 significant neutralizing titers were found with the H9 cell line infected with the Haitian isolate RF, which is ~80% homologous to III-B.25 These results suggest group-specific neutralizing activity.

One major neutralizing epitope of HIV-1 was located in the loop region of the third variable region (V3) of the GP120 protein (between amino acid residues 307 and 330). The presence of antibody directed to that region of four separate isolates of HIV-1 was tested by EIAs using peptide sequences specific to each isolate as capture antigens. The peptides and respective isolates are shown in Table 3 along with the binding activity of HIVIG preparations and a control lot of IVIG. Antibody binding was the highest with

Table 2. Subclass Distribution and Specific HIV Markers in HIVIG and IVIG

<table>
<thead>
<tr>
<th>Marker</th>
<th>Lots 1</th>
<th>Lots 2</th>
<th>Lots 3</th>
<th>IVIG</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG1 (%)</td>
<td>82.1</td>
<td>81.9</td>
<td>84.5</td>
<td>65.2</td>
</tr>
<tr>
<td>IgG2 (%)</td>
<td>13.0</td>
<td>12.3</td>
<td>10.2</td>
<td>25.7</td>
</tr>
<tr>
<td>IgG3 (%)</td>
<td>4.1</td>
<td>4.4</td>
<td>9.2</td>
<td>8.2</td>
</tr>
<tr>
<td>IgG4 (%)</td>
<td>0.8</td>
<td>1.4</td>
<td>1.0</td>
<td>1.2</td>
</tr>
<tr>
<td>p24 Antigen Negative</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>p24 Antibody Titer</td>
<td>272,500</td>
<td>243,500</td>
<td>238,500</td>
<td>&lt; 5</td>
</tr>
<tr>
<td>GP41 Antibody Titer</td>
<td>8,800</td>
<td>8,700</td>
<td>7,800</td>
<td>&lt; 5</td>
</tr>
<tr>
<td>GP120 Antibody Titer</td>
<td>3,400</td>
<td>2,910</td>
<td>3,100</td>
<td>&lt; 5</td>
</tr>
<tr>
<td>Culture</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PCR* for gag of HIV-1</td>
<td></td>
<td></td>
<td></td>
<td>Negative</td>
</tr>
</tbody>
</table>

IVIG corresponds to IV polyvalent human IgG preparation.
*Polymerase chain reaction used to detect HIV genome, experiments were performed by M. Kuhns, Abbott Laboratories.
Table 3. Blocking of HIV Cell Interaction

<table>
<thead>
<tr>
<th>Assays</th>
<th>Target</th>
<th>HIV Strain</th>
<th>HIVIG Lots</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Neutralization* of HIV infection</td>
<td>AA5</td>
<td>IIB</td>
<td>160</td>
</tr>
<tr>
<td></td>
<td>MN</td>
<td>IIB</td>
<td>&gt; 2,560</td>
</tr>
<tr>
<td>V3 loop binding†</td>
<td>Peptides</td>
<td>IIB</td>
<td>0.056</td>
</tr>
<tr>
<td></td>
<td>MN</td>
<td>IIB</td>
<td>0.447</td>
</tr>
<tr>
<td></td>
<td>WMJ</td>
<td>IIB</td>
<td>0.066</td>
</tr>
<tr>
<td></td>
<td>SC</td>
<td>IIB</td>
<td>0.193</td>
</tr>
<tr>
<td>Syncytia formation‡</td>
<td>Molt cells</td>
<td>IIB</td>
<td>900</td>
</tr>
<tr>
<td></td>
<td>CEM cells</td>
<td>RF</td>
<td>1,060</td>
</tr>
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</table>

*Expressed as titer of neutralization (described in the Materials and Methods section). Neutralization experiments with the RF isolate were also performed by Dr. David Ho of Cedars Sinai Hospital, UCLA, with a slightly different technique. Neutralizing antibody titers of 1:16, 1:32, and 1:16 were obtained with HIVIG lots 1 through 3, respectively.

†Optical density recorded with specific peptide ELISA. Concentration of peptide in the coating solution was 2 μg/mL (described in the Materials and Methods section).

‡Concentration (in micrograms per milliliter) of IgG at which viral cytopathic effect was reduced by 50%.

§No effect was noted with HIVIG at 5,000 μg/mL IgG.

the MN loop region and lowest with IIB. This reactivity was consistent with the higher titer of neutralizing antibody found with MN in two of the three lots of HIVIG studied.

Inhibition of syncytia formation. The anti-HIV activity as determined by inhibition of syncytia formation by HIVIG and control IVIG is shown in Table 3. The system involves CD4-bearing CEM cells which fuse with HIV-1-infected cells to yield giant cells. Although the significance of this assay is not fully established, HIVIG can be distinguished from the IVIG control, which had no measurable activity.

