Brief Reports

Additional Examples of Cold Autoagglutinins with M Specificity

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Examples of both anti-M and anti-N have been reported in patients possessing these antigens; however, in only two instances has anti-M been reported as an autoantibody. This paper describes two additional patients with auto anti-M. In each case the anti-M was shown to be an autoantibody by absorption and elution studies. Optimum reactivity was obtained at 4 C. Column chromatography and 2-mercaptoethanol reduction indicated that the antibody was an IgM globulin. There was no evidence of autoimmune hemolysis in either of these patients.

IT IS an extremely rare event for a cold autoagglutinin to manifest specificity other than anti-I. Recently, however, two cases of auto anti-M have been reported in the literature. Fletcher and Zmijewski[1] found an auto anti-M in the serum of a pregnant woman without evidence of hemolytic disease. Tegoli *et al.*[2] also reported an auto anti-M in a patient following liver transplantation. Two additional patients with auto anti-M will be described.

Case Report 1

A 37-year-old white female, who had been pregnant nine times, received two units of blood during an appendectomy and seven units during and after a Caesarean section four years previously. Shortly thereafter, symptoms resulting from anomalous communications between the coronary artery and the pulmonary trunk developed. She was treated by operative ligation of the abnormal vessels and received seven units of M negative blood during and after the operation.

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Results

The patient was typed as Group O, Rh_0 positive, MNs, U positive, P_1 positive. The patient's M status was verified with two human and six rabbit anti-M sera. No mixed field phenomena were observed; thus, the possibilities of chimerism or mosaicism were unlikely. The direct antiglobulin test was negative.

The tests for antibody in the serum prior to the operation were positive only at 4 C. At this temperature all red blood cells in a commercial panel were agglutinated (Table 1). The presence of anti-M was suspected since it was observed that the strongest reactions occurred with homozygous M cells and the weakest with homozygous N cells. The patient's serum agglutinated homozygous N cells from an adult but not cord cells, suggesting the possibility of anti-I in addition to anti-M. Furthermore, reactions occurred with MN cord cells, demonstrating that the presence of the I antigen was not necessary for anti-M reactivity. Absorption at 0 C with the patient's ficin-treated cells completely removed the anti-I revealing anti-M. This absorbed serum was then reabsorbed at 0 C with the patient's nonficin-treated cells.

TABLE 1. *Case 1. Reactivity* of Serum*

	1 M	2 M	3 MN	4 MN	5 MN	6 N	7 N	8 N	Auto MN
Saline 4 C	3+	3+	2+	2+	2+	2+	1+	1+	2+
Saline 25 C	1+	0	0	0	0	0	0	0	0
Albumin 37 C	0	0	0	0	0	0	0	0	0
Indirect antiglobulin test	0	0	0	0	0	0	0	0	0

* 4+ One solid agglutinate, clear background.
3+ Several large agglutinates, clear background.
2+ Uniform medium-sized agglutinates.
1+ Uniform small agglutinates, opaque reddish background.
0 No agglutination.

This procedure completely removed anti-M from the serum. Furthermore, anti-M could be recovered from the cells used for the latter absorption by eluting at 37 C for 15 minutes (Table 2). Homozygous N cells failed to absorb the anti-M. Acidification of serum (to *p*H 6.6), as suggested by Beattie *et al.*,[3] resulted in enhancement of the anti-M activity, insofar as the undiluted acidified serum reacted with M and MN cells at room temperature while unacidified serum did not. Specificity and titer were equivalent for acidified and nonacidified sera at 4 C. No other irregular antibodies were demonstrated.

Column chromatography and 2-mercaptoethanol reduction indicated this auto anti-M to be an IgM globulin. The reactivity of the patient's anti-M remained constant over an 11-month period.

Case Report 2

A 51-year-old white female had clinical and histopathologic evidence for chronic aggressive hepatitis. She had not been transfused with blood or components and had been pregnant once.

Results

The patient was typed as Group B, Rh_0 negative, MNs, U positive, P_1 negative. Her cells failed to react with the following antisera: anti-Vw, -M^g, -Mi^a, -Hunter, and -Henshaw. Two human and four rabbit anti-M sera demonstrated that the patient's cells reacted to the same titer as other MN adult and cord cells. As in Case 1, there was no evidence to suggest chimerism or mosaicism. The patient's direct antiglobulin test was negative.

