Gm Allotype Preference in Erythrocyte IgG Antibodies of Patients With Autoimmune Hemolytic Anemia

By S. D. Litwin, S. Balaban, and M. E. Eyster

IgG antibodies were isolated from erythrocytes of five patients with autoimmune hemolytic anemia (AHA) and one case of hemolytic anemia of unknown etiology. All six persons were heterozygous for human IgG1 allotypes, and thus their serum immunoglobulins were Gm (a+f+). Quantitative analysis of the antibody preparations disclosed that three patients contained only the Gm(a) allotype; the remaining samples showed a predominance of Gm(a) in comparison to the Gm(f) allele. It was concluded that erythrocyte antibodies of patients with AHA are often restricted in respect to their Gm genetic characters with a preference for the Gm(a) allotype. In a second group of experiments, sera from three cases of hemolytic anemia were incubated with Rh+ erythrocytes from a healthy donor. Subsequently, the red cell IgG coat was eluted and analyzed. One sample had only Gm(a+) antibody; a second case showed Gm(a) predominance. The last antibody preparation varied from a disproportionately high Gm(a) concentration to equal allotype concentrations, depending on the antibody/antigen ratio used during the sensitization of the erythrocytes. The latter data suggested that allelic expression was influenced by the immunologic characteristics of the antibody subpopulation tested. No association could be demonstrated between the Ig allotypes and either the Rh specificity of the eluate, or the presence of complement coating on erythrocytes. These findings are similar to a previous report from this laboratory that noted a preference for the Gm(a) allotype in many isoantibodies with Rh specificity. The data suggest an association between certain erythrocyte antibodies and Ig allotypes.

Patients with autoimmune hemolytic anemia (AHA) frequently possess erythrocytes heavily coated with IgG, complement, or both. Analysis of the IgG antibody coat has shown immunologic specificity, in many cases, for an Rh antigen or for an Rh-related portion of the red cell membrane. The AHA red cell antibody has been reported to be restricted as to the light-chain type and, less frequently, as to heavy-chain subclass. Analysis of the genetic control of IgG has been facilitated by the Gm factors, a series of genetically determined antigens on the constant regions of the heavy chains. Gm(a) and Gm(f) are genetic characters for the major IgG subclass, i.e., IgG1, and are determined in white populations by allelic genes. [The alternative numerical nomenclature for the Gm factors are: Gm(a) = (1), Gm(f) = (4), Gm(b) = (5), Gm(g) = (21), and Gm(z) = (17).]
A previous paper from this laboratory has quantitatively analyzed Gm expression in Rh isoantibodies recovered from Gm (a+f+) heterozygous individuals. Many of the antibody populations were composed of more Gm(a+) than Gm(f+) antibody. Therefore, it was thought useful to examine the Gm allotype expression in AHA red cell antibodies recovered from Gm heterozygotes in order to determine if there are unequal proportions of Gm antigens present, to determine if one allotype occupies a preferential position, and to relate allotype expression, if possible, to the specificity of the AHA antibodies or to the presence of complement on the sensitized AHA red cell.

Five patients with AHA and one individual with hemolytic anemia of unknown etiology were studied. Antibody was isolated either directly from the IgG-coated cells of the patient (coated in vivo) or from normal red cells incubated with the serum of the patient with AHA (coated in vitro).

All of the antibody preparations showed a predominance of Gm(a). The significance of this finding in respect to the genetic control of certain erythrocyte antibodies is discussed.

**MATERIALS AND METHODS**

**Clinical Observations**

Five cases of AHA and one case of hemolytic anemia of uncertain etiology were studied (Table 1). Four cases were included, in part, in a recent report and one case was included in an earlier publication. All sera were from white people who were heterozygous for IgG1 Gm factors; their serum phenotype was Gm (a+f+b+g+z+). All of the patients were female and ranged in age from 6 to 60 yr. Patients BA. and KROM. had AHA but no other major clinical problems. Both MAK. and BE. had AHA and systemic lupus erythematosus. Case PANK. presented with idiopathic thrombocytopenic purpura, hypogammaglobulinemia, and a monoclonal protein. Patient M. had

<table>
<thead>
<tr>
<th>Patient</th>
<th>Direct Coombs' Test t</th>
<th>RBC Antibody Eluate</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>IgG</td>
<td>C</td>
</tr>
<tr>
<td>PANK.</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>MAK.</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>BE.</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>KROM.</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>M</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

NT. not tested.
ND. No Gm antigens detected. Lower limit of sensitivity was 0.1 µg/ml.

* IgG-coated red cells were taken from Gm (a+f+) patients with hemolytic anemia, and the antibody was recovered by the digitonin-acid elution technique.

