Warm autoimmune hemolytic anemia associated with an IgM autoanti-Ge

T. Sererat, D. Veidt, P. A. Arndt, and G. Garratty

A 28-year-old male with a prior history of Hodgkin’s disease and a recent upper respiratory tract infection presented with autoimmune hemolytic anemia (AIHA). The patient’s red blood cells (RBCs) were spontaneously agglutinated after room temperature and 37°C washes. Dithiothreitol-treated RBCs reacted strongly with anti-C3 and were nonreactive with anti-IgG, -IgM, and -IgA; they reacted with anti-IgM (κ light chains only) by flow cytometry. The patient’s serum was nonreactive. An acid eluate was only weakly reactive, but a 56°C heat eluate strongly agglutinated untreated RBCs (3+). Ficin-treated RBCs were nonreactive. En(a−) RBCs were strongly reactive, but Ge− RBCs were nonreactive. The anti-Ge in the eluate was IgM. The patient’s untreated RBCs were shown, by flow cytometry, to be weakly Ge+. This is the first report of IgM-mediated warm AIHA associated with autoanti-Ge.10

Key Words: autoimmune hemolytic anemia, warm autoanti-Gerbich, IgM autoantibody

Autoimmune hemolytic anemia (AIHA) due to the presence of IgM (without IgG) warm autoantibodies is rare.1 Some previously reported specificities seen in hemolytic anemia associated with warm IgM autoantibodies have included anti-Ena, anti-Wrb, anti-Pr, and anti-c.1-4 AIHA associated with autoanti-Ge has been previously described.2,5-9 Those that were characterized for immunoglobulin class were shown to have IgG present in addition to IgM or IgA. We report here the first case of IgM-mediated warm AIHA associated with autoanti-Ge.10

Case Report

A 28-year-old male was admitted to the hospital because of jaundice and progressive anemia. The patient had been seen by his physician 2 to 3 weeks prior to his admission for symptoms of an upper respiratory tract infection. His hemoglobin at that time was 13.9 gm/dL; his blood smear showed atypical lymphocytes and his liver enzymes were abnormally elevated. His past medical history included Hodgkin’s disease, which had been treated 6 years previously with radiation and chemotherapy. His current medications included diltiazem (Rhone-Poulenc Rorer Pharmaceuticals Inc., Collegeville, PA) and hydrochlorothiazide (Merck & Co. Inc., West Point, PA). On admission, his physical examination showed jaundice but no organomegaly. His hemoglobin was 4.4 gm/dL; reticulocyte count, 10%; bilirubin, 4.2 mg/dL; LDH, 266 IU/L; and ALT, 477 IU/L. The patient received 4 units of crossmatch-compatible red blood cells (RBCs) without adverse reaction and was started on corticosteroids. His hemoglobin after transfusion was 13.0 gm/dL. He was discharged 6 days after admission.

Materials and Methods

All tests were performed on the patient’s pretransfusion blood samples.

Red Cell Testing

Direct antiglobulin test (DAT)

The patient’s washed RBCs were tested with anti-human IgG (The American National Red Cross, Rockville, MD), anti-C3 (in-house), anti-IgM (CLB Research Diagnostics, Inc., Flanders, NJ), and anti-IgA (Tago Inc., Burlingame, CA). A 6% bovine albumin control was tested in parallel. The anti-C3, -IgM and -IgA were standardized as previously described.11 Because the patient’s room temperature (RT)-washed and 37°C-washed RBCs showed agglutination in the 6% albumin control, the RBCs were retested after treatment with dithiothreitol (DTT)12 and also with chloroquine diphosphate (CDP).13

The patient’s untreated and DTT-treated RBCs were tested with fluorescein (FITC)-labeled antihuman-IgG, -IgM and -IgA (Cappel/Organon Teknika Corporation, Durham, NC) by a FACSort flow cytometer (Becton Dickinson Immunocytometry Systems, San Jose, CA), using a previously described method.14,15 Briefly, 0.01 mL of FITC anti-IgG, -IgM and -IgA (1:10 in phosphate-buffered saline [PBS]) was added to 0.1 mL of 5%
washed RBCs; as a background control, the same RBCs were tested without addition of FITC anti-Ig. After a 30-minute incubation at RT, the RBCs were washed (×3) and resuspended in PBS for analysis by the flow cytometer. Prior to analysis, each RBC suspension was mixed using a fine-bore pipet and by vortex. Ten thousand events were collected using logarithmic amplification.

