Intravenous Anti-D Treatment of Immune Thrombocytopenic Purpura: Analysis of Efficacy, Toxicity, and Mechanism of Effect

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The efficacy, toxicity, and mechanism of effect of intravenous Anti-D (Winrho) were studied in 43 Rh- patients with immune thrombocytopenic purpura (ITP) who had not undergone splenectomy and in three already splenectomized patients. The mean platelet increase for the 43 nonsplenectomized patients was 95,000/μL (median 43,000/μL). Children had greater acute platelet responses than did adults. Human immunodeficiency virus status and duration of thrombocytopenia did not affect response. Maintenance treatment was given to patients as needed: the average interval between infusions was 24 days. The three splenectomized patients had no platelet response whatsoever. Toxicity was minimal; infusions were completed in less than 5 minutes. The generally accepted mechanism of effect of Anti-D has been Fe receptor blockade by substitution of antibody-coated red blood cells for antibody-coated platelets. Evidence is presented suggesting that the effect of IV Anti-D is not limited to Fe receptor blockade, including: (1) no correlation of parameters of hemolysis with platelet increase; (2) a 48- to 72-hour delay before platelet increase; (3) a tendency of the change in monocyte Fe receptor 1 expression to correlate with platelet increase; and (4) increased in vitro production of antibodies to sheep red blood cells following IV Anti-D infusion.

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IMMUNE thrombocytopenic purpura (ITP) is manifested by a bleeding tendency, the severity of which is related primarily to the platelet count. The thrombocytopenia has been shown to be caused by antiplatelet antibodies that mediate platelet destruction by interacting with Fe receptors (FcRs) on macrophages. The standard treatments of ITP include glucocorticoids, splenectomy, and intravenous gammaglobulin (IVIG). Infusion of IVIG had been shown to result in so-called “Fc receptor blockade” as demonstrated by serial study of the autologous red blood cell (RBC) clearance before and after infusion.

The use of Anti-D in ITP came about as a result of the effectiveness of IVIG treatment of this disease. Salama et al. hypothesized that low levels of anti-RBC antibodies contained in the IVIG preparations might be responsible for the FeR blockade as well as the increase in the platelet count by substituting antibody-coated RBCs for antibody-coated platelets. To provide proof for this hypothesis, they injected Rh- ITP patients with Anti-D and demonstrated that the platelet count increased in the majority of Rh- patients. Clinical studies have shown efficacy in children and in human immunodeficiency virus (HIV)-infected patients. Exploration of the mechanism of Anti-D effect has been limited to showing that Rh- patients do not respond to it and to a small study indicating the lack of relationship of response to the degree of hemolysis.

This study was performed to evaluate the clinical efficacy of IV Anti-D and to explore its mechanism of effect during the treatment of 46 patients, more than twice the size of any of the previous studies. It demonstrated that: (1) children responded better than did adults; (2) HIV-infected individuals responded as well as did classical ITP patients; (3) platelet counts could be maintained at adequate levels with infusions at a mean interval of 24 days; (4) 5 of 14 children with chronic ITP were able to discontinue treatment with a mean follow-up of 13 months; (5) minimal toxicity was seen, even with infusion durations of less than 3 minutes; and (6) that the mechanism of effect may not be limited to FeR blockade based on changes in FeR1 expression on circulating monocytes and changes in in vitro Ig production to sheep RBCs. These findings imply that the FeR interactions of Anti-D–coated RBCs may have immunomodulatory effects.

METHODOLOGY

Patient Selection and Inclusion

The trial was an unblinded single treatment arm pilot study of Rh- patients with ITP (Fig 1). Thirty-eight of 46 patients had pretreatment platelet counts less than 30,000/μL. Eight patients were included at higher counts for: (1) ongoing but not major bleeding; (2) upcoming surgery; or (3) if a patient had traveled a long distance to receive treatment, had consistently needed treatment for low counts, and was currently on a treatment such as prednisone that would have temporarly elevated the platelet count.

Three additional patients beyond the 46 were treated but excluded from the analysis. Two patients were said to be Rh- by their referring physicians but were discovered to be Rh- after initial treatment. One child was subsequently discovered to have a hemangioma causing her thrombocytopenia. After the treatment of three splenectomized patients who had no platelet response to high-dose Anti-D treatment (see Results) as had been previously reported, no more already splenectomized patients were entered in the study.

