Rh Blood Group-Specific Antibodies in Immune Hemolytic Anemia Induced by Nomifensine

By A. Salama and C. Mueller-Eckhardt

Nomifensine (Merital, Alivil; Hoechst, Frankfurt, FRG), an antidepressant drug, may cause immune hemolytic anemia (IHA) of the so-called immune complex type that is believed to occur by means of an innocent-bystander mechanism. In this report we describe findings that are not consistent with this mechanism in a patient with nomifensine-induced intravascular IHA associated with renal failure. In vitro studies showed a transitory positive direct antiglobulin test (DAT) due to IgG, IgM, and C3 fixation. The causative antibodies were found to be (1) a drug-independent IgM antibody in the serum and eluate that reacted only with E-positive RBC, although the patient's RBC were E-negative; (2) an IgG antibody in the serum and initial eluates that showed a stronger reaction with E-positive than with E-negative or Rhnull RBC, but only in the presence of ex vivo antigen (ie, urine containing the drug and all its metabolites); and (3) an IgM antibody in the serum and initially also on the patient's RBC that, in the presence of ex vivo antigen as well as in the presence of known metabolites of the drug, agglutinated all RBC equally strongly, but was hemolytically more active against E-positive than E-negative cells. Within a few days of stopping the drug the hemolysis rapidly resolved without administration of prednisone, the DAT became negative with anti-IgG and anti-IgM, and the drug-independent anti-E disappeared, but both metabolite-dependent antibodies remained detectable in the patient's serum. We conclude that the production and specificity of the causative antibodies in this case were controlled by a larger antigenic site, presumably consisting of the drug and/or its metabolites plus RBC antigens, rather than by epitopes of the drug or metabolites alone.

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anticoagulated blood by a tube method using commercial antisera (polyspecific antiliglobulin serum [Ortho], anti-IgG [Behringwerke AG, Marburg, FRG], anti-IgM and anti-IgA [Diaimed, Morat, Switzerland], and anti-C3d [Dako, Copenhagen]). Antibodies were eluted from sensitized RBC by the heat technique (ten minutes at 56 °C). The detection of drug-dependent antibodies, including hemolysis assays, was performed as recently described using nomifensine, its known three main metabolites (M1, M2, M3), and its ex vivo antigens (urine collected from a healthy volunteer before (U0, Uppsala, Sweden), at 4 °C. The immunoglobulin fractions (IgM, IgA, IgG) were concentrated to the original serum volume and examined for antibody activity against RBC in the presence and absence of the drug and its metabolites.

To characterize the immunoglobulin classes of the antibodies, a serum sample of the patient obtained on day 15 was chromatographed on a Sephacryl S 300 column (Pharmacia Fine Chemicals, Uppsala, Sweden), at 4 °C. The immunoglobulin fractions (IgM, IgA, IgG) were concentrated to the original serum volume and examined for antibody activity against RBC in the presence and absence of the drug and its metabolites.

RESULTS

Demonstration of a transitory anti-E in early serum samples. The patient’s blood group was O Rh-positive (CCDee). Her serum contained an agglutinating anti-e in initial samples (Table 1), but not in specimens taken after day 6. Although this antibody had a relatively low titer, its agglutinating activity against e-positive cells did not change after washing the cells three times with large volumes of saline so that the indirect antiglobulin test (IAT) could not be performed. This antibody was drug independent since its reactivity was unaffected when the serum was dialyzed in large volumes of saline containing 0.2% bovine serum albumin (saline-BSA) or when the antibody was recovered by elution after absorption on e-positive RBC in the absence of the drug and its metabolites.

Characterization of RBC-bound antibodies in the DAT. The DAT was performed with RBC obtained from the patient before any treatment and at various times after administration of 4 units of E-negative packed RBC. The cells were washed either with saline-BSA or with saline containing ex vivo antigens (saline-U5). As shown in Table 2, IgG, IgM, and C3d could be demonstrated on the RBC in both instances, but the reactions due to IgG and IgM were more pronounced when the cells were washed with saline-U5. The strength of agglutination decreased with time (Table 2). No antibody could be demonstrated on the RBC after day 10. These findings indicated that the antibodies belonged to the IgG and IgM classes and were, at least partly, dependent on the presence of the drug and/or its metabolites.

