First example of Rh:-32,-46 red cell phenotype

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The red cells of a white male blood donor typed as Rh:-1, -2, -3, w4, w5, 6, -17, w19, -31, -32, -34, and -46. Although the donor has no history of transfusion, his serum contains an alloantibody that is weakly reactive with most red blood cells (RBCs) tested. Only Rhnull and D-- RBCs are nonreactive. Reactivity is enhanced with ficin- or papain-treated RBCs and is unaffected by AET or DTT treatment of the RBCs. Previously described Rh:-46 RBCs have been of deletion types D--; D••, and Rhnull, or Rh:32. In three multitransfused patients, the Rh46 antigen was temporarily suppressed and the phenotype eventually reverted to normal. This is the first report of RBCs of the Rh:-32,-46 phenotype that are not of a rare Rh deletion or Rhnull type. In addition, the Rh:w5, w19, -31, -34 phenotype is rarely found in whites. Immunohematology 1994;10:130-133.

The Rh blood group system is highly polymorphic, and many red blood cell (RBC) antigens have been described. The Rh antigens are thought to be encoded by two genes on chromosome 1: one gene encodes the D antigen (and associated low-incidence antigens), and another encodes C and E and associated antigens. Paired antigens exist within the system. For example, C and c, and E and e, usually behave as if they are antigenic antigens encoded by alleles. Some antigens such as Rh19 and Rh31 are associated with Rh5(e) and are thought to be part of an “e mosaic.” Therefore, RBCs that type as Rh:-5 also lack Rh19 and Rh31.

The high-incidence antigen Rh46 has so far been found on all RBCs tested except those with Rh deletions, those of the Rhnull phenotype, and those from rare Rhnull homozygotes. The latter RBCs are positive for Rh32. Issitt et al. proposed that Rh32 and Rh46 are alleles, and individuals who are Rh32 homozygous for R32. These suggestions were supported by data from a study on 18 Rhnull homozygotes by Le Pennec et al.

There are considerable differences in the Rh phenotypes between blacks and whites. For example, the Rh:-32,-46 phenotype has been described so far only in black individuals, as might be expected, since variants of e are more common in blacks than in whites.

We report the first example of the Rh:-32,-46 phenotype in a white individual whose RBCs have an apparently normal, common Rh phenotype. In contrast to three black patients, whose RBCs were transiently Rh:-32,-46, the RBCs of the propositus have remained consistently Rh:-32,-46 over a period of 3 years.

Case Report

The propositus (AD) is a 53-year-old group A, Rh negative male blood donor of Central Italian descent. He has never been hospitalized, has not served in the military, and has never been transfused. In 1990, upon the donation of his 18th unit of blood, an unexpected antibody was detected during routine donor processing. The donor antibody screening procedure had been changed between his 17th and 18th donation, from a tube method using the pooled sera of five donors, to a solid-phase technique using serum from one donor. Therefore, it is not known how long the antibody had been present. The antibody was weakly reactive with all RBCs of normal Rh phenotype, although a preference for e+ cells was observed. Since the donor's RBCs typed as e+, further investigation was undertaken.

Materials and Methods

Red cells

AD's RBCs were tested with antisera to the following Rh antigens by conventional serologic techniques: Rh1, Rh2, Rh3, Rh4, Rh5, Rh6 (D, C, F, c, e, f, respectively), Rh17, Rh19, Rh31, Rh32, Rh34, Rh46, and LW. Commercially available antisera were used for common Rh phenotyping (Gamma Biologicals, Inc., Houston, TX). Reagents for extended Rh typing and LW status were selected from our in-house inventory of rare sera. Multiple examples of each antibody specificity were tested when available. Adsorption and elution techniques were performed for each antigen found to be absent by tube typing. Details of these techniques are described elsewhere.

