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 Rh_0 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.

SEVERAL CASES of nomifensine-dependent immune hemolytic anemia (IHA) have been reported in the last 5 years.1-4 It is generally maintained that the RBC in these patients are damaged by the so-called immune-complex mechanism. In this type of reaction, drugs are believed to serve as haptons by conjugating with some soluble noncellular macromolecule, giving rise to subsequent formation of high-affinity antibodies against the drug. The antibodies could first react with the drug, and then the antibody-drug complexes could be adsorbed nonspecifically by the RBC, which act merely as “innocent bystanders.” This hypothesis is based on the assumption that immunization does not require conjugation of the drug with RBC and that the antibodies produced do not have specificity for red cell antigens.5

We have recently reported that nomifensine-dependent antibodies probably bind to target RBC via their Fab and not by their Fc portion by showing that, first, epitopes on the Fc portion of RBC-bound antibodies are still accessible to Fc-specific anti-IgG/IgM and, second, that nomifensine-dependent antibodies remain firmly attached to RBC if trace amounts of drug are added to the wash solution.6 Such findings are not consistent with the aforementioned mechanism of nonspecific adsorption of drug-antibody complexes on target RBC.

We now report a patient with nomifensine-dependent IHA who, in the course of the disease, developed various drug-induced antibodies with Rh specificity: a transient IgM pseudo-anti-E (mimicking an alloantibody), a metabolite-dependent IgG anti-e (detectable in serum and eluates), and a metabolite-dependent IgM anti-E that was hemolytic. These findings provide direct evidence that the epitopes seen by the nomifensine-induced antibodies in this case are not formed solely by the drug or its metabolites but at least in part by RBC-specific membrane antigens.

CASE REPORT

K.I., a 58-year-old female, had been treated for psychotic episodes with various drugs since 1960. Nomifensine was first given to the patient in 1982. On November 5, 1985, nomifensine was readministered at a dose of 75 mg/d. On November 19, 1985, she noticed weakness and jaundice. On the following day the patient was admitted to a local hospital for acute lumbar pain, malaise, and dark urine. At initial presentation the patient showed the typical signs and symptoms of acute intravascular hemolysis: she was somnolent and extremely pale, and hemoglobinemia and hemoglobinuria were present. Her total hemoglobin concentration was 4.3 g/dL (free hemoglobin was not measured), the lactate dehydrogenase content was 1,746 (normal, <200 IU/L), the bilirubin level was 6.1 mg/dL (of which 4.3 mg was indirect bilirubin), and haptoglobin was undetectable. Although the patient had never been transfused, the direct antiglobulin test (DAT) was found to be strongly positive, and an agglutinating anti-E was identified in the patient’s serum. On the assumption of drug-dependent IHA, nomifensine was discontinued and the patient was transfused with 4 units of compatible, E-negative packed RBC. Without further treatment, the hemolysis rapidly resolved, but transient renal insufficiency developed, requiring temporary hemodialysis. On December 4, 1985, the patient was transferred in good physical condition to a psychiatric hospital.

MATERIALS AND METHODS

Blood samples were collected from the patient on admission before any therapy and during hospitalization at times indicated in the Tables. A complete serological analysis (ABO, Rh antigens, antibody screening) was made by conventional techniques. For antibody identification the Resolve panel (Ortho Pharmaceutical Corp, Raritan, NJ) was used. Rh_0 RBC were kindly provided by Professor S. Seidl (Frankfurt). The DAT was assayed exclusively from EDTA-

From the Institute of Clinical Immunology and Blood Transfusion, Justus Liebig University, Giessen, FRG.

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Address reprint requests to Professor C. Mueller-Eckhardt, MD, Institute of Clinical Immunology and Blood Transfusion, Justus Liebig University, Langhansstrasse 7, 6300 Giessen, FRG.

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anticoagulated blood by a tube method using commercial antisera (polyspecific antiglobulin serum [Ortho], anti-IgG [Behringwerke AG, Marburg, FRG], anti-IgM and anti-IgA [Diamoed, 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 described6 using nomifensine, its known three main metabolites (M1, M2, M3), and its ex vivo antigens (urine collected from a healthy volunteer before (U0, control) and five hours (U5) after the ingestion of 200 mg nomifensine).

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).6 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,6 and were then tested against R1R2, 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 R2R2 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 R1R2 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 R2R2, R1R2, 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 R2R2 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 IHA,1,4 the hemolysis was acute, intravascular, and remitted promptly after discontinuation of the drug. Renal failure, often seen in this syndrome,4 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

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Table 1. Patient's Serum, Day 0, Tested in the Absence of Nomifensine and Its Metabolites

<table>
<thead>
<tr>
<th>Tested Against RBC</th>
<th>Whole Serum</th>
<th>Titer of Agglutination With Eluates Obtained From RBC</th>
<th>Serum Absorbed With RBC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Undialyzed</td>
<td>Dialyzed</td>
<td>R1R2</td>
</tr>
<tr>
<td>R1R2</td>
<td>8</td>
<td>8</td>
<td>4</td>
</tr>
<tr>
<td>R1R2</td>
<td>2</td>
<td>2</td>
<td>1*</td>
</tr>
<tr>
<td>rr</td>
<td>2</td>
<td>2</td>
<td>1*</td>
</tr>
<tr>
<td>Rhnull</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
</tr>
</tbody>
</table>

Key: --, negative; *, weak but stable reaction with undiluted serum; NT, not tested.
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.\(^6\)\(^-\)\(^10\)

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 RBC membrane antigens. This phenomenon became particularly obvious upon separate analysis of serum and immunoglobulin fractions in the presence of nomifensine, its main metabolites, and its 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 Rh\(_{11}\) RBC in the presence of ex vivo antigen, but not in the presence of nomifensine or its known metabolites. This reaction pattern resembles that seen with warm anti-C autoantibodies, which often react more strongly with ex vivo RBC than with metabolites or with e-negative RBC.\(^11\) Experiments with the IgM fraction of the same serum sample yielded a more complex pattern in that reactions were strong with all RBC in the presence of ex vivo antigen and M2, but weaker in the presence of M1 and M3, presumably reflecting cross-reactivity with M2.\(^6\) On the other hand, E-positive cells were found to be more susceptible to complement-mediated lysis by the same antibody under identical conditions. This phenomenon remained inexplicable because absorption and elution of antibodies could be accomplished with rr as well as R1R2 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 vivo\(^12\) 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 5. Hemolytic Activity of Metabolite-Dependent Antibodies

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

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

able that these antibodies recognize variable structural sites on the RBC surface to which they attach rather loosely in the absence, but more strongly in the presence of the drug or its metabolites. Another possible explanation for the transiently positive DAT is a simultaneous coating of RBC with residual metabolites and antibodies. In either possibility antibody binding involves defined structures on the RBC surface. This is supported by the fact that a drug-related antibody reacts only with one type of blood cell, i.e., with platelets and not with RBC.

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