Isolation of antibodies by elution. Antibodies were eluted from the patient’s RBC after washing with saline-BSA or saline-U5, respectively, and were then tested against R,R, rr, and Rhnull RBC in the presence or absence of ex vivo antigens (Table 3). Using day 0 eluates, drug-independent agglutination was observed only with R,R,R RBC. Metabolite-dependent reactions were found in the IAT predominantly with rr RBC. With day 6 eluates, only metabolite-dependent positivity in the IAT was detectable; it was clearly stronger with rr than with R,R and very weak with Rhnull RBC. After the patient had been hospitalized for 2 weeks, no antibody could be eluted from her red cells.

Immunoglobulin classes and hemolytic activity of antibodies in relation to drug dependency and Rh specificity. Whole serum from day 15 and immunoglobulin fractions (IgG, IgA, IgM) were tested concomitantly against R,R, R,R, rr, and when possible, Rhnull RBC in the presence of nomifensine, its metabolites (M1, M2, M3), and its ex vivo antigens. The results are summarized in Table 4. Two important observations were made. First, antibody activity was detectable in the presence of metabolites and/or ex vivo antigens (U5), but not in the presence of nomifensine, clearly indicating metabolite-specific antibodies. Second, at least two (possibly three) different metabolite-dependent antibodies were noticed: an IgG antibody exclusively reactive with ex vivo antigens exhibiting predominantly anti-e specificity and an agglutinating IgM antibody preferentially reactive with M2 without obvious Rh specificity. However, when the hemolytic activity of serum and immunoglobulin fractions was analyzed (Tables 4 and 5), it became evident that hemolysis was confined to IgM antibodies and occurred mainly with R,R,R RBC, possibly due to a third metabolite-specific antibody. The IgA fraction did not react with RBC.

DISCUSSION

The data clearly show that the hemolytic episode in the patient described here was the result of multiple nomifensine-induced antibodies. Like previous reports of nomifensine-dependent HLA, the hemolysis was acute, intravascular, and remitted promptly after discontinuation of the drug. Renal failure, often seen in this syndrome, was transient but required temporary hemodialysis.

From an immunologic point of view the most intriguing and as yet undescribed finding was the apparent Rh specificity of nomifensine-induced free as well as RBC-bound antibodies. This observation bears particular significance on our understanding of the underlying pathogenetic mechanism. Initially an anti-E was detectable in the serum and in eluates prepared from autologous RBC before transfusion. Its reactivity was clearly drug independent. The assumption that the antibody represented an ordinary alloantibody is very unlikely for the following reasons: the patient had until then never been transfused, the antibody disappeared quickly after withdrawal of the drug, and it could be eluted from autologous RBC although the patient had the Rh phenotype CCDee. We therefore favor the interpretation that the
Rh-SPECIFIC DRUG-DEPENDENT ANTIBODIES (-I-).

The reactivity of this antibody appeared to be directed against an E-like antigen on human RBC, a situation resembling the pseudo-anti-E specificity described in patients with autoimmune hemolytic anemia.8-10

The antigenic determinants of the other antibodies, either bound to RBC or free in the serum, appeared to consist of compound structures related both to metabolites of nomifensine and to ex vivo antigens (presumably comprising additional as yet unidentified metabolites) against RBC with different Rh phenotypes. The IgG fraction was strongly reactive with rr, less with R1R2, and weak with R2R2 or Rhnull RBC in the presence of ex vivo antigen, but not in the presence of nomifensine or its known metabolites. This reaction pattern resembles again that seen with warm anti-c and that the antibodies remained recognizable on patient’s RBC longer than six days after discontinuation of nomifensine and after transfusion of 4 units of packed RBC. It is therefore unlikely that residual metabolites were a prerequisite for the positive DAT.