The main subgroups of patients analyzed were the 20 children and 17 HIV+ patients; 14 of the children had had ITP for more than 6 months before IV Anti-D treatment and three of the children were HIV+. The 23 adults included 14 HIV+ patients.

Approval for this study was obtained from the New York Hospital Institutional Review Board and consent was obtained from each patient and/or their parents before initiation of treatment.

There were two sets of seven patients each analyzed in the pilot studies of monocyte and neutrophil FeR expression and of in vitro
Ig production. The patients involved were chosen according to availability of the testing as a pilot study of the mechanism.

Preparation

The Winrho preparation of Anti-D was used (Winnipeg Rh Institute of the University of Manitoba, Winnipeg, Canada). It is the only preparation of Anti-D in North America that can be administered IV. The plasma is obtained from 35 sensitized donors whose plasmaphresed weekly. The initial processing step is the only preparation of Anti-D in North America that can be received IV. The plasma is obtained from 35 sensitized donors whose plasmaphresed weekly. The initial processing step is added instead of the combination of antihuman Ig and complement as with the standard plaque assay. Controls were used with saturating concentrations of monoclonal antibodies (MoAbs). FITC-conjugated antimouse IgG (Cappel) was added to the washed cells before flow cytometry. The MoAbs used were: 3G8 (anti-FcRII), neutrophils only; “IV.3” (anti-FcRII); and “32.2” (courtesy of Paul Guyre, Dartmouth Medical Center, Hanover, NH) and “27.17,” both anti-FcRI. In addition, both autofluorescence and irrelevant IgG1 and IgG2a monoclonals were used as controls. Results were expressed as (changes in) units of mean channel fluorescence.

Dose

Two protocols of dosing Anti-D in nonsplenectomized patients were followed during induction, ie, the first week of treatment. The first 13 patients received an initial dose of 10 μg/kg followed by daily doses of 20 μg/kg/d until either the platelet count increased by a minimum of 20,000 to 30,000/μL or the hemoglobin decreased by greater than 2 g/dL. After the first 13 patients were treated, it became clear that the platelet response did not occur within 24 hours and that no patient developed significant anemia after receiving 30 μg/kg during the first 2 days. Subsequently patients 14 through 49 received 25 μg/kg on day 1, with the same dose repeated as needed on days 3 and 4 using the same criteria as described previously for cessation of treatment. More than 90% of the patients received two doses and there was not a significant difference in the quantity of Anti-D infused between the two groups.

The three splenectomized patients were treated differently in view of their lesser likelihood of response; they received a total dose of 100 to 150 μg/kg each.

Based on the treatment model developed using IVIG, maintenance treatment was administered as a single dose infused as needed to keep the platelet count greater than 20,000 to 30,000/μL. Single maintenance doses were chosen, depending on the clinical situation, to be between 50% and 100% of the total induction dose. Patients requiring repeated maintenance treatments had their doses systematically alternated in an attempt to define the optimal maintenance dose. Because the initial dose varied based on individual response, the actual infused maintenance doses varied considerably and ranged between 25 and 60 μg/kg/infusion. Several patients were infused enough times so that a third dose of Anti-D could be compared with the optimal of the two initially compared doses.

Routine Laboratory Measurements

Complete blood counts, liver function tests, direct and indirect Coombs test, and IgG, IgM, and IgA levels were measured by the routine hospital laboratories. Blood counts were performed with a Coulter S+2; reticulocyte counts and white blood cell (WBC) differential counts were performed manually. Because the RBC number was not available for all blood counts at the time of analysis, the reticulocyte count was corrected for a hemoglobin of 13 g/dL rather than determining the absolute reticulocyte count using the formula: corrected retic count = measured retic count × patient hgb/13. Platelet counts were verified by examination of the peripheral smear and measured manually in the case of discrepancies. Ig levels were assayed by nephelometry.

Flow Cytometry

Measurements of FcR expression by circulating monocytes and neutrophils were performed as previously described in paired studies before Anti-D infusion and 5 to 7 days after the initial infusion. In brief, neutrophils and monocytes were isolated using Ficoll-Hypaque, and incubated in phosphate-buffered saline (PBS) with saturating concentrations of monoclonal antibodies (MoAbs). FITC-conjugated antimouse IgG (Cappel) was added to the washed cells before flow cytometry. The MoAbs used were: 3G8 (anti-FcRII), neutrophils only; “IV.3” (anti-FcRII); and “32.2” (courtesy of Paul Guyre, Dartmouth Medical Center, Hanover, NH) and “27.17,” both anti-FcRI. In addition, both autofluorescence and irrelevant IgG1 and IgG2a monoclonals were used as controls. Results were expressed as (changes in) units of mean channel fluorescence.

