Use of Human Immunodeficiency Virus (HIV) Human Hyperimmune Immunoglobulin in HIV Type 1–Infected Children (Pediatric AIDS Clinical Trials Group Protocol 273)

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The clinical, immunologic, and virologic effects and the pharmacokinetics of human immunodeficiency virus (HIV) human hyperimmune immunoglobulin (HIVIG) were assessed in 30 HIV-infected children aged 2–11 years. All had moderately advanced disease with an immune complex–dissociated (ICD) p24 antigen >70 pg/mL and were on stable antiviral therapy. Three groups of 10 children received 6 monthly infusions of 200, 400, or 800 mg/kg of HIVIG, and serial immunologic and virologic assays were performed. HIVIG doses as high as 800 mg/kg were safe and well tolerated. The half-life of HIVIG, determined by serial p24 antibody titers, was 13–16 days, the volume of distribution was 102–113 mL/kg, and clearance was 5.6–6.0 mL/kg/day. Plasma ICD p24 decreased during the infusions, but CD4 cell levels, plasma RNA copy number, cellular virus, immunoglobulin levels, and neutralizing antibody titers were minimally affected by the infusions. Clinical status did not change during the 6-month infusion and 3-month follow-up periods.

The use of antibody in the treatment of ongoing viral infection is occasionally efficacious but rarely curative [1, 2]. It has been used alone (e.g., in parvovirus infection) [3] and in conjunction with antiviral therapy (e.g., in cytomegalovirus infection) [4]. Antibody also has been used in human immunodeficiency virus type 1 (HIV-1) treatment with HIV-1 human hyperimmune immunoglobulin (HIVIG), immune plasma, porcine immunoglobulin, and monoclonal antibody (MAb) [5, 6]. Of 4 controlled studies of passive HIV immunotherapy [7–11, 2000;181:548–54]

Materials and Methods

Study design. The 30 children in the study were HIV-1 infected, aged 2–11 years, part of the Pediatric AIDS Clinical Trials Group (PACTG), and on stable antiviral therapy (no change for 3 months before entry or during infusions without approval by the protocol chair), weighed <45 kg, and had CD4 cell counts ≥200/μL (age 2–5 years) or ≥100/μL (age ≥5 years). All children had ≥70 pg/mL HIV p24 ICD plasma antigen levels that were stable or increasing. At entry all patients were receiving ≥1 reverse-transcriptase inhibitors;
none was receiving protease inhibitors. The children had not received intravenous immune globulin (IVIG) in the 60 days before study entry and did not have hypogammaglobulinemia or a protein-losing state.

The children were randomly assigned to 1 of 3 treatment groups: group 1 received 6 monthly HIVIG infusions of 200 mg/kg; group 2, 400 mg/kg; and group 3, 800 mg/kg. Although the number of subjects was determined by product availability, a sample size of 10 per group provided 80% power to detect toxicity rate differences between dose groups that were >60% and to detect a linear dose response of virus load, given that the true correlation coefficient is as high as 0.45. Patients were infused at a rate of 1.2 mL/kg/h, increasing at 20-min increments to a rate of 4.8 mL/kg/h. Treatment of infusion-related adverse experiences was specified in the protocol.

**Product description.** HIVIG (North American Biologicals, Boca Raton, FL) is purified human IgG containing high titers of antibody to HIV structural proteins [15]. HIVIG is prepared from plasma of HIV-seropositive donors from diverse regions of the United States. Donors were asymptomatic; had >400 CD4 cells/µL; were negative for HIV-1 p24 antigen, hepatitis B surface antigen, and antibody to hepatitis C virus; and had a normal alanine aminotransferase (ALT) test.