Table 2. DAT on the Patient’s Red Cells

<table>
<thead>
<tr>
<th>Day</th>
<th>Therapy</th>
<th>IgG</th>
<th>IgA</th>
<th>IgM</th>
<th>C3d</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>None</td>
<td>2-3+</td>
<td>/-</td>
<td>1-2+</td>
<td>2-3+/2+</td>
</tr>
<tr>
<td>1, 2</td>
<td>Btt (4U), hemodialysis</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>3</td>
<td>Hemodialysis</td>
<td>2-3+/4-</td>
<td>/-</td>
<td>-1-2+</td>
<td>2-3+/2+</td>
</tr>
<tr>
<td>6</td>
<td>Hemodialysis</td>
<td>1-2+</td>
<td>/-</td>
<td>/-</td>
<td>1-2+/2-3+</td>
</tr>
<tr>
<td>10, 15, 22</td>
<td>None</td>
<td>/-</td>
<td>/-</td>
<td>/-</td>
<td>1+/1+</td>
</tr>
</tbody>
</table>

DAT was performed with cells washed either in saline containing 0.2% BSA or saline containing ex vivo antigen (U5) of nomifensine, respectively (-/-). Key: Btt, blood transfusion; /-, negative; 4+ > 3-4+ > 3+ > 2-3+ > 2+ > 1-2+ > 1+.

remained inexplicable because absorption and elution of antibodies could be accomplished with r as well as Rhnull RBC irrespective of whether the IgM fraction or whole serum was used (data not shown).

Although the in vitro reaction of these antibodies seemed to be dependent solely on the presence of metabolites or the drug (augmented or exclusive reactivity in the presence of ex vivo antigen), the antibodies remained attached to RBC in vivo even in the absence of the drug or its metabolites. This notion that the positive DAT is drug independent is supported by the fact that nomifensine is quickly metabolized in vivo12 and that the antibodies remained recognizable on patient’s RBC longer than six days after discontinuation of nomifensine and after transfusion of 4 units of packed RBC. It is therefore unlikely that residual metabolites were a prerequisite for the positive DAT. Indeed, a serum sample obtained from the patient ten hours after the last drug ingestion and a urine specimen of the following day did not seem to contain nomifensine or metabolites as indicated by their failure to be utilized as ex vivo antigens with known nomifensine-induced antibodies (sera 1 and 7). How, then, can the enhanced positivity of the DAT and of the eluates in the presence of ex vivo antigen be explained? It is conceiv-

Table 3. Reactions of Eluates Prepared From the Patient’s Red Cells

<table>
<thead>
<tr>
<th>Patient’s RBC</th>
<th>Titre of Eluted Antibodies Tested by Agglutination/IAT® against RBC with Rh Antigens</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absent</td>
<td>Present</td>
</tr>
<tr>
<td>saline-BSA</td>
<td>2/SA</td>
</tr>
<tr>
<td>saline-U5</td>
<td>NT</td>
</tr>
<tr>
<td>saline-BSA</td>
<td>/-</td>
</tr>
<tr>
<td>saline-U5</td>
<td>/-</td>
</tr>
</tbody>
</table>

Before elution the cells were washed in the presence (saline-U5) or absence of ex vivo antigens (saline-BSA) and tested as indicated. *Using polyspecific antoglobulin serum. †In the presence or absence of ex vivo antigen (U5). ‡Strong agglutination unchanged after washing.
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Considering all these findings, there is now suggestive evidence that target cells in patients with drug-induced immune cytophenias of the so-called immune-complex type do not act as innocent bystanders, but rather serve as "carrier" surfaces. Although conjugation of drugs and cells in vitro does not appear stable, it might still suffice to render this complex immunogenic in some patients. Hence, it is not surprising that the antibodies in such patients, like those in animals immunized with hapten-protein conjugates, will be quite heterogeneous with regard to their binding sites on the conjugates. A similar suggestion related to drug-induced immune cytophenias has recently been made by Habibi and further corroborated by a number of reports that indicate the presence of receptors for certain drug-dependent antibodies on target blood cells.

ACKNOWLEDGMENT

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REFERENCES


Table 5. Hemolytic Activity of Metabolite-Dependent Antibodies

<table>
<thead>
<tr>
<th>Red Cell Rh Phenotype</th>
<th>Whole serum*</th>
<th>IgM fraction</th>
<th>IgG fraction</th>
</tr>
</thead>
<tbody>
<tr>
<td>RPR1</td>
<td>40†</td>
<td>12</td>
<td>—</td>
</tr>
<tr>
<td>RPR2</td>
<td>45</td>
<td>10</td>
<td>—</td>
</tr>
<tr>
<td>rr</td>
<td>8</td>
<td>16</td>
<td>—</td>
</tr>
</tbody>
</table>

*Day 15 serum and fractions.
†Percent hemolysis in the presence of ex vivo antigen (US) and complement.
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