TABLE 2. *Case 1. Differential Absorption of Anti-I and Anti-M and Subsequent Elution of Anti-M*

	Reactions at 4 C								
	1 M	2 M	3 MN	4 MN	5 MN	6 N	7 N	8 N	Auto MN
Unabsorbed serum ↓	3+	3+	2+	2+	2+	2+	1+	1+	2+
Autoabsorbed with ficinized cells ↓	2+	2+	1+	1+	1+	0	0	0	1+
Further autoabsorbed with nonficinized cells ↓	0	0	0	0	0	0	0	0	0
Eluate of autoabsorbed nonficinized cells	2+	2+	1+	1+	1+	0	0	0	1+

TABLE 3. *Case 2. Reactivity of Unabsorbed Serum*

	1 M	2 M	3 M	4 MN	5 MN	6 N	7 N	8 N	Auto MN
Saline 4 C	4+	4+	3+	4+	3+	3+	3+	3+	4+
Saline 25 C	1+	0	2+	3+	1+	3+	2+	0	0
Albumin 37 C	0	0	0	0	0	0	0	0	0
Indirect antiglobulin test	0	0	0	0	0	0	0	0	0

Reaction of the patient's unabsorbed serum with a commercial panel of red blood cells revealed a strong cold autoagglutinin (Table 3). Autoabsorption with ficin-treated cells revealed anti-M reactive only in saline at 4 C (Table 4). This antibody resisted further absorption with the patient's ficin-treated cells, whereas two absorptions with nonficin-treated cells completely removed it. As in the previous case, anti-M was recovered in eluates prepared from nonenzyme-treated cells used for autoabsorption.

The anti-M was shown to be an IgM globulin by column chromatography and 2-mercaptoethanol denaturation. This patient's serum was studied from April through December 1971, and on each occasion anti-M having the same characteristics was present.

Discussion

In addition to the cases of Tegoli *et al.*[2] and Fletcher *et al.*[1] and the two examples of auto anti-M described in this report, a retrospective study from the University of Michigan Blood Bank revealed another example of anti-M occurring in an M positive patient. This 67-year-old white female, who had been pregnant but never transfused, was admitted for cardiac catheterization in January of 1965. The patient was typed as Group O, Rh_o positive, MN. A strong cold autoagglutinin was found reacting equally with all members of the panel in saline at low temperature. Two months later, a further specimen of this patient was examined and, on this occasion, cells containing the M antigen, including the patient's cells, reacted stronger than those M negative. Since the patient had been typed as MN, specimens were sent to three reference laboratories. Each of them confirmed the presence of a cold antibody having M specificity.

Several factors seem remarkably similar among the auto anti-M antibodies which have been described. All five cases have occurred in white females. The immunoglobulin nature of four of the antibodies has been investigated and found to be composed largely of IgM. While the thermal amplitude of auto anti-M is somewhat variable, this antibody characteristically reacts optimally at 4 C. The auto anti-M reported by Tegoli *et al.*[2] occurred in a 13-year-old girl shortly after hepatic transplantation. The antibody was significantly attenuated four weeks after its initial identification and was absent eight weeks later at the time of the patient's death. Somewhat similarly, the auto anti-M described by Fletcher *et*

TABLE 4. *Case 2. Reactivity of Serum After Autoabsorption With Ficin-Treated Cells*

	1 M	2 M	3 MN	4 MN	5 MN	6 MN	7 N	8 N	Auto MN
Saline 4 C	3+	3+	3+	2+	2+	2+	0	0	2+
Saline 25 C	0	0	0	0	0	0	0	0	0
Albumin 37 C	0	0	0	0	0	0	0	0	0
Indirect antiglobulin test	0	0	0	0	0	0	0	0	0

al.[1] required more precise conditions for its detection as time passed until at five months after discovery it could not be demonstrated in saline without serum acidification. The thermal amplitude of the auto anti-M antibodies described by Tegoli *et al.*[2] and Fletcher *et al.*[1] was such that they were initially apparent at 37 C and 20 C, respectively. The auto anti-M antibodies demonstrated in the sera of our patients consistently required a temperature of 4 C from the time of discovery. Furthermore, throughout the follow-up periods, 11 months and eight months respectively, these antibodies were detectable at 4 C with routine saline incubation. There appears to be no correlation between auto anti-M and a specific pathologic process.

References

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