Clinical evaluation for lymphoproliferative disease prompted by finding of IgM warm autoanti-I^T in two cases

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Anti-I^T is an unusual specificity originally described as a naturally occurring cold agglutinin. The antibody reacts strongly with cord RBCs, weakly with adult I RBCs, and most weakly with the rare adult i RBCs. IgG anti-I^T in patients with hemolytic anemia has been associated with Hodgkin's lymphoma. Difficulties in blood grouping tests and the presence of a warm reactive agglutinin in samples from two patients with hemolytic anemia led to further serologic studies and the identification of anti-I^T. In both cases, the anti-I^T was a rarely encountered IgM warm reactive agglutinin; in one case, the IgG component was also anti-I^T, whereas in the second case the IgG antibody was broadly reactive. The unusual serologic finding of anti-I^T prompted further clinical evaluation for lymphoproliferative disease in these two patients. ***Immunohematology* 2009;25:60–62.**

Key Words: IgM warm autoantibody, autoanti-I^T, hemolytic anemia

Anti-I^T was originally described as a benign, naturally occurring, IgM cold agglutinin in Melanesians[1] and later in Venezuela Indians.[2] The anti-I^T agglutinin did not demonstrate classical I or i specificity but reacted strongly with cord RBCs, weakly with normal adult I RBCs, and most weakly with the rare adult i RBCs. It was thought the agglutinin recognized a transition state of i to I, thus the designation I^T (T for transition).[1] Subsequent data do not support this hypothesis; I^T is expressed nearly as well or as well on fetal RBCs ranging in age from 11 to 16 weeks as on cord RBCs.[3] The biochemical structure of I^T is still unclear.

The first four examples of IgG anti-I^T were in sera of patients with autoimmune hemolytic anemia (AIHA) and Hodgkin's lymphoma.[3,4] Others reported a similar association.[5] Hafleigh et al.[6] found three examples of IgG autoanti-I^T in patients who did not have Hodgkin's disease or AIHA. One example of IgM warm anti-I^T and one IgM cold plus IgG anti-I^T in patients with AIHA were not associated with Hodgkin's disease, but one of the cases was associated with non-Hodgkin's lymphoma.[7,8] We report an unusual 37°C reactive IgM agglutinin plus an IgG anti-I^T[9] and a 37°C reactive IgM agglutinating anti-I^T plus broadly reactive IgG autoantibody in two patients with hemolytic anemia who were subsequently evaluated for lymphoproliferative disease owing to the specificity of the antibodies reported.

Case Reports

Case 1

A 69-year-old Hispanic woman presented with pancytopenia (WBC = 2.3 × 10^9/L; Hb = 6.9 g/dL; Hct = 19.1%; platelets = 64,000/μL), splenomegaly but no lymphadenopathy, and hemolytic anemia (spherocytosis; LDH = 672 U/L [normal 355–630]; total bilirubin = 2.8 mg/dL [normal 0.1–1.2]; direct bilirubin = 0.7 mg/dL [normal 0–0.4]). The direct antiglobulin test (DAT) was positive. Lymphoma was not suspected until after the anti-I^T was identified. Computed tomography (CT) scans showed evidence of large lymphoid infiltrates composed of a mixture of small and large lymphocytes. Bilateral bone marrow aspiration showed increased lymphocytes but was not diagnostic of lymphoma. The patient was empirically treated with steroids and had improved hemoglobin and sense of well-being. Steroids were tapered. Two and a half months later the patient returned for a splenectomy, which was pathologically negative for lymphoma. Sections showed lymphoid hyperplasia with a benign phenotype consisting of T and B cells with no aberrant expression of CD5, CD23, or bcl-2. Serologic studies at that time showed similar reactivity of anti-I^T. Three months later, the patient exhibited mild thrombocytopenia with slightly elevated LDH. A repeat bone marrow showed lymphocytosis suggestive of low-grade B-cell lymphoproliferative disease. A year later, after rituximab and other chemotherapy, the bone marrow showed atypical lymphoid infiltrates. The infiltrates were composed primarily of small mature lymphocytes with occasional larger lymphocytes noted, most likely representing a lymphoproliferative disorder. Flow cytometry showed an entire B-cell population that did not express CD20. Less than 3 years after original presentation, the patient was transfusion-dependant and diagnosed with aplastic anemia. She was discharged to hospice care with end-stage liver disease, lymphoproliferative disorder, and hemolytic anemia.