There are multiple steps in the manufacture of HIVIG to inactivate HIV, including solvent/detergent treatment. These procedures reduce infectious virus concentrations in starting plasma by a factor of >14 log₁₀. The final product is a 5% (50 mg/mL) solution that contains >98% monomeric plus dimeric IgG. It has an endpoint anti-p24 titer of 1 x 1000 at 50 mg/mL (5%) by the Abbott (Abbott Park, IL) HIVAB p24 (rDNA) EIA. HIVIG is negative for HIV-1 p24 antigen and for HIV-1 RNA by polymerase chain reaction (PCR), is unable to infect activated human lymphocytes, and is negative for hepatitis C virus RNA by PCR. Against HIV-1and other laboratory-adapted strains, the product binds strongly to related V3 loop peptides, inhibits syncytia formation at 357–1800 µg/mL, and has antibody-dependent cellular cytotoxicity activity at 2.5–250 µg/mL [15]. HIVIG can neutralize primary isolates, including 50% neutralization of 10 of 12 infant isolates and 90% neutralization of 3 of 12 infant isolates [16, 17].

**Specimen collection.** Blood obtained before study entry was tested for complete blood count (CBC), chemistry panel, T cell subsets, immunoglobulin levels, and ICD p24 antigen. The chemistries and CBCs were repeated at entry and on day 56 (before infusion 3), day 140 (before infusion 6), and day 224 (12 weeks after the final infusion). The virologic studies were performed at the local site (peripheral blood mononuclear cell [PBMC] HIV coculture) or at a single laboratory (ICD p24, RNA PCR, neutralizing antibody).

**Virologic assays.** Quantitative HIV-1 PBMC cocultures were done before study entry, at entry, and on days 14, 28, 84, 140, 168, and 224 by use of an ACTG protocol and were reported as infectious units per million mononuclear cells (IUPM) [18]. Virus-containing supernatants were saved for autologous neutralizing antibody titers.

Blood for plasma HIV-1 RNA and serum HIV-1 p24 ICD antigen was obtained before and at entry and on days 1, 7, 14, 28, 84, 140, 141, 147, 154, 168, and 224. These samples were separated, frozen at −70°C, and assayed at a single ACTG laboratory (Y.B., UCLA). HIV-1 RNA was measured by an HIV-1 PCR assay (Amplicor; Roche Molecular Systems, Alameda, CA) [18] and HIV ICD p24 by the Abbott EIA sandwich assay [19]. Serial ICD p24 results were available for 2 children in group 1, 6 in group 2, and 7 in group 3.

Neutralizing antibody studies were done on 12 of the 30 patients, using entry plasma and day 147 plasma (1 week after infusion 6): 4 patients in each treatment group, 2 who had decreased HIV RNA copy numbers and 2 who had stable or increased HIV RNA copy number levels during their infusions. The virus isolates from PBMC cocultures at entry and at day 147 were expanded by culture with phytohemagglutinin (PHA)-activated normal PBMC; the supernatants were frozen and titered for virus [16]. Two-fold dilution of the patient plasma (1 : 10 to 1 : 160) or HIVIG (7.8–1000 µg/mL) in triplicate were incubated with 50 TCID₅₀ of virus in 96-well microtiter plates. PHA-stimulated PBMC were added, and plates were incubated at 37°C for 7 days. HIV p24 antigen was determined by use of a Coulter kinetic assay on the supernatants. Neutralization was defined as a 50% or 90% reduction of p24 viral antigen in the supernatant of the wells, compared with p24 antigen in the supernatant of virus incubated with HIV-1 antibody–negative plasma and PHA-stimulated PBMC.

**Immunologic assays.** Immunoglobulin assays (assessed at entry and on days 140 and 224) were done by quantitative nephelometry. T cell subsets (CD3, CD4, and CD8—at entry and on days 56, 140, and 224) were assessed by flow cytometry in ACTG-approved laboratories. For pharmacokinetics analysis, HIV-1 p24 antibody assays were performed on serum samples obtained before infusion

