Detection of anti-D following antepartum injections of Rh immune globulin

M.S. KENNEDY, J. MCNANIE, AND A. WAHEED

Antepartum prophylaxis using Rh immune globulin (RhIG) at 28 weeks of gestation is routine in unsensitized Rh-negative women. As various sources state that anti-D may be detected up to 6 months after administration, we reviewed the medical and laboratory records of all Rh-negative women who delivered at our institution during 1995. For 385 evaluable women, only 137 (35.6%) had anti-D demonstrable in their sera at delivery; 97.8 percent of these delivered within 75 days after administration of RhIG. Of 248 women (64.4%) who delivered in < 76 days after administration of RhIG, 134 (54%) had demonstrable anti-D. For 125 women who delivered between 76 to 95 days after RhIG, only 3 (2.4%) had demonstrable anti-D. Of 14 women who delivered more than 96 days after RhIG, none had anti-D at delivery. These data show that the 300-μg dose used in the United States may not be adequate for antepartum protection and that the detection of anti-D more than 100 days after the administration of RhIG should be viewed with suspicion.

Key Words: anti-D, Rh immune globulin

Antepartum administration of a 300-μg dose of Rh immune globulin (RhIG) at 28 weeks gestation has resulted in a decrease in the incidence of Rh immunization in D− women from about 1–1.5 percent, with postpartum administration only, to about 0.1–0.16 percent.1 Anti-D is demonstrable in the sera of these women within a week of injection. The American Association of Blood Banks’ Technical Manual2 states that anti-D due to RhIG may remain detectable for as long as 6 months. In addition, Konugres3 states that injected RhIG may be detected 5 to 6 months using some sensitive methods.

We reviewed the test results of 385 D− females who received RhIG at 28 weeks gestation to see if we could detect anti-D in third trimester antibody screens and whether anti-D could be detected at delivery in these females.

Materials and Methods

The medical and laboratory records of all D− women who delivered at the Ohio State University Medical Center during 1995 were reviewed to determine the date of RhIG (RhoGAM™ Rh0 (D) Immune Globulin, Ortho-Clinical Diagnostics, Raritan, NJ) administration, the dates and results of the ABO and Rh type and antibody screen, and the intervals between these events. For patients seen in the outpatient clinics, the RhIG administration logs were matched with the delivery logs of the hospital. For patients seen in physicians’ offices, records were retrieved and reviewed. Patients were included regardless of the ABO and Rh type of their newborns and the parity of the mothers.

The ABO, Rh, and antibody detection tests were performed by tube techniques using monoclonal antisera (Gamma Biologicals, Houston, TX) according to the manufacturer’s instructions. All nonreactive anti-D results were tested for weak D with anti-human globulin (AHG). Only AHG-negative women were included. Antibody detection was performed by the addition of polyethylene glycol (PEG; PeG, Gamma Biologicals) with conversion to an AHG test using an anti-IgG reagent (Gamma Biologicals).

For sensitivity studies, 0.1 mL was removed from the 300-μg vial (0.7 mL) of RhIG and used to make serial dilutions to 1:4,000,000 in 0.9% sodium chloride (saline) and was tested using PEG, saline to AHG, and albumin to AHG techniques.

Results

For 385 confirmed D− women, the date of RhIG injection was reliably established by log entries. Only 137 women (35.6%) had anti-D demonstrable in their sera at delivery (Table 1) and 134 (97.8%) of these 137 women with anti-D delivered within 75 days after RhIG. For 248 women (64.4%) who delivered within 75 days of RhIG, only 134 (54%) had demonstrable anti-D at delivery (Table 1). For 123 women (31.9%) who delivered 76 to 95 days after administration of RhIG, only 3 (2.4%) had demonstrable anti-D at delivery (Tables 1 and 2). Of 14 women (3.6%) who delivered more than 95 days after
RhIG injection, none had detectable anti-D at delivery (Table 1). For the women who delivered between 75 and 105 days after RhIG administration, the data in 5-day intervals are shown in Table 2.

Table 1. Detectable anti-D in relation to days from Rh immune globulin administration until delivery.