In Vitro Ig Production

In vitro Ig production was performed as previously described using the reverse-plaque assay. In brief, percoll isolated mononuclear cells were incubated in wells at a density of 10⁵ cells per well in triplicate and stimulated with pokeweed mitogen, staphylococcus protein A (Cowan strain), and Epstein-Barr virus. After incubation for 5 to 7 days, guinea pig complement and rabbit antihuman IgG were added as the test reagents and the number of “plaques” were counted. “Specific” antibody production (to sheep RBCs) was assayed by the use of sheep RBCs with only complement added instead of the combination of antihuman Ig and complement as with the standard plaque assay. Controls were evaluated in conjunction with the ITP patients and showed no changes over the time course of study.

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**Fig 1. Outcome of IV Anti-D-treated patients. Flow diagram of the 49 patients who received IV Anti-D.** Note that six patients were removed from analysis (top of the figure), including three who had been splenectomized before treatment, and that three additional patients were lost to follow-up. In addition, patients did not receive maintenance for either of two reasons: sustained response to initial treatment or failure to respond to initial treatment.
Data Analysis

The data were described using means, medians, ranges, and standard deviations. Spearman correlation coefficients and t-tests were used to ascertain the probability of correlation and of differences, respectively.

A response was defined as a platelet increase ≥20,000/μL. In analyzing the effects of different doses of IV Anti-D during maintenance treatment, no patient was included unless they had two consecutive pairs of alternate doses available from a period during which their disease was stable; this limited the evaluable patients to eight from an initial group of 12.

Long-term outcome was divided into the categories of: remission; stable without therapy, i.e., a platelet count less than 150,000/μL but greater than 20 to 30,000/μL on no treatment; maintenance, which was divided into patients receiving infusions as needed at greater than or less than 3-week intervals between infusions; and refractory.

RESULTS

Induction Treatment

Increases in Platelet Counts

The 43 Rh⁺ ITP patients who had not undergone splenectomy form the basis of this report. The mean initial platelet count was 22,000/μL with a range of 6 to 45,000/μL. The mean increase in the platelet count was 55,000/μL with a median increase of 43,000/μL (see Table 1). Only 9 of 43 cases (21%) failed to have a response, defined as a platelet increase ≥20,000/μL. Ten patients, nine of which were children, had a platelet increase greater than 100,000/μL.

Platelet increases were greater in children, median 92,000/μL, than in adults, median 26,000/μL (Table 1). Regression analysis showed that the increase in the platelet count diminished with age (P < .02, Fig 2). There was no difference in platelet response between HIV⁺ and HIV⁻ patients (Table 1) and between patients with acute and chronic disease (data not shown).

The platelet count after infusion of Anti-D required 72 hours to become significantly different from the pretreatment count (see Fig 3). The peak platelet count occurred at a mean of 8 days following the initial infusion.

Signs and symptoms of hemorrhage, such as petechiae and purpura, gradually disappeared as the platelet count increased. Eleven patients were able to undergo minor and major surgical procedures without hemorrhage after receiving Anti-D treatment.

Dose

Two different dose-response regimens were used during the period of study (see Methodology). The first 13 patients received a dose of 10 and then daily doses of 20 μg/kg/d until either the platelet count increased by greater than 20 to 30,000/μL or the hemoglobin decreased by greater than 2 g/dL. The subsequent patients (14 through 49) were infused with 25 μg/kg on day 1 with additional doses if needed on days 3 and 4. The mean dose of IV Anti-D infused during induction was 51.7 μg/kg. Children and adults and HIV⁺ and HIV⁻ patients received similar induction doses (Table 1).

There was no relationship between the dose of Anti-D and the subsequent platelet increase (Fig 4). This lack of correlation persisted even when splenectomized patients and nonresponders were removed from the analysis. How-

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Table 1. Platelet Increase and Hemoglobin Decrease in Response to IV Anti-D

