Autoimmune hemolytic anemia caused by warm-reacting IgM-class antibodies

R.J. Sokol, D.J. Booker, R. Stamps, S. Sobolewski, and A. P. Haynes

Warm IgM autoantibodies occur in association with IgG-class and/or IgA-class immunoglobulins in approximately 30 percent of patients with warm-type autoimmune hemolysis. They may be classified as agglutinins or hemolysins, which may be incomplete or complete, depending on in vitro serology; they almost always bind complement. Autoimmune hemolytic anemia solely due to warm IgM autoantibodies is exceedingly rare. We report two cases of the incomplete agglutinin type. The autoantibodies were confirmed as IgM by their ability to rebind to normal red blood cells (RBCs) after elution; the absence of small increases in RBC-bound IgG and IgA was shown by a sensitive enzyme-linked antiglobulin test.

Case Reports

Patient 1 was a 64-year-old female who presented with a short history of increasing weakness and fatigue. Physical examination revealed gross pallor, mild icterus, hepatomegaly (4 cm below right costal margin), splenomegaly (10 cm below left costal margin), tachycardia, and an ejection systolic murmur. The hemoglobin level was 50 g/L and RBC, reticulocyte, leukocyte, and platelet counts were $1.4 \times 10^{12}/L$, $120 \times 10^9/L$, $1.2 \times 10^9/L$, and $100 \times 10^9/L$, respectively. The bone marrow showed marked erythroid hyperplasia and a few small densely stained lymphocytes; a trephine biopsy confirmed a diagnosis of non-Hodgkin's lymphoma of the centrocytic-centroblastic type. The serum bilirubin level was $32 \mu mol/L$ (normal, $\leq 17 \mu mol/L$) and the haptoglobin was < 0.1 g/L. The direct antiglobulin test (DAT) was positive for IgM, C3d, and C3c. A diagnosis of warm-type AIHA secondary to malignant lymphoma was made. Pulsed combination chemotherapy (cyclophosphamide, vincristine, adriamycin, and prednisolone) was started and blood transfusion support was given. The patient responded well, her general condition gradually improved, and the splenomegaly decreased; transfusions were no longer needed after 6 months, although the DAT remained positive for IgM and C3d. Eighteen months after presentation, she complained of a decline in her general health and progressive weakness and lethargy. These symptoms were due to a relapse of the AIHA: hemoglobin 79 g/L, reticulocyte count $211 \times 10^9/L$, serum bilirubin $41 \mu mol/L$, haptoglobin < 0.1 g/L, and strongly positive DAT for IgM, C3d, and C3c. Treatment with high-dose steroids, intravenous immunoglobulin, and blood transfusion was instituted, and subsequent splenic irradiation was given; splenectomy was eventually performed at 23 months. The pulsed chemotherapy continued and the patient slowly recovered. She was reasonably well when last seen 39 months after presentation; her blood counts were normal, but the DAT was still positive for C3d.

Patient 2 was a 65-year-old female who presented

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Red blood cell (RBC) autoantibodies of the IgM class can be found in approximately 30 percent of patients with warm-type autoimmune hemolysis if sensitive direct antiglobulin techniques are used; these autoantibodies almost always occur in association with antibodies of the IgG and/or IgA class. Patients in whom autoimmune hemolytic anemia (AIHA) is solely due to warm-reacting IgM antibodies are exceedingly rare. We report two such cases; the autoantibody nature of the two IgM antibodies was confirmed by their ability to rebind to normal RBCs after elution; the absence of small increases in RBC-bound IgG and IgA was shown by a sensitive enzyme-linked antiglobulin test.
with a 2-month history of anemia. There had been no symptoms suggestive of cardiovascular or lymphoproliferative disease or of malignancy. Physical examination revealed pallor and a degree of icterus but was otherwise unremarkable. The hemoglobin level was 78 g/L and reticulocytes were 244 × 10⁹/L. The blood film was consistent with hemolytic anemia, showing spherocytes and polychromasia; bone marrow aspiration confirmed erythroid hyperplasia but revealed no other pathology. The serum levels of bilirubin, lactic dehydrogenase, and haptoglobin were 50 μmol/L, 733 IU/L (normal: 230–460 IU/L) and < 0.1 g/L, respectively. The DAT was strongly positive for IgM and C3d. A diagnosis of idiopathic warm-type AIHA was made and treatment with prednisolone (40 mg/day) was started. The initial response was good and the hemoglobin level rose significantly over 2 weeks. However, an attempt to reduce the dose of prednisolone was unsuccessful and azathioprine (100 mg daily) was added to the treatment regime. One month after presentation, she was readmitted to the hospital with a hyperosmolar nonketotic diabetic coma associated with a Salmonella septicemia and she required treatment with insulin and intravenous antibiotics; blood transfusion also was necessary as the hemoglobin level had fallen to 61 g/L. Three weeks later she was again admitted, on this occasion suffering from pneumonia, and was once more treated with intravenous antibiotics; blood transfusion also was given even though the hemolytic anemia appeared to be well controlled with continued treatment. Further blood transfusions were given 2 and 6 weeks after this episode, but the patient became lost to follow-up thereafter and apparently died at home within a short period. Details of the cause of death were not available.