The patient’s RBCs were tested in parallel with DAT-negative RBCs (untreated and DTT-treated) from “normal” donors. Results were expressed as (1) the percentage of positive events (% pos), which is arrived at by electronic subtraction of the background fluorescence histogram from the test fluorescence histogram by LYSYS™ II software (Becton-Dickinson); and (2) median relative intensity (RI):

\[ \text{RI} = \frac{\text{median fluorescence of test RBCs}}{\text{median fluorescence of background RBCs}} \]

The FITC anti-IgG, -IgM and -IgA had been tested previously with RBCs sensitized with IgG, IgM, IgA, and/or C3 to determine their reactivity and to control for cross-reactivity.

**Ge antigen typing**

The patient’s untreated RBCs were incubated with an anti-Ge and then with an FITC-labeled Fab fragment of anti-IgG (Cappel) and analyzed by flow cytometry. Ge+ and Ge− RBCs were tested in parallel.

**Eluates**

Eluates were prepared from the patient’s RBCs using a commercial acid elution kit (Gamma Biologicals, Inc., Houston, TX) and by 56°C heat elution into 6% albumin. Testing of the acid eluate included the use of polyethylene glycol (PEG; in-house) and anti-IgM. Titration and thermal amplitude studies were performed on the 56°C eluate as previously described. Treatment of the 56°C eluate with an equal volume of 0.01 M DTT for 2 hours at 37°C resulted in gel formation, so the immunoglobulin class of the antibody was determined by treatment with 0.005 M DTT.

**Test RBCs**

Commercial reagent RBCs were tested untreated and after treatment with ficin or sialidase (neuraminidase; Calbiochem, San Diego, CA). En(a−) RBCs were from ERP; Ge:−1,−2,−3 RBCs were from a commercial source (Immucor, Inc., Norcross, GA), and Ge:−2,−3 RBCs were from a Los Angeles Red Cross donor. The RBCs were stored in liquid nitrogen.

**Serum Testing**

The patient’s serum was tested by a previously described serum screen method used to characterize antibodies in AIHA. This method includes testing sera after acidification (to pH 6.5–6.8), and the addition of fresh normal serum as a source of complement, against untreated and ficin-treated RBCs at 37°C (prewarmed) and at 20°C. Testing was also performed using albumin and PEG (Gamma Biologicals, Houston, TX).

**Results**

**Direct antiglobulin test**

RBCs from the patient spontaneously agglutinated (1+) following washing at RT and even when washed at 37°C. Following treatment of the RBCs with 0.01 M DTT and CDP, no spontaneous agglutination occurred. The DTT-treated RBCs were strongly reactive (3+) with anti-C3 and were serologically nonreactive with anti-IgG, -IgM, and -IgA. The negative result of the treated RBCs with anti-IgM was expected, even if RBC-bound IgM was present in vivo, because DTT breaks the IgM pentamer molecule into monomers. Similarly, CDP-treated RBCs reacted strongly (4+) with anti-C3.

Flow cytometry results are shown in Table 1. Visual analysis of the forward scatter versus side scatter cytograms of the patient’s untreated RBCs (not shown) showed no gross agglutination, but positive results with FITC anti-IgG and -IgA versus untreated RBCs (see Table 1) that became negative when tested versus DTT-treated RBCs suggested that these positives were false positives because of agglutination of the untreated RBCs. In contrast, the patient’s untreated RBCs were strongly positive with anti-IgM (a median RI of 19 means that RBCs + FITC anti-IgM were 19 times more fluorescent than background RBCs [not incubated with FITC anti-IgM]); DTT-

**Table 1.** Flow cytometric analysis of the patient’s untreated and DTT-treated red blood cells (RBCs) after incubation with FITC anti-IgG, -IgM and -IgA.