### Table 1. Baseline characteristics (mean) of 3 groups of human immunodeficiency virus (HIV)–positive children receiving human immunoglobulin.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Group 1 (200 mg/kg)</th>
<th>Group 2 (400 mg/kg)</th>
<th>Group 3 (800 mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>6.1 (3–11)</td>
<td>6.5 (4–10)</td>
<td>5.3 (2–8)</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>22.4 ± 7.1</td>
<td>21.3 ± 5.1</td>
<td>19.1 ± 4.0</td>
</tr>
<tr>
<td>Boys/girls</td>
<td>9/1</td>
<td>3/7</td>
<td>5/5</td>
</tr>
<tr>
<td>CD4 cells/µL</td>
<td>477 ± 30</td>
<td>569 ± 193</td>
<td>855 ± 242</td>
</tr>
<tr>
<td>CD4, %</td>
<td>20.4 ± 1.7</td>
<td>25.1 ± 2.7</td>
<td>25.1 ± 2.9</td>
</tr>
<tr>
<td>IgG, mg/dL</td>
<td>1974 (1147–2809)</td>
<td>2300 (1170–2066)</td>
<td>2269 (1090–5320)</td>
</tr>
<tr>
<td>HIV p24 antigen, pg/mL</td>
<td>260 (152–401)</td>
<td>425 (107–1063)</td>
<td>298 (119–620)</td>
</tr>
<tr>
<td>HIV RNA, log₁₀ copies/mL</td>
<td>4.5 ± 0.65</td>
<td>4.89 ± 0.51</td>
<td>4.59 ± 0.81</td>
</tr>
<tr>
<td>HIV, infectious U/10⁶ cells*</td>
<td>15 (0.5–303)</td>
<td>63 (16–420)</td>
<td>61 (0.2–2503)</td>
</tr>
</tbody>
</table>

* Median (range).

**NOTE.** Data are mean (range) or mean ± SD unless noted.
Figure 1. Pharmacokinetics data (mean ± SD reciprocal p24 antibody titer values vs. time) in 3 groups of children treated with human hyperimmune immunoglobulin (HIVIG). Data are for children given 200 (●), 400 (▲), and 800 (▲) mg/kg. HIVIG infusions were administered on day 0 and then every 28 days for a total of 6 infusions.

1; at 1 h, 24 h, and 4, 7, and 14 days after infusion 1; before and after infusion 2; before infusions 3–6; and 1 h, 24 h, and 4, 7, 14, 28, 56, and 84 days after infusion 6. All HIV-1 p24 antibody titers were measured in triplicate by Abbott HIVAB p24 (rDNA) EIA over 8 5-fold serial dilutions (1:5 to 1:390,625) in a batched fashion. Values were reported as an end-point titer. These assays were done in a single laboratory (Quest Diagnostics, Baltimore) and were analyzed at the Baylor PACTG Pharmacology Core Laboratory (Houston).

Statistical methods. We used Kruskal-Wallis tests to compare differences across the 3 dose groups (HIVIG 200, 400, and 800 mg/kg) for demographic data (age, weight, baseline CD4 cells, and baseline virus load) and to detect dose differences in the immunoglobulin levels and quantitative coculture measures between 2 time points. Adverse events were categorized as grades I or II (mild), grade III (moderate to severe), and grade IV (life threatening). Longitudinal changes in lymphocyte markers and virus load (as measured by HIV RNA copy number) were analyzed by use of linear mixed-effects models. In addition to fitting a linear mixed-effects model that used all available data, we used Kruskal-Wallis tests to evaluate differences in the CD4 cell and HIV RNA copy number changes from baseline to selected time points. Two-sided Wilcoxon signed rank tests assessed the statistical significance of changes from baseline to days 140 and 224 for each immunoglobulin level. For the pharmacokinetics studies, HIV-1 p24 antibody titers were analyzed, as described elsewhere [13]. Distribution (T1/2, α) and elimination (T1/2, β) phase half-lives, steady-state volume of distribution (Vdss), area under the curve, and total body clearance were calculated by standard pharmacokinetics equations.

Results

Demographics. Baseline comparisons of the groups are shown in table 1. Of the children studied, there were 17 boys and 13 girls. By race, 18 were black, 5 white, 6 Hispanic, and 1 Asian, and were distributed randomly.