† IgG refers to a positive test for IgG. C refers to a positive test for the third component of complement.

† Anti-Rh refers to eluate that reacted with all Rh-positive test cells but not with Rh null cells. Anti-non-Rh refers to eluate that reacted with both Rh-positive and Rh null cells.
Coombs' positive hemolytic anemia and had been receiving long-term therapy with α-methyldopa.

**Gm Typing and Gm Quantitative Assay**

The methods and the reagents used are detailed in a previous paper.\(^\text{13}\)

**Preparation of Eluates**

The acid elution method from digitonin stoma was used as previously described.\(^\text{14,15}\) Eluates from two sources were tested. In the first group of experiments (Table I), 3–20 ml of blood were drawn from each patient. The cells were washed and were lysed with digitonin, and the IgG antibody was eluted from stroma using a glycine-HCl buffer. These red cells are referred to as coated in vivo. In a second group of studies, 2–2.5 ml of erythrocytes from a single normal Rh-positive donor were incubated with varying volumes of serums from patients with hemolytic anemia. The coated antibody was then recovered by digitonin lysis and subsequent elution. These preparations are referred to as in vitro-coated red cells. Previous limited studies showing elution of approximately equal quantities of allotype (+) IgG from red cells coated with antibody of Gm(a) and Gm(f) homozygotes argue against selective elution of one allotype.

Indirect Coombs' tests were performed on eluates as previously described.\(^\text{11}\) Eluates that reacted with all cells in a commercial cell panel but failed to react with Rh null cells were termed Rh specific. Eluates that reacted with all cells tested, including Rh null cells, were termed non-Rh.

**RESULTS**

**Gm Concentrations of Serums of Six Patients**

Gm allotype concentrations of the serums of the six patients studied were all within the normal range for a control population.\(^\text{16}\) Gm(f)/(a) ratios of these serums varied from 0.41 to 1.92.

**Gm Concentrations in Antibody Eluates Recovered From Red Cells Coated With IgG In Vivo**

Table 1 shows the Gm allotype constitution of eluates recovered directly from the IgG-coated erythrocytes of five patients with positive direct Coombs' tests. Three of the eluates had no detectable Gm(f+) molecules. Since these individuals all had a Gm(a+f+) serum phenotype, the data indicate "allelic exclusion" within the limits of detection of the Gm assay. Two antibody preparations were predominantly Gm(a+) with a minority of Gm(f+) Ig molecules. Since the trend in the five samples was always in the same direction, it was difficult to ascertain whether the presence of complement in addition to the IgG coating, or the specificity of the eluate, was related to the expression of the Gm allotypes. The antibody preparation from patient M., the only one who did not have AHA, had the smallest disproportion between Gm concentrations (approximately threefold).
Table 2. Gm Allotypes of Red Blood Cell Antibody Preparations Isolated From Erythrocytes Coated In Vitro

<table>
<thead>
<tr>
<th>Patient</th>
<th>Packed ABC (ml)</th>
<th>Serums (ml)</th>
<th>Gm(f) (μg/ml)</th>
<th>Gm(a) (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAK</td>
<td>2.0</td>
<td>0.3</td>
<td>ND</td>
<td>1.50</td>
</tr>
<tr>
<td></td>
<td>2.0</td>
<td>1.3</td>
<td>ND</td>
<td>3.92</td>
</tr>
<tr>
<td>BA.</td>
<td>2.0</td>
<td>0.3</td>
<td>0.42</td>
<td>3.44</td>
</tr>
<tr>
<td></td>
<td>2.0</td>
<td>1.0</td>
<td>1.20</td>
<td>4.44</td>
</tr>
<tr>
<td>M.</td>
<td>2.5</td>
<td>0.5</td>
<td>1.8</td>
<td>2.2</td>
</tr>
<tr>
<td></td>
<td>2.5</td>
<td>4.5</td>
<td>1.0</td>
<td>3.6</td>
</tr>
</tbody>
</table>

ND. no Gm antigens were detected. Lower limit of sensitivity was 0.1 μg/ml.

Reactant volumes for sensitization of erythrocytes are shown. Serums from patients with hemolytic anemia were incubated with Rh-positive red cells of a single normal donor; the cells were washed and then subjected to digitonin lysis and acid elution as described.

Gm Concentrations in Antibody Eluates Recovered From Normal Red Cells Coated With IgG In Vitro (Table 2)

By exposing Rh-positive cells of a normal donor to serums from three patients with hemolytic anemia, the erythrocytes were sensitized with IgG in vitro. The red cells from two of these patients (MAK. and M.) were previously studied in vivo. The third case (BA.) was only studied in vitro. The antibody eluate of patient MAK. showed allelic exclusion for Gm(a). The same allelic distortion was observed when patient MAK.-coated red cells were tested directly (Table 1).