<table>
<thead>
<tr>
<th>RBCs</th>
<th>FITC anti-IgG</th>
<th>FITC anti-IgM</th>
<th>FITC anti-IgA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>% pos Median RI</td>
<td>% pos Median RI</td>
<td>% pos Median RI</td>
</tr>
<tr>
<td>Patient</td>
<td>68.2 2.69</td>
<td>99.7 19.09</td>
<td>64.5 2.46</td>
</tr>
<tr>
<td>DTT-treated</td>
<td>59.0 2.46</td>
<td>48.2 1.95</td>
<td>52.3 2.16</td>
</tr>
</tbody>
</table>

**NOTES:** RBCs from three normal donors were tested in parallel, and a median RI of 19 means that RBCs + FITC anti-IgM were 19 times more fluorescent than background RBCs (not incubated with FITC anti-IgM); DTT-

The high fluorescence results seen with normal RBCs can be explained by (1) RBC autofluorescence, (2) cytophilic Ig, and (3) the dilution of the FITC anti-Ig used.
treated RBCs were less positive with FITC anti-IgM but still clearly demonstrated the presence of IgM (monomers) on the patient’s RBCs. The Toledo Hospital (Toledo, OH) also used flow cytometry to show that the antibody on the patient’s RBCs was IgM (λ light chains; results not shown).

**Antigen typing**

The patient’s DTT-treated RBCs typed as group A, D+. Using flow cytometry, the patient’s RBCs were positive with anti-Ge, although weaker than two Ge+ controls.

**Eluate testing**

An acid eluate prepared from the patient’s RBCs only weakly agglutinated untreated RBCs. Tests using PEG–anti-IgG and PEG–anti-IgM were negative. A 56°C eluate from the patient’s RBCs directly agglutinated (3+) untreated RBCs; ficin-treated and sialidase-treated RBCs were nonreactive. En(a−) RBCs were strongly reactive (3+); Ge− RBCs were nonreactive. The eluate no longer reacted after DTT treatment; thus, the antibody appeared to be IgM.

Dilutions of the 56°C eluate gave titers of 4, 8, 4, and 4 when tested against Ge+ RBCs at 37°C, 30°C, 23°C, and 4°C, respectively. Ge− RBCs were nonreactive at all temperatures. Thus, the eluate appeared to contain an IgM autoanti-Ge that reacted best at 30°C.

**Serum Testing**

The patient’s serum was nonreactive by all techniques.

**Discussion**

Although this patient had an obvious hemolytic anemia and the hematologist strongly suspected it to be AIHA, the results of routine serologic tests were not impressive. The DAT was strongly positive due to RBC-bound complement, but no antibodies were detected in the serum. Spontaneous agglutination of the RBCs was found to be due to an IgM autoanti-Ge reacting best at 30°C, which could be eluted from the RBCs (preferentially by heat elution). The presence of RBC-bound IgM was not detected by a routine antiglobulin test but was detected easily by flow cytometry. We have found flow cytometry to be a useful technique for detecting IgM (pentamers and monomers) on RBCs. In some cases, spontaneous agglutination occurs only after centrifugation, and untreated RBCs can be analyzed by flow cytometry without interference by agglutination. In other cases, RBCs will show spontaneous agglutination even without centrifugation, so DTT treatment is required for flow cytometric analysis of these RBCs to avoid falsely positive results (single-cell suspensions are required for flow cytometry). The results in this case support findings in other reports of AIHA associated with IgM warm autoantibodies, that, even in cases of severe hemolytic anemia, the routine serology is often not dramatic, and the association with IgM antibody is not always obvious. If the RBCs of a patient with AIHA show spontaneous agglutination when centrifuged, especially in the absence of potentiliators, then RBC-bound IgM should be suspected. Spontaneous agglutination is not synonymous with autoagglutination due to cold agglutinins (i.e., when such RBCs are centrifuged, the agglutination is obviously due to serum antibodies; the RBCs are not spontaneously agglutinating).

It is of interest that the specificity was anti-Ge, as the AIHA appeared to be associated with an upper respiratory tract infection; however, this association may have been coincidental.

**References**


Attention SBB and BB Students: You are eligible for a free one-year subscription to Immunoheematology. Ask your education supervisor to submit the name and complete address for each student and the inclusive dates of the training period to Immunoheematology, P.O. Box 40325, Philadelphia, PA 19106.

Attention: State Blood Bank Meeting Organizers—If you are planning a state meeting and would like copies of Immunoheematology for distribution, please contact Mary H. McGinniss, Managing Editor, 3 months in advance, by phone or fax at (301) 299-7443.