ADCC. The ADCC activity of HIVIG as compared with control IVIG is shown in Fig 1. All lots of HIVIG directed broadly reactive ADCC activity against the three infected target cell lines. Activity expressed as the minimum IgG concentration that induces measurable ADCC activity (20% lysis) ranged from ~0.5 μg/mL with the RF-infected target cells to 25 μg/mL with IIB. The 20% lytic activity with the MN infected target cells was achieved with lots 2 and 3 at concentrations ranging from 13 to 250 μg/mL concentration. The 250-μg/mL level would be achieved in vivo at a HIVIG dose of ~25 mg/kg, well within planned human protocols.

Inhibition of HIV cytopathic effect in T cells. The three lots of HIVIG and a control preparation of polyvalent IVIG were tested at dilutions of 1:10, 1:100, 1:1,000, and 1:10,000 for the ability to block syncytia formation of HTLV-III-B-infected ATH-8 cell line. With all three lots of HIVIG, 75% inhibition was observed at a dilution of 1:100 corresponding to 500 μg/mL HIVIG. At a dilution of 1:1,000, 8%, 24%, and 16% of inhibition was observed with lots 1 through 3, respectively. In contrast, no dilution of IVIG showed inhibition. The inhibitory activity of HIVIG was enhanced in combination with AZT (data not shown), suggesting that the combination of HIVIG and AZT may allow antiviral effect at reduced and more tolerable AZT doses.

Effect of HIVIG in HIV-1-infected M/M. The inhibition of HIV replication in M/M is a well-defined in vitro assay for detection of antiviral drugs. Testing of HIVIG in this assay showed that complete inhibition of viral replication was achieved in all M/M tested at an HIVIG concentration...
CHARACTERIZATION OF AN ANTI-HIV IMMUNE GLOBULIN

Fig 2. Inhibition of HIV replication in M/M by anti–HIV-1 antibody (solid circles) HIVIG lot 2, and IgG prepared from HIV-seronegative donors (solid squares).

≥500 μg/mL (Fig 2). In one experiment, complete inhibition was obtained at 50 μg/mL when the M/M were exposed to GM colony-stimulating factor (GM-CSF). No toxicity related to HIVIG was detected in any M/M population up to 40 days. In all studies, HIVIG was added at the time of infection, removed after 2 days, and not replaced. As already shown for anti-HIV antibody preparations, HIVIG can enhance viral replication in M/M. This occurs at concentrations (5 to 500 pg/mL) far below (5 to 500 pg/mL) those attained in vivo conditions, however. Control IVIG did not interfere with viral replication.

Animal safety. Experimental safety was established by administration of a 1,000-mg/kg dose of HIVIG in two healthy uninfected seronegative chimpanzees, and results were compared to a control animal infused with the same total dose of IVIG. The lack of HIV infectivity of HIVIG observed in vitro was confirmed in vivo. Monitoring for 6 months showed no evidence of HIV infection, as assessed by plasma and lymphocyte culture. There was no development of an IgM response to HIV and no evidence of HIV DNA by PCR applied to lymphocytes and plasma 6 months and 1 year postinfection. The biologic half-life (t½) of HIV antibody to p24, gp41, and gp120 ranged from 11 to 17 days in the two animals. Titers of antibody to hepatitis-A were monitored as control. A t½ of 15 to 19 days was found.

DISCUSSION

In addition to developing a HIVIG, we studied the effects of long-term plasmapheresis of HIV-seropositive donors. Because of a concern that plasmapheresis could cause a depletion of protective antibody, we studied stored specimens obtained from eight donors who had previously donated plasma for production of hepatitis-B Ig. Analysis of stored samples established that five of the eight were infected between June 1982 and September 1984. Four of these donors were plasmapheresed until November 1986, donating from 30 to 100 L plasma in this period; they continued to show an increase in anti-p24 titers while remaining HIV plasma antigen negative and maintaining their healthy status. This profile of high-titer anti-p24 antibody, HIV antigen negativity, and healthy status was previously reported. To date, no donor has had a decrease in p24 titers below 1:128 and all have remained HIV antigen negative. The decrease in CD4+ cell count of 1% a month in the donors is similar to the rate of decrease reported by DeWolf et al. in a group of HIV antibody-positive, antigen-negative individuals monitored for 30 months. In another study, 1,827 HIV-seropositive men monitored for 2 years had an average decrease in CD4+ cell count of 53 cells in a 6-month period. Using this predicted rate of decrease and estimating from baseline values, 13 of the 14 donors had actual cell counts significantly higher than the predicted value. These data suggest that plasmapheresis is not detrimental to HIV-infected individuals.