Titration studies were performed with commercial anti-c and anti-e (Gamma) to detect possible dosage of the antigens. Doubling dilutions of the antisera were made in 6 percent bovine serum albumin. These dilu-
Rh:-32,-46 red cell phenotype

Titration reactions were scored according to Marsh.9

Flow cytometry

Flow cytometry studies were performed with anti-c and anti-e. Although the methodology has been described in detail elsewhere, it can briefly be described as follows: AD's RBCs were incubated with the test serum, washed, then incubated with FITC-conjugated anti-human globulin. The RBCs were washed again and analyzed using the Spectrum III (Ortho Diagnostic Systems, Inc., Raritan, NJ) and FACScan (Becton-Dickinson & Co., Mountain View, CA) flow cytometers.

Serum

AD's serum was tested with a selected panel consisting of RBCs of normal Rh phenotypes, rare Rh deletions, and Rh null cells, by a LISS indirect antiglobulin test (IAT) as well as IAT tests utilizing protease-treated (ficolin and papain) RBCs, and RBCs chemically modified with either dithiothreitol (DTT), 2-amino-ethylisothiouronium bromide (AET), or chloroquine diphosphate (CDP). RBCs were obtained from various commercially available panels (Gamma, Ortho, Immucor, Inc., Norcross, GA, and Organon Teknika, Inc., Durham, NC) as well as from a frozen RBC inventory. To conserve serum, testing by a capillary IAT was also performed. All tube and capillary techniques are described in detail elsewhere.8,11

To determine whether the antibody was an autoantibody, the serum was adsorbed four times with papain-treated autologous RBCs, and then tested with a panel of RBCs. In addition, an acid eluate (Eli-Kit II, Gamma) was prepared from the first aliquot of adsorbing RBCs and tested for reactivity. Further characterization of the antibody was attempted by adsorbing the serum with R2R2 RBCs and testing the adsorbed serum with a panel of RBCs. Again, an acid eluate was prepared from the first postadsorption aliquot of adsorbing RBCs.

Results

Red cells

AD's RBCs typed as Rh:-1,-2,-3,w4,-5,6,-17,w19,-31,-32,-34, and -46 with at least two examples of each specificity. Negative results were confirmed by adsorption and elution tests. Confirmatory antigen typing was performed in at least three different laboratories using multiple sources of antisera. AD's RBCs were confirmed as Rh:-46 using the ET and MIF sera (two patient-derived anti-Rh46 sera that have been extensively shared) in three laboratories, and the Cou serum (another patient-derived anti-Rh46) in one of two laboratories. The Cou serum reacted weakly with AD's papain-treated RBCs in one laboratory. Other sources of anti-Rh46 were nonreactive with AD's papain-treated RBCs either by direct testing or by adsorption and elution tests.

Titration studies utilizing anti-c and anti-e showed no appreciable difference between AD's RBCs and control RBCs (Table 1). Flow cytometry analysis of the c and e antigens on AD's RBCs differed from the titration studies (Table 2). In the flow cytometry studies, AD's RBCs were sensitized with dilute single-donor antibodies (anti-c and anti-e + -Ce), and the differences in antigenicity were quite distinct. In the analysis with potent anti-c, the results are expressed as a mean of the counts for each sample (Table 2). The values observed with AD's RBCs quite clearly match those of the Cc control RBCs. The flow analysis performed with anti-e + -Ce gave more ambiguous results, and there is poorer differentiation (and greater overlap) between the three control populations. However, values determined for AD's RBCs fall between those of the negative control (EE) and those of the single-dose control (Ee).