Case 2

A previously healthy 19-year-old Korean man presented to the emergency room with insidious onset of fatigue, shortness of breath, dyspnea on exertion, and pallor. Laboratory data revealed thrombocytopenia and hemolytic anemia:

WBC = 6.8 × 10^9/L; Hb = 4.6 g/dL; Hct = 12%; platelets = 20,000/μL; spherocytes on the peripheral smear; total bilirubin = 2.9 mg/dL; direct bilirubin = 0.4 mg/dL; and LDH = 539 U/L. The haptoglobin was less than 5.8 mg/dL. There was no splenomegaly or lymphadenopathy. The DAT was positive. An autoanti-I^T agglutinin plus a broadly reactive IgG autoantibody were identified. After a few days on pulse dexamethasone (Decadron) therapy the hemoglobin improved only mildly, and the patient was started on a 4-day course of IVIG with recovery of his hemoglobin to 9.3 g/dL. Because of the association of lymphoma with anti-I^T the patient underwent CT scans, bone marrow biopsy, and positron emission tomography. There was no evidence of malignancy or lymphoproliferative process. During the period of a year, the patient had multiple relapses of the hemolytic anemia (but not thrombocytopenia), necessitating further treatment with steroids. Rituximab, given for the hemolytic process, had no sustained benefit. The patient underwent a splenectomy, and both the hemoglobin and platelet count remain normal. There has been no evidence of Hodgkin's disease, lymphoma, or other malignancy in several years of follow-up.

Materials and Methods

The DAT was performed with anti-human IgG (Ortho Clinical Diagnostics, Raritan, NJ) and anti-human C3 (in-house reagent). A 6% bovine albumin control was tested in parallel. The in-house anti-C3 was prepared by injecting rabbits with purified proteins and standardized as previously described.[10] Before testing, the patients' RBCs were treated with 0.01 M dithiothreitol (DTT) to break IgM bonds that cause spontaneous agglutination.[11] Eluates were prepared from the patients' RBCs using a commercial acid elution kit (Gamma Elu-Kit II, Gamma Biologicals, Houston, TX); cold LISS was substituted for the kit wash solution.[12]

For immunoglobulin classification, samples were treated with 0.01 M DTT. The agglutinin titer and thermal amplitude was determined at 37°C (prewarmed), 30°C, 22°C, and 4°C with group O adult I, cord, and adult i RBCs,[10] and for Case 1, with DTT-treated autologous RBCS. The tests at each temperature were set up separately (i.e., using separate sets of dilutions) to eliminate potential carryover of agglutination. Patients' sera were also tested by a previously described serum screen method[10] used to characterize antibodies in AIHA. Briefly, serum was tested with and without acidification and the addition of fresh normal serum as a source of complement, against untreated and enzyme-treated RBCs at 37°C (prewarmed) and 20°C. Agglutination results were graded and scored as previously described.[10]

Results

Case 1

The DAT on DTT-treated RBCs was 3+ with anti-IgG and anti-C3; the 6% albumin control was nonreactive. The initial titer and thermal amplitude studies showed a 37°C agglutinin that reacted to a higher titer with cord RBCs than with adult I RBCs. Titration results with adult I, cord i, and adult i RBCs (to determine specificity) are shown in Table 1. Anomalous results (unexplained high titer) were obtained with DTT-treated autologous RBCs: titers of 512 at 37°C, 1024 at 30°C, 2048 at 22°C, and 8000 at 4°C. An apparent anti-I was detected at 4°C (adult I RBC titer of 256, cord RBC titer of 64). An acid eluate prepared from the patient's RBCs reacted more strongly with cord RBCs than with adult I RBCs at 37°C. After treatment with DTT, the patient's serum and the eluate did not agglutinate cord RBCs, but reacted 1+ and 4+, respectively, at the antiglobulin test with anti-IgG. The dilution control (for the abolished agglutination) reacted 3½ to 4+ with the serum and 1½ + with the eluate. Thus, IgM plus IgG anti-I^T was demonstrated in both the serum and the eluate. In the AIHA serum screen, agglutination was observed at 22°C and 37°C similar to that in the titration and thermal amplitude studies; a hemolysin was also observed with enzyme-treated RBCs at both temperatures, but without a clear preference for either temperature.