Safety. All 30 patients completed 6 infusions; 1 child withdrew because of an increasing HIV RNA level at 28 weeks, 8 weeks after infusion 6. No changes in antiviral therapy occurred during the infusions, but 15 patients (6 in group 1, 5 in group 2, and 4 in group 3) had changes after day 140. With 1 exception, the infusions were well tolerated. One child in group 3 developed decreased capillary refill and mottled skin during infusions 4 and 6 but was able to complete the infusions when the rate was decreased and diphenhydramine and acetaminophen were administered.

Twenty-two (73%) of the 30 children, distributed equally in the 3 groups, developed a grade I chemistry toxicity (usually a slight elevation of aspartate aminotransferase or ALT). Twelve (40%) developed a hematologic grade I and 10 a grade II toxicity (leukopenia or anemia), none thought to be related to HIVIG, and distributed equally in the groups. One grade III clinical toxicity occurred in group 1 (otalgia), 2 in group 2 (rhinitis, skin blisters), and 3 in group 3 (fainting, vomiting, and hypotension); only the hypotension was thought to be related to an infusion.

T cell subsets. As noted in table 1, baseline CD4 cell levels in group 3 were higher than in groups 1 and 2. There was a
Table 2. Pharmacokinetics of human hyperimmune immunoglobulin in human immunodeficiency virus-infected children at 3 dose levels.

<table>
<thead>
<tr>
<th>Group (dose)</th>
<th>Half-life (T½, days)</th>
<th>Volume of distribution, mL/kg</th>
<th>Clearance, mL/kg/day</th>
<th>Area under curve, titer* day/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (200 mg/kg)</td>
<td>16 ± 4.8</td>
<td>113 ± 33</td>
<td>6 ± 2.6</td>
<td>127,877 ± 43,999</td>
</tr>
<tr>
<td>2 (400 mg/kg)</td>
<td>13 ± 3.2</td>
<td>102 ± 17</td>
<td>6 ± 1.2</td>
<td>230,367 ± 40,654</td>
</tr>
<tr>
<td>3 (800 mg/kg)</td>
<td>16 ± 5</td>
<td>108 ± 16</td>
<td>5.6 ± 2.3</td>
<td>536,676 ± 156,360</td>
</tr>
</tbody>
</table>

* Excludes 1 patient because of very high baseline p24 antibody level.

slight but significant overall decrease (P = .04) of CD4 cells over time; this was greatest in group 2, decreasing from a mean of 596 cells/µL at entry to 444/µL at week 20 (26% decrease). In group 1 there was a mean increase of 10% and in group 3 a mean decrease of 3% over the same period, but these were not statistically significant. A further analysis of entry CD4 cell counts indicated no trend in changes of T cells among children with <400, 400–600, or >600 CD4 cells/µL. CD3 and CD8 cell counts paralleled the CD4 cell counts.

Immuno globulin levels. The initial serum immunoglobulin assessments (table 1) showed diffuse hypergammaglobulinemia in the 3 groups. The median IgG for all groups was 2002 mg/dL; and IgA, 238 mg/dL (range, 32–623 mg/dL). Two-sided Wilcoxon signed rank tests showed that changes from baseline to day 140 (after 5 infusions) and to day 224 (12 weeks after the final infusion) were not statistically significant for any of the immunoglobulins. These data indicate no increase of immunoglobulin in the serum in the patients’ circulation.

ICD p24 antigen levels. All patients had HIV ICD p24 levels ≥70 pg/mL before the infusions. After the infusions, the HIV p24 antigen decreased to <6 pg/mL in 12 of 13 children, usually within 24 h, and remained low or undetectable during the infusions. One child in group 3 with an initial p24 antigen level of 119 pg/mL had detectable p24 antigen throughout the infusions (22–372 pg/mL). p24 antigen increased to >6 pg/mL in 6 patients on day 226, 3 months after the final infusion.

