An Autoantibody with U-specificity in a Patient with Myasthenia Gravis

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Lack of reactivity of IgG autoantibodies with Rh_{null} red blood cells does not necessarily imply specificity directed towards the Rh system. Recent reports have indicated that occasionally the autoantibodies of warm antibody type hemolytic anemia may have U-specificity. This report describes an example of auto-anti-U occurring in a patient with myasthenia gravis who had no evidence of hemolysis.

Although the majority of autoantibodies associated with warm antibody type autoimmune hemolytic anemia appear to be "non-specific," blood group specificity occasionally can be recognized. This first was demonstrated by Weiner[15] in 1953, and confirmed in subsequent reports.[5] With rare exceptions,[6, 7, 14, 17] such specificity is directed towards the Rh system; moreover, many of the apparently "non-specific" autoantibodies recognize antigenic material intimately involved in the Rh determinants.[4, 16]

Recently, Nugent *et al.*[11] reported a patient with autoimmune hemolytic anemia whose autoantibody reacted in a "non-specific" manner with cells of common Rh types, more weakly with -D- cells and not at all with Rh_{null} cells. Subsequent studies revealed the antibody specificity to be anti-U rather than anti-Rh.

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It is now evident that this case does not represent an isolated instance of auto anti-U. Marsh[9] and his colleagues found complex autoantibody specificities involving Rh and U in three of a series of 50 patients with warm antibody autoimmune hemolytic anemia. Blajchman *et al.*[3] described two members of a family found to have idiopathic autoimmune hemolytic anemia. The propositi's serum and eluate contained anti-U.

This report describes a further example of autoimmunity involving U-specificity. In this patient, however, no evidence of *in vivo* hemolysis could be found.

Case Report

A 23-year-old Caucasian man first was seen in 1970 complaining of weakness, diplopia and fatigability. This led to a diagnosis of myasthenia gravis. Serologic studies were not performed until one year later when he was readmitted for thymectomy. A strong positive direct antiglobulin test, found at that time, was due to IgG sensitization. No free antibody was demonstrated in his serum other than a weak, cold-reactive anti-I, no stronger than that encountered in many normal sera. An eluate prepared from the patient's cells yielded anti-U.

Studies performed one week later confirmed the original finding and suggested the additional presence of a "non-specific" autoantibody. Further tests of the patient's serum, performed three months later, revealed a strong "non-specific" component which, following absorption with U-negative cells, left

anti-U. At no time during the period of observation was the patient anemic. His hemoglobin consistently was above 15.0 g/100 ml, and his hematocrit averaged 44 per cent. Reticulocytes comprised no more than 0.9 per cent of the erythrocytes.

Materials and Methods

Standard blood typing technics were used throughout. Eluates were prepared by the method of Rubin[12] from EDTA anticoagulated blood. Red blood cells used for antibody identification studies either were from commercially prepared panels or from our own frozen panels.

Results of Serologic Studies

The direct antiglobulin test was strongly positive with a broad spectrum anti-human globulin serum. The prior addition of human IgG was inhibitory, showing sensitization to be due to IgG globulins. The patient's cells were not agglutinated by rabbit anti-C'3. The patient was typed as O, Rho (D) positive, MS. The anti-s and anti-U typing sera available were reactive only by the indirect antiglobulin technic, thereby precluding their use.

The patient's serum contained a cold-reactive anti-I agglutinin. An eluate prepared from his cells yielded a very strong antibody which reacted with all members of a commercial panel by the indirect antiglobulin technic. No difference in strength of reaction between the various cell samples was noted with either undiluted eluate or eluate diluted 1:25 with saline solution. Two U-negative cell samples that had been stored frozen in glycerol failed to react; a third U-negative cell sample from a commercial source also was non-reactive. In addition, two cell samples of the Rh_{null} phenotype were not agglutinated by the patient's eluate.

A panel of cells, selected for the absence of high incidence antigens was tested with the eluate by the indirect antiglobulin technic. The following cells were all strongly reactive: Lu(b—), Fy(a—b—), K+k—, Kp(a+b—), Js(a+b—), K_o, Tj(a—), Vel negative, Cs(a—), Chido negative, Ge negative, and Lan negative. An adult i cell and ten cord blood samples also were strongly reactive. Weaker reactions were obtained with an example of each of the following cell types: -D-, CD-, and $rh^{G}rh^{G}$. The results of titration of the eluate against various red blood cells are shown in Table 1.

TABLE 1. *Reactions of Patient's Eluate against Various Red Blood Cells*

Red blood cell type	Dilution Reciprocal							Score
	1	2	4	8	16	32	64	
Rh1, Rh2, U+	+++	+++	+++	+++	++	+	0	53
Rh2, Rh2, U+	+++	+++	+++	+++	++	0	0	48
-D-/-D-, U+	+++	+++	++	±	0	0	0	31
CD-/CD-, U+	+++	+++	+++	+	0	0	0	35
rh^{G} rh^{G}, U+	+++	+++	+++	±	0	0	0	33
U-	0	0	0	0	0	0	0	0
Rh_{null}	0	0	0	0	0	0	0	0

Subsequent serologic investigations three months after the initial studies revealed a change in the specificity of the autoantibody. Whereas the autoantibody initially was monospecific, subsequent studies revealed a "non-specific" component. Beck *et al.*[2] have reported sequential changes in autoantibody specificity in a patient with autoimmune hemolytic anemia, and the present patient manifested similar features. Although the "non-specific" antibody reacted only weakly with Rh_{null} cells, it did not have U-specificity. The concurrent presence of anti-U was demonstrated by absorption of the "non-specific" component with U-negative cells; the absorbed serum had anti-U specificity.

Discussion

The antibody responsible for the positive direct antiglobulin test in this patient represents the second reported example of auto anti-U. Nugent *et al.*[11] recently reported anti-U as a cause of autoimmune hemolytic anemia in an elderly woman, Although the cells of our patient were strongly sensitized, there was no evidence of anemia. In the absence of red blood cell survival studies, we cannot exclude the possibility of a compensated hemolytic process; however, the patient never manifested reticulocytosis.

The observations in this patient support the finding of Nugent *et al.* that antibodies reacting with all cells other than Rh_{null} do not necessarily have Rh-specificity. Nugent *et al.*[11] and Marsh *et al.*[9] noted that failure of reactivity of autoantibodies with Rh_{null} cells may occasionally be a result of the aberrant U status of these cells. Schmidt *et al.*[13] have postulated that the Rh and U aberrations found in Rh_{null} cells are a result of abnormality of sequential genes controlling common terminal sugars, giving various specificities depending upon the basic precursor.

We have confirmed the observation of Nugent *et al.*[11] that -D- cells react more weakly with auto-anti-U than do U-positive red blood cells of normal Rh phenotypes. This observation of weakened reactivity was extended to include other U-positive cells having the Rh phenotypes CD- and rh^Grh^G.

The significance of the weak reaction of anti-U with rh^Grh^G cells is difficult to assess. The red blood cells used in this study had been frozen in glycerol for nearly two years but their reactivity with other antisera was not modified by storage. We have no reason to believe that prolonged storage alters U antigen activity.[10] Weak reactions of various anti-Rh sera with fresh rh^G cells has been noted by others.[1] If, as Schmidt suggests,[13] there is precursor material common to both Rh and U, it is conceivable that there is weakened expression of the U gene in rh^Grh^G cells.

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