<table>
<thead>
<tr>
<th>Platelet Increase</th>
<th>% All (N = 43)</th>
<th>% Children (20)</th>
<th>% Adults (23)</th>
<th>% HIV⁺ (17)</th>
<th>% HIV⁻ (26)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 20</td>
<td>21 (9)</td>
<td>15 (3)</td>
<td>26 (6)</td>
<td>18 (3)</td>
<td>23 (6)</td>
</tr>
<tr>
<td>20–50</td>
<td>30 (13)</td>
<td>10 (2)</td>
<td>48 (11)</td>
<td>41 (7)</td>
<td>23 (6)</td>
</tr>
<tr>
<td>51–100</td>
<td>26 (11)</td>
<td>30 (6)</td>
<td>22 (5)</td>
<td>24 (4)</td>
<td>27 (7)</td>
</tr>
<tr>
<td>101–150</td>
<td>9 (4)</td>
<td>15 (3)</td>
<td>4 (1)</td>
<td>6 (1)</td>
<td>12 (3)</td>
</tr>
<tr>
<td>&gt; 150</td>
<td>14 (6)</td>
<td>30 (6)</td>
<td>0 (0)</td>
<td>12 (2)</td>
<td>15 (4)</td>
</tr>
</tbody>
</table>

Mean hemoglobin decrease 1.9* 1.5 2.2 1.9 1.9

Platelet count increase × 10⁻³/μL. There was no difference between patients with acute and chronic disease.

Hemoglobin decrease in grams per deciliter.
ever, there was a weak correlation between the dose of Anti-D and the decrease in the hemoglobin (r = .35, n = 43, P = .05).

**Change in Hemoglobin and Reticulocyte Counts**

The mean decrease in hemoglobin was 1.9 g/dL. The hemoglobin decreased greater than 3.0 g/dL in eight patients, six of whom were adults with poor responses to Anti-D. The mean decrease in the hemoglobin by patient groups is presented in Table 1: HIV+ patients did not have greater hemoglobin decreases than did HIV- patients. The nadir in hemoglobin concentration occurred on day 6 (median), followed by a return to baseline on day 12. The mean peak increase in the corrected reticulocyte count was 1.1%, which also occurred on day 6. Neither the change in hemoglobin levels (A) nor the peak corrected reticulocyte counts (B) had any relationship to the platelet increase (both P > .30, Fig 5).

**Splenectomized Patients**

The three splenectomized patients were analyzed separately. They received an average of 9.1 mg per patient, greater than 100 μg/kg/patient, without any platelet response. One patient had a positive direct Coombs test before starting treatment; her hemoglobin was the only one of the three patients' to decrease (patient 3, Table 2). A positive indirect (as well as direct) Coombs test developed in these three patients; none of 20 consecutive nonsplenectomized patients, all of whom became direct Coombs positive, developed a positive indirect Coombs test indicating the higher dose received by the splenectomized patients.

**Maintenance**

**Long-term Outcome**

Thirteen of 43 patients (Fig 1 and Table 3) did not receive maintenance treatment. Eight were refractory to treatment and five patients entered remission (3) or became stable without therapy (2) with induction treatment alone.

Thirty of 43 patients received maintenance treatment (Fig 1). Three HIV+ adult patients were lost to follow-up after a single maintenance infusion. The remaining 27 patients received maintenance infusions for a mean of 13 months. The mean and median interval between treatments was 24 days.

Of the 27 patients on maintenance, two achieved remission and six became stable without therapy. The remaining 19 patients required continued treatment, 15 of which received treatment at intervals greater than 3 weeks. Thirteen of the 19 patients continue to receive maintenance infusions. Six patients discontinued IV Anti-D for splenectomy in four cases and prednisone in two cases. In this series only one patient initially responding to IV Anti-D subsequently became refractory to it; however, several
patients required an increase in their maintenance dose during treatment.

In overall summary of long-term outcome, eliminating the two children with acute ITP treated at onset because of their high rate of spontaneous remission and the three patients lost to follow-up immediately after starting maintenance, the outcomes in 38 patients were: 3 (8%) remission; 8 (21%) stable without therapy; 19 (50%) maintenance; and 8 (21%) refractory. Therefore, combining patients achieving remission with those who became stable without therapy, 8% + 21%, or 29%, of all patients were able to eventually discontinue all treatment.

Subgroup Analysis of Long-Term Outcome

Children with chronic ITP (Table 3). Of the 14 children with chronic disease, two achieved remission and three became stable without treatment for a rate of discontinuation of treatment of 5 of 14 or 36% for the children with chronic ITP. These results were based on a mean follow-up of 13 months.

HIV+ patients. Among the 14 HIV+ patients evaluable for long-term response, four were able to discontinue treatment; eight of nine were maintained with repeated infusions at intervals greater than 3 weeks; and only one was refractory. Four HIV+ adults who had required treatment for more than 1 year showed substantial improvement on Anti-D: two discontinued therapy and two eventually required maintenance at 3-month intervals. Four patients who changed from IVIG maintenance to IV Anti-D had intervals between infusions that were a median of 10 days longer while on IV Anti-D than while on IVIG (range 7 to 16 days longer).