Materials and Methods
The immunohematologic investigations carried out at this Centre have been described elsewhere.2–5 The DATs employed standard agglutination and enzyme-linked methods, both techniques using heavy chain-specific anti-human IgG, IgA, and IgM; the agglutination method also used anti-human C3d and -C3c. The agglutinating reagents were produced at this Centre by immunizing rabbits with purified human serum protein (e.g., IgM), and made specific by selective absorption. The enzyme-linked reagents were obtained commercially (Sigma Biosciences, Poole, UK); this assay was extremely sensitive and could measure the small amounts of immunoglobulins on normal RBCs and, in the case of IgG, the results in absorbance units could be converted to molecules per cell.3 Indirect antiglobulin tests (IATs) were used to examine the autoantibodies in the serum and in eluates prepared by standard heat and acid methods.5,6 Tests for warm and cold hemolysins were carried out as described previously and involved the use of acidified serum at 18°C and 37°C, papainized RBCs at 37°C, and the indirect Donath Landsteiner procedure.5 Serum haptoglobin levels were measured by their hemoglobin binding capacity7 (normal range: 0.4–2.0 g/L).

Results
Patient 1 was group A, D+. The standard agglutination DAT was strongly positive for IgM, C3d, and C3c, and negative for IgG and IgA. The enzyme-linked DAT was strongly positive for IgM and negative for IgG and IgA; it showed that the amount of cell-bound IgG was, in fact, reduced (< 10 mols/red cell). The RBC eluate contained autoantibody of the IgM class only, and showed no specificity within the routinely identified blood group systems (ABO, Rh, Kell, MNS, Duffy, and Kidd). A weak IgM-class autoantibody with stronger reactions against autologous red cells was identified in the serum using a LISS IAT. Tests for warm and cold hemolysins were negative. This serologic pattern remained virtually unchanged throughout the course of her illness and was still evident when the AIHA relapsed 18 months after presentation.

Patient 2 was group A, D+. The standard agglutination DAT was positive for IgM and C3d and negative for IgG and IgA. The enzyme-linked test was strongly positive for IgM, whereas the level of cell-bound IgG (< 10 mols/red cell) was reduced. Autoantibody of the IgM class only was eluted from the RBCs; it showed no evidence of routine specificity. The IAT employing LISS demonstrated that no free autoantibodies were present in the serum. Tests for warm and cold hemolysins were negative. The serologic pattern was similar in tests carried out 1 and 2 months after presentation, but at 3 months, the enzyme-linked DAT showed weak positive results for IgG and IgA (in addition to the strong IgM), the IgG being detected by the agglutination method at 4 months. These changes were accompanied by the development of a weakly reacting serum autoantibody; the haptoglobin levels remained normal during this period.

Discussion
Patients with warm IgM autoantibodies as the sole immunoglobulin bound to their RBCs are exceedingly rare and account for well below 1 percent of cases with AIHA.1,2,8–10 These autoantibodies are, however, more commonly seen in association with autoantibodies of
other immunoglobulin classes, in which quoted incidences range from approximately 2–8 percent using conventional DATs to about 30 percent if sensitive enzyme-linked methods are employed, and as such are more frequent than warm autoantibodies of the IgA class. Because warm IgM autoantibodies are rare, their literature tends to be biased toward interesting and spectacular cases; this should be kept in mind when considering, for example, details of age, sex-associated diseases, and clinical manifestations. Particularly interesting cases also may be featured in more than one article.

Our patients were both female and in their middle 60s, which is in keeping with previous reports in adults of 16 females and 12 males, age 22–78 years (median age 57 years) and 15–78 years (median age 58 years), respectively. In the one published series of warm IgM autoantibodies in children, there were 7 females and 5 males, and their ages ranged from 2 days to 6 years.

In Patient 1, the condition was secondary to non-Hodgkin’s lymphoma, whereas Patient 2’s condition was idiopathic. A number of the previous cases also were secondary to a variety of diseases, including non-Hodgkin’s lymphoma, Hodgkin’s disease, chronic myeloid leukemia, primary biliary cirrhosis, systemic lupus erythematosus, polyarthritis, rheumatoid arthritis, collagen vascular disease, infection, and methyl dopa therapy; whereas in others, the condition was idiopathic.

The autoantibodies in our cases were predominantly cell bound and showed no obvious specificity within the routinely identified blood group systems; however, it should be noted that they were not tested for sialidase-sensitive determinants (anti-En, Pr, Wp, or Ge), which have been particularly associated with warm-reacting IgM-class autoantibodies and severe, sometimes fatal, hemolysis. Other reported specificities have included anti-c, anti-e, anti-H, anti-I, and anti-IT.

