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A Relationship of Direct Coombs Test Pattern to Autoantibody Specificity in Acquired Hemolytic Anemia. (32211)

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In the past 15 years evidence has accumulated(1,2) that "warm-type" autoantibodies in acquired hemolytic anemia are frequently reactive with components of the Rh complex on the human red blood cell (RBC). Independently, it has been learned that these "warm-type" autoantibodies are always or virtually always γG (IgG) globulins (3,4). The presence of such autoantibodies on the surface of a patient's RBC is readily demonstrated *in vitro* by producing agglutination with an anti-γG Coombs serum. In some cases the patient's RBC can also be agglutinated by specific antiserum to human complement (C′) components; in other cases anti-C′ serum gives no reaction(1,4,5). Thus, human RBC autosensitization by "warm-type" γG autoantibodies may or may not be accompanied by bound C′ components. From these observations it seemed reasonable to assume that some γG autoantibodies were C′-fixing while others were not(cf. 4). This difference might, in turn, depend on differences in specificity of the autoantibodies(6), by analogy to observations with blood group isoantibodies. Since it is well known that human Rh *iso*antibodies seldom are C′-fixing (7), a survey was begun in 1965 to evaluate the possibility that, among γG autoantibodies, Rh-related specificity might be found more often in patients whose RBC gave positive direct antiglobulin (Coombs) reactions with anti-γG alone. Conversely, autoantibodies from patients whose RBC were autosensitized with both γG globulin and C′ might, by this reasoning, be less likely to have Rh specificity.

TABLE I. Relationship of Direct Antiglobulin Pattern to Reactivity of Autoantibody with Rh_{null} Erythrocytes.

Direct antiglobulin (Coombs) reaction	No. of patients	Ability of eluted autoantibodies to sensitize: "Normal" RBC*	Rh_{null} RBC
Agglutination by anti-γG globulin alone	10	+++†	0
	2	+++	trace +
	5	+++	+++
Agglutination by both anti-γG and anti-C′	13	+++	+++
	1	+++	0

* Includes O ce/ce (rr) and O DCe/ce (R^1r) red cells.

† Agglutination observed in indirect antiglobulin reaction using an optimal dilution of anti-γG serum.

This report summarizes the results of our initial test of this concept. Autoantibodies, eluted from the RBC of individual patients, were tested for their reactivity with human RBC of common Rh phenotypes and with Rh_{null} RBC. "Rh_{null}" (- - - / - - -) is the rare human RBC phenotype in which none of the antigens of the Rh complex is detectable(8,9).

Materials and methods. The preparation of specific rabbit antisera to human γG globulin and to human C′ components and the procedures used in direct antiglobulin (Coombs) reactions have been described(4). Autoantibodies were eluted at pH 3.0-3.2 from thoroughly washed RBC stromata and concentrated by ultrafiltration, as described elsewhere(10). More recently, the acid elution procedure utilized the modifications of Kochwa and Rosenfield(11). Human RBC of phenotypes O D- -/D- - (L.H.) and A_1 Rh_{null} (- - -/- - -) (L.M.)(9) were generously donated by Drs. F.H. Allen and Arthur Rowe, New York Blood Center. These RBC had been frozen and stored in liquid nitrogen until used. Frozen RBC were rapidly thawed at 37°C in 0.15 M NaCl containing 0.45 M sucrose and 0.45 M dextrose. The recovered RBC were promptly washed twice in isotonic saline at room temperature for serological work. In early experiments, group O DcE/DcE (R^2R^2) RBC, which had also been recovered from liquid nitrogen storage, were used as "normal" RBC. They behaved no differently than freshly drawn RBC of the same phenotype. Thereafter, the RBC of 2 healthy donors, either freshly drawn or stored for a few days at 5°C in Alsever's solution, served as "normal" human RBC. Their apparent genotypes were O ce/ce (rr) and O DCe/ce (R^1r), respectively. All tests of eluted autoantibodies against various RBC phenotypes were carried out by indirect antiglobulin tests. In practice, 0.1 ml of a 2.5% saline suspension of each test cell was incubated at 37°C for 60 minutes with 0.1 ml of an appropriate dilution of a given eluate. After 3 washings the sensitized RBC were tested for macroscopic agglutination with a previously standardized dilution of anti-γG Coombs serum.

Results and discussion. Erythrocyte autoantibodies from 31 patients were studied. Seventeen of these patients exhibited RBC autosensitization with γG globulin alone. In 10 of these 17 cases, the eluted autoantibodies gave no reaction with Rh_{null} RBC (Table I). In 2 additional cases, reactions with Rh_{null} RBC were weaker than reactions with "normal" RBC. In contrast, 5 of these 17 cases gave equal reactions with both "normal" and Rh_{null} phenotypes (Table I).

Fourteen of the 31 patients studied had RBC autosensitization with both γG globulin and C′. In all but one of these cases the eluted autoantibodies reacted strongly with both "normal" and Rh_{null} RBC (Table I).

From present concepts of Rh antigens(9, 12), it is reasonable to conclude that autoantibodies capable of sensitizing Rh_{null} RBC are not reactive with known antigens of the Rh system. By this criterion it is evident from the data in Table I that autoantibodies eluted from RBC which had given positive direct Coombs reactions with both anti-γG and anti-C′ sera have a very low incidence of Rh-related specificity. The specificity of these

TABLE II. Effect of Absorption of Selected Eluates with Rh_{null} RBC.