Adults. Among the nine HIV- adults, three of four with acute ITP had good responses to Anti-D while four of five patients with chronic disease were refractory. All of the adults refractory to Anti-D were HIV-.

Dose

The mean initial maintenance dose was 43 μg/kg infusion or 82% of the total induction dose. HIV+ patients received

Table 2. Lack of Effect of IV Anti-D in Splenectomized Patients

<table>
<thead>
<tr>
<th>Day of Treatment:</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pt. 1 (53 kg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dose*</td>
<td>1,200</td>
<td>2,400</td>
<td>2,400</td>
<td>—</td>
<td>2,400</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Platelet</td>
<td>15</td>
<td>13</td>
<td>15</td>
<td>14</td>
<td>18</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hgb</td>
<td>13.7</td>
<td>13.3</td>
<td>13.7</td>
<td>13.2</td>
<td>13.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pt. 2 (84 kg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dose</td>
<td>1,200</td>
<td>800</td>
<td>2,700</td>
<td>2,700</td>
<td>—</td>
<td>2,700</td>
<td></td>
</tr>
<tr>
<td>Platelet</td>
<td>17</td>
<td>20</td>
<td>16</td>
<td>21</td>
<td>21</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hgb</td>
<td>13</td>
<td>12.1</td>
<td>12.8</td>
<td>12.7</td>
<td>12.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pt. 3 (61 kg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dose</td>
<td>1,500</td>
<td>15</td>
<td>1,500</td>
<td>1,500</td>
<td>1,500</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>Platelet</td>
<td>37</td>
<td>34</td>
<td>31</td>
<td>32</td>
<td>41</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>Hgb</td>
<td>13.2</td>
<td>13.2</td>
<td>12.5</td>
<td>12.1</td>
<td>11.3</td>
<td>10.4</td>
<td></td>
</tr>
<tr>
<td>Average platelet count</td>
<td>23</td>
<td>22</td>
<td>21</td>
<td>22</td>
<td>21</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Dose of Anti-D in micrograms. All patients received more than 100 μg/kg bodyweight.
†Platelet count × 10^-3 /μL.
‡Hgb is hemoglobin in grams per deciliter.
§Patient 3 already had a positive direct Coombs test before an IV Anti-D.
IV ANTI-D FOR ITP

Significantly larger doses of Anti-D during maintenance than did the HIV- patients ($P < 0.05$).

Twelve of the 19 patients receiving repeated maintenance infusions had their infusion doses alternated to investigate the effects of different doses (eg, see Fig 6). Eight of the 12 patients did not have changes in their disease and had at least four consecutive infusions available to compare the effect of different doses. Increasing the dose of IV Anti-D in maintenance lead to both a significantly increased peak platelet count and to an increased interval between infusions; $P < 0.01$ for both differences. These differences were seen individually in seven of eight and in six of eight patients, respectively.

The optimal dose varied in the different patients from 25 to more than 50 µg/kg and needed to be individually determined. Anemia became the dose-limiting toxicity in that a plateau of the platelet response was not reached even at 50 to 70 µg/kg/infusion (Fig 6).

**Mechanism of Effect**

Two parameters were investigated in a limited number of patients before and after infusion to investigate possible immunomodulatory effects of IV Anti-D: (1) expression of FcRs on circulating mononuclear cells and neutrophils, and (2) in vitro Ig production. These two parameters were chosen because they had been previously investigated with IVIG.

In the seven patients tested before and after IV Anti-D, there were no significant changes in overall expression of FcRs I, II, and III on monocytes and neutrophils. However, there appeared to be a relationship between a greater increase in FcRI expression on monocytes and a smaller platelet count increase ($0.05 < P < 0.10$); this relationship is illustrated in Fig 7 using the results seen with MoAb “32.2.” Similar relationships were seen with the other MoAbs specific for FcRI, “27.17,” and with the IgG1 control (which could bind to FcRI via its Fc region).

In vitro Ig production was investigated with the reverse-plaque assay using stimulation with pokeweed mitogen, Epstein-Barr virus, staphylococcus protein A, and specific antibody production to sheep RBCs. There was no significant change in any of the first three parameters, although the response to pokeweed mitogen showed a tendency to increase postinfusion. However, there was a consistent and statistically significant increase in the production of antigen-specific (anti-sheep RBC) antibodies after IV Anti-D (see Fig 8).