The last two samples presented relatively complex data. In the antibody preparations from the serum of patient BA., the total yield of Gm(+) Ig increased when a larger volume of this AHA serum was used to sensitize the same number of erythrocytes (higher antibody/antigen ratio). The increase consisted of approximately equal amounts of Gm(a) and Gm(f), although a Gm(a) predominance remained (Table 2). In contrast, the antibody preparations of patient M. had close to equal values for each allotype when 2.5 ml of packed erythrocytes were reacted with 0.5 ml of patient M. serum but shifted to a Gm(a) predominance when a 4.5 ml of serum was employed. The data point out the critical role of the proportions of immune reactants in examining allotype expression.

DISCUSSION

Previous observations have indicated that the light and heavy chains of erythrocyte-bound IgG antibodies in AHA patients may be restricted in respect to their structural features. In 20 cases examined by Leddy and Bakemeier, 12 cases of the AHA serums had only one detectable light-chain type, and four cases had one predominant light-chain type. Homogeneity as to heavy-chain IgG subclass was found to be less marked. However, Eyster et al. reported that AHA antibodies were bitypic for light chains when direct Coombs' tests were performed on the cells of nine patients.

In the present studies, all of the patients were Gm heterozygotes and had relatively balanced concentrations of Gm(a) and (f) allotypes in their serums. The predominance, and in some cases exclusivity, for a single allotype in the
erythrocyte eluates indicates that there is restricted expression of IgG1 allotype genes in these red cell antibodies.

Earlier experiments have demonstrated the high specificity of the detection systems for Gm antigens. Control experiments show that Rh isoantibody eluates, recovered from Gm(a−) or (f−) individuals, fail to inhibit the respective Gm assays. The question of nonspecifically adsorbed IgG in the antibody preparations should be considered. The methodologic studies of Kochwa and Rosenfield suggest that this is a small factor. Even if nonantibody IgG were present, it would dilute the data in favor of equal Gm concentrations, since there is no reason to expect that Gm(a+) Ig would selectively adsorb to the erythrocyte surface.

The quantitative imbalance between the allotypes was consistently in favor of a preferential position for Gm(a). Even if it is assumed that molecular homogeneity is the rule for AHA antibodies, the random selection of one allotype in the six samples would appear unusual. The significance of the Gm(a) predominance is reinforced by several additional facts. (1) A previous investigation, employing the same techniques as the present one, reported a predominance of Gm(a) among Rh isoantibodies. (2) Allelic exclusion or preference for Gm(a) was found for IgG antibody coating red cells both in vivo and in vitro. (3) The Gm constitution of the erythrocyte eluates could be manipulated by changing the conditions of sensitization. The data are interpreted to indicate that AHA erythrocyte antibodies are often associated with the Gm(a) allotype.

Several of the factors influencing allotype expression were explored. No correlation was found between Ig allotypes and the presence or absence of complement on red cells, or the specificity of the red cell eluate.

By changing the ratio between the volume of sera and erythrocytes during red cell sensitization, shifts in allotype expression were induced. It is presumed that different subpopulations of Rh antibody were being selected for. One view of the data is that Rh antibody includes the products of a number of clones. The majority of these clones, or alternatively those producing higher avidity antibody, would have to be associated with Gm(a) according to the data presented. Any differences in binding avidity between Gm(a+) and Gm(f+) antibodies and red cell antigens cannot be directly attributed to the primary structure of the allotype antigens, which are on the constant region of the IgG1 heavy chains, but rather must be due to an association between Gm genes and those genes determining the variable regions for Rh antibody.

The cumulative data, from this and a previous report, suggest that the Gm(a) allotype is preferentially associated both with Rh isoantibody and AHA autoantibody. Other red cell antibodies have not been analyzed. Thus, the unequal genetic expression could be related to the Rh site or it could be a more general property of erythrocyte antigens.

In certain cases, associations between structural features of the constant region of human heavy chains are known. Many of the blood group antibodies consist predominantly of one class of Ig. Yount et al. showed variations in subgroups and genetic markers in subfractions of various human antibodies. Antibodies to dextran, levan, and techoic acid contained a high fraction of IgG2 molecules and in some cases allelic exclusion for a genetic marker.

It is not clear whether an interrelationship between antibody specificity and
allotype will prove true for antibodies in general. The answer awaits study of a wider number of antibody specificities, preferably under conditions designed to select for a relatively homogeneous antibody population or subpopulation. In limited data available from homogeneous rabbit antibody against streptococcal antigens and mouse antibody to p-azophenylarsonate, experimental evidence has recently been presented for linkage between Ig allotypes and antibody specificity.

ACKNOWLEDGMENT

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