Warm IgM autoantibodies almost always bind complement, as in Patients 1 and 2, and can be conveniently classified by their in vitro serologic reactions into agglutinins and hemolysins, which may be either incomplete or complete.

The present patients can be placed in the incomplete agglutinin group in which the IgM autoantibodies are detected by antiglobulin techniques. RBC destruction is most often extravascular and takes place mainly in the liver via macrophage complement receptors. There are few reported cases in this category, and the severity of the hemolysis in Patients 1 and 2, as judged by the nadir of hemoglobin levels (50 and 78 g/L), was similar to the 48–71 g/L in the other cases. The clinical course has varied dramatically and includes examples of transient hemolysis responding well to treatment, acute fulminating and rapidly fatal hemolysis, and chronic hemolysis, which is difficult to treat, as in our patients. It should be recalled that Patient 2 died within a few months of presentation.

Complete agglutinins agglutinate the patient’s RBCs in saline and give rise to severe hemolysis, with hemoglobin levels ranging from 33 g/L to 72 g/L. The response to treatment has varied; some patients experience a good recovery with steroids, whereas others experience a rapidly fatal hemolysis that was refractory to intensive treatment.

The vast majority of warm IgM hemolysins fall into the incomplete group; these react only with enzyme-treated RBCs in vitro. They frequently occur in association with autoantibodies of other immunoglobulin classes (mostly IgG), being detected in 259 out of 1,999 cases (13%) with warm autoantibodies and in 18 out of 340 patients (5.3%) with warm-type autoimmune hemolytic anemia. Clinically, incomplete hemolysins are relatively benign and only occasionally are associated with moderate hemolysis in vivo. If treatment is required, steroids and blood transfusions are usually effective. However, in some patients with complicated clinical histories, fatalities have occurred and were associated with severe hemolysis and hemoglobin levels as low as 46 g/L.

Complete hemolysins lyse nonenzyme-treated RBCs in vitro and are typically associated with severe intravascular hemolysis; this may be fatal, sometimes within 24 hours of diagnosis. Fortunately, these antibodies are rare, with only 4 out of 340 examples of warm hemolysins reacting with untreated RBCs. Hemoglobin levels are typically low (e.g., < 50 g/L), and patient treatment, based on blood transfusion and high-dose steroids, can be difficult, although sometimes a good response has been reported; plasma exchange and complement inhibitory agents have been suggested on theoretical grounds. Occasional cases may have a more benign prognosis and two patients in which the in vitro hemolytic activity was ionic-strength dependent had no evidence of hemolysis in vivo.

Some patients do not fit into these groups and a series of children were described with warm IgM RBC autoantibodies that were nonagglutinating and noncomplement binding. Hemolysis was generally severe.
(hemoglobin levels ranging from 30–94 g/L), but tended to respond well to steroids and blood transfusions. How these IgM autoantibodies caused RBC destruction (other than by the undetected activation of complement) is not known, as macrophages are said to lack specific receptors for IgM. However, in other circumstances, evidence has been presented that IgM coating can act synergistically with IgG in promoting RBC destruction independently of complement. In a study using strictly defined population groups with no detectable RBC-bound complement, a significantly higher proportion of patients with small amounts of IgM in addition to IgG on their RBCs had haptoglobins of < 0.1 g/L compared with patients with IgG alone on their cells (p < .0005).

The autoantibody nature of the cell-bound IgM in the present patients was shown by the elution studies whereby the IgM could be rebound onto normal RBCs and detected by an indirect antiglobulin technique; this rebinding distinguishes it from immunoglobulin attached to the red cells as part of an immune complex or from nonspecific adsorption. It can be difficult to obtain IgM autoantibodies in eluates and in some early studies all attempts failed. An investigation that addressed these problems showed that heat elution was the best method, and when 42 patients with increased amounts of cell-bound IgM were examined, IgM was detected in 38 out of 53 eluates (72%), and its presence was unrelated to whether the autoantibodies were of the cold or warm type. In another study involving 124 patients with warm-reacting IgA class autoantibodies, concomitant IgM antibodies were detected in 61 cases and were elutable in 31. IgM was identified in 44 of 106 eluates and subjected to sodium dodecyl sulphate–polyacrylamide gel electrophoresis and immunoblotting. The IgM was pentameric in 43 instances, but in one case, an elderly patient with chronic lymphocytic leukemia, only oligomeric IgM was identified.

In conclusion, warm-type AIHA solely due to IgM-class autoantibodies is an exceedingly rare condition but should always be considered when the clinical evidence is in keeping with this diagnosis and tests have ruled out the presence of RBC autoantibodies of the IgG and IgA class.

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References


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