**Toxicity**

In general there were few adverse effects of infusion of IV Anti-D. Only five reactions occurred during or immediately after the first 344 infusions. Two reactions were severe: one occurred in a patient with known hypersensitivity to plasma products, the other in a patient who had

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**Table 3. Outcome of IV Anti-D Treatment**

<table>
<thead>
<tr>
<th></th>
<th>All Patients</th>
<th>Age</th>
<th>Chronic</th>
<th>Duration†</th>
<th>HIV Status‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Remission</td>
<td>5</td>
<td>55</td>
<td>0</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Stable without therapy</td>
<td>8</td>
<td>3</td>
<td>5</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Maintenance: &gt;3-wk intervals</td>
<td>15</td>
<td>7</td>
<td>8</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>&lt;3-wk intervals</td>
<td>4</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Refractory</td>
<td>8</td>
<td>3</td>
<td>5</td>
<td>0</td>
<td>4</td>
</tr>
</tbody>
</table>

Lost to follow-up: three patients.

*Outcome of children < 18 years old with chronic ITP (duration of ITP > 6 months). Five of 14 children with chronic ITP were able to discontinue treatment.

†There was no difference in outcome from chronic versus acute patients.

‡HIV+ patients appeared to achieve remissions less frequently but also appeared less likely to be refractory than HIV- patients.

§Two children achieving remission after Anti-D had acute ITP treated at diagnosis, which has a high rate of spontaneous remission.

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**Fig 6. Example of the relationship between the dose of Anti-D in maintenance to the interval between infusions and the peak platelet response.** The left y-axis represents (□) the mean number of days between treatments with IV Anti-D infused as needed to maintain the platelet count greater than 30,000/µL in this individual patient as an illustration; the right y-axis (●) represents the mean peak increase in the platelet count after each treatment dose. The x-axis represents three different doses in micrograms of IV Anti-D per kilogram of bodyweight used as single infusions in maintenance. This patient had more than four infusions at each of the three dose levels; the standard error of these determinations is large in the individual patient. However, in the overall analysis, increasing the dose of Anti-D lead to a significantly increased peak platelet count as well as to an increased number of days between infusions reflecting a greater duration of effect (both $P$ values $< 0.05$). Note that there was no upper limit reached to the effect of increasing the dose of Anti-D to greater than 50 to 60 µg/kg. The dose-limiting toxicity became the development of postinfusion (transient) anemia.
Fig 7. Change in monocyte FcRI expression in relation to platelet increase. The y-axis represents the change in circulating monocyte FcRI expression expressed as the change in channels (pre-post) of fluorescence of monoclonal anti-FcRI antibody "32.2." The x-axis represents the increase in the platelet count. The least squares line represents a linear regression analysis with \(0.05 < P < 0.10\), suggesting a relationship between decrease in monocyte FcRI expression and increase in the platelet count. Similar results were obtained with the other MoAb directed at FcRI "27.17" as well as with the IgG1 control, which could have bound to FcRI via its Fc piece.

received IV Anti-D before and numerous times since without any reactions. Both resulted in shaking and chills with gradual recovery over a period of 1 hour. There were no significant changes in vital signs with infusions. The mean changes were: temperature -0.14, pulse -2.3, blood pressure systolic +1.7 and diastolic +1.5, and respiratory rate -0.35.

The results of the liver function tests, where available, have been previously published\(^9\); no patient developed hepatitis after infusion of IV Anti-D. IgG, IgM, and IgA did not appear to change with infusion; IgG levels decreased during the first few months of treatment in several patients, presumably because they had recently received IV gamma-globulin.

Twenty-four patients who were HIV- were tested for antibody to HIV-1 at least 2 months after receiving their initial dose of IV Anti-D; no patient seroconverted. Twelve of these 24 patients were tested more than 6 months after their initial infusion of IV Anti-D.

DISCUSSION

As demonstrated by this pilot study, IV Anti-D is a useful treatment of ITP in appropriately selected patients because it is effective, safe, simple to administer, and relatively inexpensive (estimated 10% the cost of IVIG). In addition to direct saturation of macrophage Fc receptors with antibody-coated RBCs, infusion of Anti-D may also work to increase the platelet count by as of yet not well-defined immunomodulatory effects.