Patient	Direct antiglobulin test pattern	Prior absorption* of eluate	Reactions† with RBC of phenotype: "Normal"‡	D--/D--	Rh_{null}
Po.	γG + C′	None	+++	++	++++
"		Rh_{null} RBC	0	0	0
Ad.	γG	None	+++	+++	+++
"		Rh_{null} RBC	0	0	0

* A single absorption of 0.5 ml of eluate with 0.25 ml of packed Rh_{null} RBC at 37°C for 60 min. Unabsorbed aliquots of eluate received 0.1 ml of saline to control the effect of dilution in the aliquots receiving Rh_{null} RBC and were incubated in parallel.

† Indirect antiglobulin reactions using an optimal dilution of anti-γG serum.

‡ "Normal" RBC in this experiment were O ce/ce (rr).

autoantibodies will therefore be designated as "non-Rh-related." As shown in Table I, "non-Rh-related" specificity as just defined was also encountered in 5 patients whose RBC gave positive direct Coombs reactions only with anti-γG serum. No suggestion is made that all cases of "non-Rh-related" specificity do, in fact, have a common specificity for some other red cell antigen or system of antigens. There could well be considerable heterogeneity among the "non-Rh-related" cases. The nature of this "non-Rh-specificity" is a subject of continuing investigation in this laboratory.

In contrast, the negative or very weak reactions with Rh_{null} RBC exhibited by 13 of the 31 autoantibodies (Table I) are consistent with specificity for one or more components of the Rh complex. Such negative or weak reactions with Rh_{null} RBC were encountered almost exclusively in cases exhibiting positive direct Coombs reactions only with anti-γG globulin. These autoantibodies will tentatively be designated as having "Rh-related" specificity. It must be admitted, however, that only one Rh_{null} cell was available for these tests and that specificity for some other antigen not represented in this particular Rh_{null} cell has not been excluded. The single example of apparent "Rh-related" specificity in the group exhibiting RBC autosensitization with both γG globulin and C′ (Table I) may be interpreted in the light of occasional instances of γG *iso*antibodies in the Rh system which have been found to be C′-fixing, *e.g.*, anti-CD(5).

It was still possible that the group of cases with "non-Rh-related" specificity might have autoantibodies with "Rh-related" specificity *in addition* to autoantibodies reacting with non-Rh determinants. The results of further studies (Table II) on 2 representative cases are against this interpretation. Absorption of these 2 eluates with Rh_{null} RBC left no detectable antibody reactive with D--/D-- or "normal" RBC phenotypes. The other cases have not yet been studied in this manner.

Further studies are in progress to characterize "Rh-related" specificity, as defined above, more explicitly. All of the autoantibodies in this group have behaved as "pan-antibodies," reacting with all human RBC of several standard test panels. Several cases have been studied more fully by titration against or by absorption with RBC of various phenotypes. Preliminary evidence has been obtained for the presence of anti-e (hr″) or anti-c (hr′) specificities among other undefined but "Rh-related" specificities in these eluates. This agrees with the earlier observations of many other workers(cf. 1) and lends support to the interpretation(2) that autoantibodies in this group are reactive with Rh-related determinants.

A breakdown of cases of idiopathic autoimmune hemolytic anemia according to the red cell antigen system involved may well prove to be a useful step in unravelling the pathogenesis of these autoantibodies. It is probably no accident, for instance, that the recently discovered Coombs positive hemolytic anemia occurring during therapy with α-methyldopa has been characterized by RBC autosensitization with γG globulin alone and Rh-related specificity of eluates(13).

Summary. Of 31 cases of acquired hemolytic anemia with "warm" γG autoantibodies,

17 exhibited positive direct antiglobulin reactions only with anti-γG globulin (Group 1) while 14 gave positive reactions with both anti-γG globulin and anti-complement serum (Group 2). As a preliminary test of Rh-related specificity, autoantibodies eluted from the RBC of patients in both groups were studied for their reactivity with Rh_{null} RBC. Twelve of 13 eluates showing negative or feeble reactions with Rh_{null} RBC were from patients in Group 1. Conversely, 13 of 18 eluates which gave reactions of equal intensity against Rh_{null} RBC and "normal" RBC were from patients in Group 2. The correlation of these apparent differences in antibody specificity with differences in the potential of γG autoantibodies for *in vivo* complement fixation has been discussed.

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Development of Chronic Low Plasma Volumes in Long-Term Adrenalectomized Dogs.* (32212)

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The plasma volume (PV) of intact and adequately maintained adrenalectomized dogs can be markedly reduced by various experimental means and held at the diminished values by feeding diets containing little sodium(1-5). The PV of intact animals, so treated, rapidly returns to normal upon resumption of the usual dietary regimen. Dogs lacking adrenals require glucocorticoids, *e.g.*, cortisone, dehydrohydrocortisone to accomplish the same result since mineralocorticoids such as aldosterone and desoxycorticosterone are not efficient for rapidly restoring the lowered PV and retaining it at normal levels(6). Long-term adrenalectomized dogs also appear to require periodic treatment with glucocorticoids in order to maintain normal plasma volumes. When they are kept for weeks or months on the usual mineralocorticoid and salt replacement therapy they eventually exhibit a gradual decline in PV which may fall to levels characteristic of animals suffering from severe adrenal insufficiency despite continuation of treatment. The ab-

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