Case 2

The DAT on DTT-treated RBCs was 4+ with anti-IgG and anti-C3; the 6% albumin control was nonreactive. In the initial titer and thermal amplitude studies, an agglutinin reacted at 37°C with cord RBCs; adult I RBCs did not react at 37°C or 30°C. Titration results with adult I, cord i, and adult i RBCs are shown in Table 1. Sufficient autologous RBCs were not available. DTT treatment of the patient's serum abolished agglutination with cord RBCs; however, dilutions of DTT-treated serum tested against adult I and cord RBCs did not show a difference in reaction strength of the IgG component. Thus, an IgM anti-I^T plus a broadly reactive IgG antibody was demonstrated in the serum. The eluate contained an IgG antibody suggestive of anti-I^T (titer/score versus adult I RBCs was 32/42 and versus cord RBCs was 128/55). In the AIHA serum screen, untreated RBCs were weakly agglutinated at 22°C and weakly sensitized at 37°C; enzyme-treated RBCs were agglutinated and hemolyzed at both temperatures.

Discussion

Table 1. Titer and thermal amplitude of two examples of anti-I^T

RBCS	Case 1 titer (score) at:				Case 2 titer (score) at:			
	37°C	30°C	22°C	4°C	37°C	30°C	22°C	4°C
Adult I	1 (4)	1 (6)	2 (11)	256 (78)	0	0	1 (4)	8 (28)
Cord i	32 (37)	128 (61)	256 (68)	64 (56)	64 (54)	64 (52)-128 (60)	64 (49)	32 (48)
Adult i	1 (6)	2 (10)	2 (10)	NT*	0	1 (4)	1 (5)	NT

*NT = not tested

IgM warm autoagglutinins reactive at 37°C are an unusual subcategory of warm autoimmune hemolytic anemia.[10,13] Typically, the patient's RBCs are spontaneously agglutinated, requiring treatment with a sulfhydryl reagent such as DTT to resolve ABO and Rh typing and to interpret the DAT. The two cases presented here were referred to the immunohematology research laboratory for further diagnostic testing and characterization of the agglutinin when these characteristics were noted by our reference laboratory. In both cases the warm agglutinin demonstrated I^T specificity. Both of our cases also demonstrated an IgG autoantibody component; in Case 1, the IgG autoantibody was anti-I^T.

We have no explanation for the high titers obtained after DTT-treatment of the group O autologous RBCs in Case 1. This may indicate that the patient's RBCs had greatly increased I^T expression. When group O untreated, DTT-treated, and ficin-treated cord RBCs were tested in parallel with dilutions of this patient's anti-I^T and another sample of anti-I^T, no enhancement of reactivity was observed with DTT treatment whereas reactivity with ficin-treated RBCs was increased as expected by three to four dilutions (data not shown). Thus, the DTT treatment should not have affected the autologous RBC test results. The previous sample of warm IgM anti-I^T associated with AIHA did not agglutinate that patient's untreated autologous RBCs at 37°C (the optimal temperature of reactivity).[7]

Anti-I^T is an unusual specificity and may not even be suspected unless dilutions of the sera are tested with cord i and adult i RBCs in parallel with adult I RBCs. In our cases, additional titrations to identify the specificity were only pursued after the initial increased reactivity with cord RBCs was observed. Examples of anti-I^T have been reported to be IgM cold agglutinins or IgG antibodies optimally reactive at 37°C. We are only aware of one reported example of IgM warm anti-I^T, and that case was not associated with Hodgkin's disease or lymphoma.[7] In more than 20 years, we have encountered only two other examples of IgM warm reactive anti-I^T; neither had an IgG component, but both were associated with a history of lymphoma before the serologic workup.

Determining the specificity of autoantibodies is typically of academic interest only. In general, serologic results do not necessarily prompt a clinical evaluation for lymphoma. In both of these cases, the unusual RBC antibody and the historic association of this specificity with lymphoma, together with the presenting findings of AIHA, led to the lymphoma workup. For patients who do not progress to lymphoma, one might speculate that rituximab (anti-CD20) given for the AIHA could have eradicated a malignant clone of B cells.

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