Effectiveness

Infusion of IV Anti-D at an initial dose of 25 to 50 \(\mu\)g/kg lead to a platelet increase greater than 20,000/\(\mu\)L in 34 of 43 or 79% of patients. The platelet increase was less dramatic than that seen with IVIG; only 10 patients had peak platelet count increases greater than 100,000/\(\mu\)L. The great majority of patients receiving maintenance treatment (15 of 19) maintained an adequate platelet count with infusion of Anti-D at greater than 3-week intervals (mean of 24 days between infusions).

The data regarding the dose of IV Anti-D were surprisingly complex. This study did not randomize the amount of the initial dose but rather used a dose-response model. This approach, combined with the 4- to 7-day time until the peak response to Anti-D was seen, contributed to the inability to demonstrate a relationship of dose with response in induction. However, in the intra-patient studies of alternating

Fig 8. Change in in vitro Ig production with IV Anti-D infusion. The y-axis represents the number of plaque-forming cells (PFC) per 100,000 cells plated. The x-axis represents the pre Anti-D and post Anti-D values in response to three different mitogens (pokeweed mitogen, Epstein-Barr virus, \textit{S aureus} Cowan strain) and specific antibody production to sheep RBCs cells (SRBC). The two values were obtained 1 week apart with the first test performed on blood drawn immediately before the first infusion of IV Anti-D. The increase in PFC to SRBC was significant; controls from those days did not increase significantly and there was no change in the control value in these assays over the period of study. As can be seen by the medians, all parameters tended to increase after IV Anti-D.
doses during maintenance treatment, it was clear in the majority of patients that increasing the size of the dose lead to both a greater platelet increase and more lasting effects.

Children

IV Anti-D was especially effective in children, who had a median platelet increase of 96,000/μL. The only other study of Anti-D treatment in children also showed that children respond well to this treatment. Eight of 10 chronic and two of five acute patients had platelet increases greater than 100,000/μL. Maintenance treatment of children with chronic disease lead to 36% (5 of 14) being able to discontinue all therapy during the first year of treatment. These preliminary findings appear similar to pilot data of Anti-D treatment in children also showed that children had a median platelet increase of 96,000/μL. In children with chronic ITP, in both of which there was a 60% rate of being able to discontinue treatment during the first 2 years of treatment.21

HIV

Thrombocytopenia is a frequent complication of HIV infection. The HIV+ thrombocytopenic patients had good acute platelet increases in response to Anti-D in this series; two recent studies initially described this effect.1,12 Regarding maintenance treatment, the preliminary results reported here suggest that: (1) IV Anti-D appears superior to IVIG in the interval between infusions; (2) development of anemia was not a problem; and (3) lasting improvement occurred in four HIV+ adult patients with long-standing ITP.

Adults

Surprisingly, four of five HIV− adult patients with chronic ITP were refractory to Anti-D treatment. Previously published results were clearly superior:22 22 of 29 patients had peak platelet counts greater than 50,000/μL and only four patients did not respond at all. A possible explanation of this difference is that our refractory patients had been previously treated with IVIG and, in part, were available for this trial because of limited response to IVIG.23 This may have selected patients unresponsive to “Fc receptor blockade” for treatment with Anti-D. However, the degree of correlation of responsiveness and of resistance to these two treatments remains to be determined.

Safety

The reaction rate to IV Anti-D was very low. No transmission of viral infections was noted, no seroconversion to HIV was seen in the 24 tested patients and no “transaminitis” occurred.18 Hemolysis was well tolerated; no patient needed a blood transfusion because of anemia caused by the IV Anti-D. Recent patients receiving only 25 mg/μL averaged a decrease in hemoglobin of less than 1 g/dL instead of the 1.9 g/dL seen in this study.

Note that this description of product safety applies only to the Winrho preparation. Licensed preparations of intramuscular Anti-D Ig available in the United States contain IgG aggregates, and severe reactions may occur if they are infused IV.

Ease of Administration

IV Anti-D was usually infused in 3 minutes. It reconstituted quickly (<5 minutes). These two properties facilitated a short time interval from the decision to treat until completion of infusion.

Cost

Anti-D, 25 μg/kg, appears to be clinically equivalent to 1 g/kg of IVIG in ITP, even though the platelet count increases more rapidly and usually to a greater level with IVIG. In Canada, where both products are available, an equivalent dose of Anti-D costs approximately 10% as much as IVIG. The short infusion time, lack of posttransfusion headaches, and absence of other side effects with Anti-D also minimize time missed from school or work.