In AIDS patients, plasmapheresis and replacement of plasma proteins can be beneficial. Plasma used to manufacture lots of HIVIG tested negative for HIV by EIA and tissue culture; however, because virus can be present as an immune complex and for added safety, the plasma pool was chemically inactivated. TNBP/TWEEN 80 inactivates at least four log 10 units of infectious HIV, the Cohn-Onley cold ethanol procedure inactivates at least 12 log 10 units, and additional inactivation is provided by the ion-exchange process. The safety of the overall process of plasma screening, chemical inactivation, and viral partitioning is supported by data obtained in chimpanzees as well as by HIVIG product testing negative by EIA, tissue culture, and PCR.

The distribution of the IgG subclasses shown in Table 2 indicates a much higher content of IgG1 in the HIVIG preparations than was observed in the IVIG prepared from normal plasma. This distribution was reported in HIV-infected individuals by Reimer et al. The presence of all four subclasses can be attributed to the combination of cold-ethanol fractionation and ion-exchange purification, because IgGs produced by the original method of Condie are devoid of IgG3. In that procedure alcohol fractionation is not used and globulin plasma proteins are absorbed...
with silica dioxide. Maintenance of a complete IgG subclass profile could be important if HIVIG is used to treat infected pregnant women, because certain subclasses cross the placenta more efficiently than others; eg, IgG2 subclass is poorly transferred.15

The biologic 1/2 of 11 to 19 days of the index-specific antibodies HIV p24 and anti-hepatitis-A virus offers a distinct advantage for therapeutic treatment as opposed to the short-acting antiviral agents or short-lived immunomodulating agents such as CD4 (serum 1/2 ~ 45 minutes).16

More recently, however, CD4 fused with part of an IgG molecule has shown an increased 1/2 in circulation.13 Sustained and accumulated anti-HIV levels have been observed in human safety studies with dosing at a 4-week interval (data not shown).

Although donors were not selected on the basis of neutralization titers, the preparations still possessed neutralizing activity, as shown in Table 3. The significance of having neutralizing activity is still unknown. An HIV Ig prepared by Prince et al from donors with high-titer neutralizing antibody failed to protect chimpanzees when challenged with HIV.8 Earlier studies with 5% IgG solutions obtained from donors preselected for anticore and antienvelope proteins indicated a lack of correlation of neutralizing activity with a specific antibody titer.

An epitope-specific measurement of neutralizing activity was obtained by determining the binding activity to the hypervariable loop peptides of four HIV isolates. The recombinant peptides correspond to a region of the GP120 loop that is a major site of HIV neutralization for all isolates. The loop binding activity correlates with the neutralization data with strongest activity against the MN isolate and with lot 1. The difference in neutralization activity between lot 1 and 2 may have resulted because more donors entered the plasma pool (17 in the latter as opposed to 9 in lot 1), suggesting that both neutralizing activity in culture and in vitro recognition of the major neutralization epitope of a range of isolates could be maximized by increasing the number of donors, preferably from different geographic locations.

Recently, Rossi et al reported that pregnant women infected with HIV-1 who have antibodies to peptides close to or part of the hypervariable loop of HTLV-III-B gp120 did not transmit the HIV infection to their child.19 Therefore, monitoring antibodies to the loop peptides as well as complete subclass distribution could be critical for predicting the efficacy of HIVIG for perinatal immunotherapy.

In all functional assays examining the antiviral activity of HIVIG, inhibitory activity was noted at antibody concentrations that can be achieved and sustained in recipients with monthly dosing; eg, inhibition of syncytia formation was observed at concentrations of 375 to 1,800 μg/mL IgG, ADCC activity occurred at 2.5 to 250 μg/mL, inhibition of cytotoxic effect in T cells occurred at 500 μg/mL, and inhibition of HIV replication in M/M occurred at 50–500 μg/mL. Assuming two-compartment distribution (plasma and extra cellular), plasma IgG concentrations from a 20-mg/kg dose would provide a HIVIG plasma level of 2,085 μg/mL. Assumming a 15-day 1/2 and monthly dosage, levels >1,000 μg/mL could be maintained at all times. Thus, unlike many antiviral agents, in vitro activity is achieved with HIVIG at levels well tolerated and easily achievable in humans. More important, the long 1/2 of HIVIG is a major advantage for studies that will involve treatment of infected pregnant women and/or infected infants. The synergistic effect with antiviral or immunomodulation agents also provides opportunities that must be investigated.

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