<table>
<thead>
<tr>
<th>Subjects (%)</th>
<th>Anti-D (%)</th>
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<tbody>
<tr>
<td>1–75 days</td>
<td>248 (64.4%)</td>
</tr>
<tr>
<td>76–95 days</td>
<td>123 (31.9%)</td>
</tr>
<tr>
<td>96–105 days</td>
<td>14 (3.6%)</td>
</tr>
<tr>
<td>Totals</td>
<td>385 (100.0%)</td>
</tr>
</tbody>
</table>

Table 2. Number of women with anti-D when interval between RhIG administration and testing was > 75 days

<table>
<thead>
<tr>
<th>Interval</th>
<th>With anti-D</th>
<th>Total subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>76–80</td>
<td>0</td>
<td>39</td>
</tr>
<tr>
<td>81–85</td>
<td>1</td>
<td>35</td>
</tr>
<tr>
<td>86–90</td>
<td>1</td>
<td>31</td>
</tr>
<tr>
<td>91–95</td>
<td>1</td>
<td>18</td>
</tr>
<tr>
<td>96–105</td>
<td>0</td>
<td>14</td>
</tr>
</tbody>
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In the dilution studies, our routine antibody detection technique could detect a 1:64,000 dilution using PEG, a 1:32,000 dilution in albumin to AHG, and a 1:16,000 dilution in saline to AHG. Assuming the intended dose is 300 μg in 0.5 mL (perhaps an overestimation as overfill of vials is generally 10%), the starting volume represents 60 μg. The 1:64,000 dilution would represent 1.8 ng, the 1:32,000 would represent 3.6 ng, and the 1:16,000 would represent 7.3 ng. Thus, PEG at 1:64,000 could detect a concentration of about 18 ng/mL (1.8 ng in 0.1 mL). As our starting concentration may have been overestimated, the PEG technique may actually detect lower concentrations.

Discussion

Our data as well as that of other investigators strongly suggest that detecting circulating anti-D using routine immunohematology methods is unlikely 6 months after injection of RhIG. The “six months” statement in the Technical Manual is unreferenced and has been present in the 10th and subsequent editions. In a study of volunteers who were injected with a 1000-μg vial of RhIG, the concentration of anti-D was 207 ng/mL 48 hours after injection and 2.5 ng/mL at 6 months. Bowman and Pollack report values of 0.4–0.8 ng/mL at 88 to 98 days after a 300-μg dose. Thus, a 300-μg dose (in the United States) would be undetectable at 6 months (<1 ng/mL). It is much more likely that anti-D detected at 6 months postinjection is the result of active, rather than passive, immunization.

Early studies by Pollack et al. demonstrated that primary immunization can be prevented by injecting as little as 20 μg of anti-D for each mL of D+ RBCs. Additional studies reported that as little as 14.6 μg of anti-D per mL of RBCs is immunosuppressive when the amount of D+ RBCs is 200 mL. However, 20 μg/mL appears to be the lower limit when small volumes of RBCs are involved. In addition, studies by Contreras and Mollison appeared to indicate augmentation of primary immunization when 1 μg of anti-D was injected for 0.8 mL RBCs, in that 9 of 13 subjects developed anti-D compared with 4 of 12 who received only 1 mL RBCs and no anti-D. Mollison cites a larger study by Ascari (personal communication) in which 103 of 134 developed anti-D after receiving 7 mL RBCs and 1.4 μg/mL of anti-D. Mollison speculated that at least 25 μg of anti-D should be present just before delivery. A 300-μg dose at 28 weeks would give this level, assuming a T 1/2 of 26 days and that all the anti-D is circulating. However, studies by Bowman and Pollack have shown these assumptions to be incorrect, as the amount of IgG with T 1/2 of 7 days reduces the estimations by about 20 to 25 percent. Our studies, detecting 18 ng/mL (54 μg/3000 mL), would thus suggest that those women without detectable anti-D between 1 and 70 days postinjection may not be adequately covered.

In conclusion, anti-D detected more than 4 months after RhIG administration is most likely due to active immunization. At the minimum, an additional dose should be given 12 weeks after the antenatal dose. Further studies are required to assess the need for additional doses.

References

3. Konugres AA. Rh immune globulin. In: Churchill WH,


Melanie S. Kennedy, MD, Associate Professor; Department of Pathology, The Ohio State University, 125 Hamilton Hall, 1645 Neil Avenue, Columbus, OH 43210; Jamie McNamie, BA, Research Associate, Department of Pathology, The Ohio State University, Columbus, OH; and Abdul Waheed, MS, MT(ASCP)SBB, Lead Technologist, Department of Pathology, The Ohio State University Medical Center, Columbus, OH.

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