HIV-1 RNA PCR. The median baseline log10 HIV RNA copy numbers in the 3 groups (table 1) did not differ statistically from one another. The changes in RNA copy numbers at various times throughout the study are shown in figure 1. There was no appreciable change in RNA copy numbers throughout the treatment period across dose groups (P = .52), nor was there evidence of dosage effect (P = .37). Furthermore, when patients were stratified by age, entry CD4 cell count, and initial RNA levels, there were no discernable treatment effects on changes in RNA copy number (P = .44). A Student’s t test performed on the difference between the log10-transformed RNA levels on days 140 and 224 showed a suggestive drop (P = .07) of RNA levels during the postinfusion follow-up period (day 224) among all patients, 12 of whom switched antiviral medication during this period, including those who received a protease inhibitor.

Quantitative cultures. The PBMC quantitative coculture titers showed a considerable variation at entry but were similar in the 3 groups (table 1). There was no significant change after the 6 infusions (week 24), nor was a dosage effect noted (P = .7). The number of children who had decreases, increases, or unchanged levels of IUPM was equivalent in the 3 groups.

Pharmacokinetics. By use of serial HIV p24 antibody titers, the pharmacokinetics of HIVIG were well described by a 2-compartment model. Figure 2 shows the p24 antibody levels over time in the 3 groups. Table 2 summarizes the pharmacokinetics parameters. The p24 antibody concentrations at baseline and before and after HIVIG infusions are shown in table 3. There was a good correlation between the HIVIG dose and the peak and trough antibody levels achieved.

Neutralizing antibody. The HIVIG used in the study could neutralize in vitro the entry virus of all 12 children studied when a 50% neutralization end point (median titer, 1 : 825) was used and in 11 of 12 children with a 90% neutralization end point (median titer, 1 : 18). The HIVIG also neutralized day 147 virus isolates of all children with a 50% neutralization end point (median titer needed, 1 : 51) and 6 of 12 virus isolates with a 90% neutralization end point (median titer needed, 1 : 630). The increase in the titer of HIVIG necessary to neutralize the day 147 viruses suggested development of more neutralization-resistant strains.

Three of 12 preentry plasma specimens had neutralizing antibody to their autologous entry virus isolates at a 50% neutralizing antibody end point and 2 of 12 at a 90% neutralizing antibody end point. By day 147 (after 6 infusions), the plasma showed neutralizing antibody in 9 of 12 patients (at a 50% neutralizing antibody end point) and in 1 of 12 patients (at a 90% neutralizing antibody end point). suggesting a significant increase in neutralizing antibody titer with infusions, when the 50% neutralizing antibody end point (but not the 90% neutralizing antibody end point) was used.

The neutralizing antibody titers of 6 children (2 in each treatment group) who had significant decreases in HIV RNA copy numbers during the infusions were examined to determine whether they developed higher neutralizing antibody titers during the HIVIG infusions than those of the 6 children who had an unchanged or increasing virus burden. There was no apparent relationship.

Table 3. Human hyperimmune immunoglobulin p24 antibody concentrations (in pg/mL) at baseline and average peak and trough levels.

<table>
<thead>
<tr>
<th>Group (dose)</th>
<th>Baseline</th>
<th>Peak</th>
<th>Trough</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (200 mg/kg)</td>
<td>183 ± 537</td>
<td>11,370 ± 2,363</td>
<td>1826 ± 849</td>
</tr>
<tr>
<td>2 (400 mg/kg)</td>
<td>35 ± 110</td>
<td>23,092 ± 5,635</td>
<td>2879 ± 1,054</td>
</tr>
<tr>
<td>3 (800 mg/kg)</td>
<td>31 ± 43</td>
<td>50,174 ± 8,310</td>
<td>7884 ± 3,973</td>
</tr>
</tbody>
</table>

* Excludes 1 patient because of high baseline p24 antibody titer.
Figure 2. RNA copy no. during human hyperimmune immunoglobulin (HIVIG) infusions. Mean ± SD of plasma log_{10} RNA copy vs. time in 3 treatment groups. HIVIG infusions were administered on day 0 and then every 28 days for total of 6 infusions. There were no significant changes during treatment periods or differences in treatment groups.