Appropriate patients

At least four Rh− patients, including two in this study, have been infused with Anti-D without any increase in their platelet counts.8,9 Plasma containing Anti-c and Anti-e has been shown to be effective in three Rh+ patients,11 confirming the essential nature of the FcR interaction. The requirement that the patient be Rh− could be overcome in the future by including Anti-c and Anti-e in the starting plasma pool.

Studies of splenectomized patients are more equivocal. Salama et al9 initially reported that splenectomized patients did not respond as well to Anti-D. However, responses were seen in two or three patients. The difference in response between splenectomized and unsplenectomized patients, unlike that seen with IVIG,24 may be explained by the relatively low dose of Anti-D infused when this is compared with the levels of Anti-D achieved clinically in sensitized patients who may hemolyze Rh+ cells, even if they have been previously splenectomized. Despite the higher dose infused and development of an indirect Coombs test in the splenectomized patients, the great majority of “D” sites on RBCs remain unsaturated with the doses of Anti-D used in this (and other) studies. Whether the fact that Anti-D is thought to fix complement poorly25 contributes to its inability to “block” phagocytic cells outside the spleen26 remains to be determined.

In summary, appropriate patients for IV Anti-D treatment would have ITP, be Rh−, and not have undergone splenectomy. The delayed response of at least 48 hours in the great majority of responders would suggest that this treatment not be used when an urgent increase in the platelet count is required.

Preparation

Different preparations of Anti-D have been used successfully in the published reports. The Winrho preparation was chosen for this study because it is the only one available for IV use in North America.
Mechanism

FcR blockade by substitution of antibody-coated RBCs for antibody-coated platelets has been the postulated mechanism of Anti-D effect. In exploring the mechanism, platelet-associated IgG was not measured in this study because it is believed to be nonspecific.22,29

Several findings in this study suggest that the mechanism of effect of IV Anti-D is not limited to FcR blockade. The apparent relationship of the change in FcRI to the platelet response (Fig 7) is not likely to be a direct effect on monocyte FcR by antibody-coated RBCs because circulating monocytes are not known to phagocytose antibody-coated RBCs in vivo. Therefore, it may represent an immunomodulatory effect resulting from IV Anti-D infusion; this may or may not be representative of similar changes/relationships of tissue macrophages. Further support for immunomodulation as a mechanism of therapeutic effect comes from both the 2- to 3-day delay in the increase in the platelet count (unlike IVIG and which would be surprising if direct FcR saturation was the sole mechanism, Fig 3) and failure to correlate parameters of hemolysis with response (here Fig 5 and reference 13).

The increase in specific antibody production after IV Anti-D (Fig 8) is also consistent with an effect of Anti-D not limited to direct FcR blockade. Substantial effects on in vitro Ig production have been reported following infusion of IVIG, although the demonstrated changes were most marked in increases in pokeweed mitogen stimulation after IVIG.9 The significance of changes seen in one assay of in vitro Ig production versus another is unclear.

Further studies must be performed before a unifying hypothesis of the mechanism of effect of IV Anti-D can be clearly defined. The therapeutic effect of Anti-D appears to require FcR interaction because Rh- patients do not respond, but these patients will respond to infusions of plasma containing Anti-c or Anti-e. The hypothesis that immunomodulatory events could be initiated by FcR interactions has been postulated for other therapies of ITP, including infusion of a monoclonal anti-FcRIII antibody11,32 and danazol; the latter also had effects on FcRI. The details of the interactions in any of these examples including Anti-D are complex and remain obscure. Another consideration is that the "immunosuppressive" effect of Anti-D in the prophylaxis of Rh disease has not yet been completely defined.

IV Anti-D and IVIG do not appear to share an identical mechanism of effect. The most striking difference is that IVIG is equally effective in splenectomized patients, apparently irrespective of patient blood type, and increases the platelet count in 48 hours.35

Ethics

Ethical considerations have been raised concerning potential depletion of Anti-D if it becomes a widely used treatment of ITP.36 If material were limited in supply, prophylaxis of Rh disease clearly would have priority over treatment of ITP. It should be possible to overcome this in the future through the use of human monoclonal Anti-D or other RBC antigen-directed antibodies.37

IV Anti-D appears to be a useful treatment of ITP. It is not a treatment for refractory patients who have failed to improve after splenectomy. Rather, it may contribute to the management of patients before splenectomy, especially in children and HIV-infected patients. There may be clinically significant immunomodulatory effects following Anti-D infusion, but their existence and nature require further study.

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