Discussion

This study indicates that HIVIG is safe and usually well tolerated by children with moderately advanced HIV infection, even in doses of 800 mg/kg, which is 4-fold the dose used in prior studies of HIVIG [7–11]. Only 1 patient had a grade III (serious) reaction to the infusions. Minor side effects were readily controlled by medication or by decreasing the rate of infusion.

The HIVIG half-life of 13–16 days with a volume of distribution of 102–113 mL/kg in all 3 treatment groups is in general agreement with 2 prior studies of HIVIG pharmacokinetics in HIV-infected men [12] and HIV-infected pregnant women [13]. The half-life is somewhat shorter than the normal half-life of IVIG of 20–25 days [20], which may be secondary to the patients’ hypergammaglobulinemia with increased IgG catabolism. This increased catabolism may be responsible for the absence of an increase in the total IgG levels and the low neutralizing antibody titers at the 90% end point.

In all patients studied serially, there was a rapid decrease or disappearance of ICD p24 antigen from the serum following the HIVIG infusion, as observed in previous studies [7, 10, 11, 13]. This probably represents complexing of endogenous p24 with the infused p24 antibody. Indeed the plasma HIV RNA copy number and the cellular IUPM titers were not altered by the infusions, even at the highest HIVIG dose. Although p24 antibody titers increased as a result of the HIVIG infusion, a decrease in circulating virus was not noted. There were no dramatic increases in HIV copy number, indicating no enhancement of viral replication. Although all 30 children remained clinically stable during the infusions, several had a slow decrease in CD4 cell counts and an increase in plasma HIV RNA copy numbers. For 12 patients, their physicians changed antiretroviral medications during the follow-up period.

In previous controlled studies, Jacobson et al. [8] gave 250 mL of HIV immune plasma (or control plasma) every 4 weeks to adult AIDS patients but found no difference in plasma or cell virus titers, patient survival, or time to a new opportunistic infection. Vittecoq et al. [7, 11] gave 300 mL of hyperimmune plasma (or control plasma) every 2–4 weeks to HIV-infected adults with <200 CD4 cells/mm³. They found fewer AIDS-defining events in the treatment group but no survival benefit or change in the rate of CD4 cell decline. Levy et al. [10] gave 500 mL of immune plasma at monthly intervals to HIV-infected adults with 50–200 CD4 cells/mm³ and noted a slight increase...
in CD4 cells and improved survival. These studies were in adults with advanced disease and thus are not comparable with our studies in less ill children. The 800 mg/kg HIVIG dose was considerably higher than the amount of IgG in the immune plasma given to the adults; furthermore, the donor pool for HIVIG is larger than in the plasma-treated patients. Nevertheless, no antiviral effect as measured by RNA PCR levels was observed. This is best explained by the low neutralizing antibody titers against autologous virus achieved in the study patients after the HIVIG infusions.

Wolfe et al. [21] and Günthard et al. [22] gave HIV neutralizing MAbs to HIV-infected adults at doses of 100–500 mg/m² and 50–200 mg, respectively. A half-life of 13–16 days was noted, similar to that of HIVIG in this study. There was persistence of the antibody in the blood for 21 days but minimal reduction in virus burden.

Clinical studies that compared the efficacy of respiratory syncytial virus (RSV) hyperimmune globulin with RSV MAb [23] and in vitro studies that compared the neutralizing ability of HIVIG with HIV MAbs [16] indicate that MAbs are 2–3 log more potent than a polyvalent immunoglobulin. Thus, a dose of HIVIG equivalent to a MAb would be 5–50 g/m², a prohibitively large dose.

The modest levels of HIV neutralizing antibody achieved do not exclude a clinical benefit of passive antibody administration. Such a beneficial effect could derive from nonneutralizing antibodies effective in antibody-dependent cellular cytotoxicity or to antibodies to opportunistic organisms [6, 24]. Without an IVIG control group, we could not determine whether HIVIG was of clinical benefit. IVIG without HIV antibody has some clinical benefit in both adults and children [5]. It would be of interest to determine whether various MAbs given at a dose sufficient to maintain a high neutralizing antibody titer would have an antiviral activity [24, 25].

References