Table 1. Titration studies on AD's red blood cells (RBCs)

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<thead>
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<th>Reagents</th>
<th>AD's RBCs</th>
<th>Control RBCs</th>
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<tr>
<td>cc</td>
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Table 2. Flow cytometry analysis of AD's red blood cells (RBCs)

<table>
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<th>Reagents</th>
<th>AD's RBCs</th>
<th>Control RBCs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-c</td>
<td>Titer</td>
<td>Score</td>
</tr>
<tr>
<td>Cc</td>
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<td>144</td>
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<tr>
<td>EE</td>
<td>58-95</td>
<td>80-146</td>
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<tr>
<td>Anti-e (+ anti-Ce)</td>
<td>Spectrum III, mean</td>
<td>FACScan, mean</td>
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<td>EE</td>
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Serum

Initial testing performed with AD's serum demonstrated a weak antibody that was reactive with all e-positive RBCs at LISS-IAT. RBCs negative for e reacted variably from negative to weakly positive. Reactivity was
enhanced when test RBCs were pretreated with papain, ficin, or trypsin prior to testing. No anti-e-like reactivity was demonstrable with enzyme-treated RBCs. Reactivity was not diminished when the antibody was tested with RBCs pretreated with either DTT, AET, or CDP. By capillary IAT the serum was nonreactive with two examples of D−, three examples of Rh:−46, and 1 hr8− example. It reacted with three examples of hr5− RBCs, and one example of RBCs documented as an "hr8 variant, hr5−.

The antibody reactivity was unaffected following autoadsorption. However, four allogenic adsorptions with phenotypically similar RrRr RBCs removed all reactivity, and antibody recovered in the eluate prepared from the first adsorption reacted in a similar manner to the serum. Tests with untreated RBCs demonstrated anti-e-like specificity. Following ficin-pretreatment of RBCs, the eluate reacted with all RBCs except D−, Rhnull, Rhmod, and CwD−/CwD− cells. Rh:32−46 RBCs were reactive.

In addition to the Rh antibody, autoanti-H was weakly demonstrable at 4°C.

Discussion
AD's RBCs are most unusual because they lack four high-incidence antigens in the Rh system, as confirmed by the participating laboratories. The anomalous result obtained with the Cou serum in one laboratory remains unresolved; however, the Cou serum is known to contain a weak anti-Rh6 (or anti-D). Since AD's RBCs are Rh:6, it is possible that the discrepancy may lie with the additional serum specificity.

The results of the flow cytometry analysis suggest that AD's RBCs express c and e antigens more weakly than other rr RBCs. RBCs of the Rh:32−46 phenotype have an enhanced D, weakened C and e, and no c antigen, quite unlike the reactivity seen with AD's RBCs. Possible explanations for these unusual findings may be that—

- The propositus is heterozygous for the silent allele F.
- The propositus has inherited an unlinked Rh suppressor gene.
- The propositus is an Rhmod.

Rhmod and Rhnull individuals typically have a compensated anemia, reticulocytosis, and stomatocytes evident on a blood film. Since AD was hematologically normal and had a reticulocyte count well within the normal range (0.4%), the third possible explanation can be excluded.

It is possible that AD is heterozygous for the C/E gene only (this possibility is suggested by the results of the flow cytometry) and lacks the D gene.1 However, it seems very unlikely that a lack of one C/E allele would give rise to the absence of so many high-incidence antigens. It is also possible that the phenotype is due to some translational event that has caused an alteration in the gene interpretation and thus in the antigens expressed.

Another possible explanation is the existence of an unlinked Rh suppressor gene, which is suppressing the products of one allele, including Rh19, Rh31, Rh34, and Rh46. Since the lack of any of these antigens in a white individual is very unusual, this may be the most likely explanation.

We have observed three other examples of the Rh:−32−46 phenotype. All examples have been in multi-transfused black women, all of whom produced an apparent alloantibody to a high-incidence antigen in the Rh system. However, subsequent testing determined that the Rh:−46 status was transient and the RBCs of all three patients regained their antigens. In one, DM, the anti-Hr/hr, antibody produced appeared to truly be an alloantibody, and the patient remained Rh−17 upon subsequent testing. Transient suppression of an antigen has been described before. For example, in a transiently LW2− individual, anti-LW2 is a common observation.12-15 In these cases, the autoreactive nature of the antibody can be demonstrated as the antigen returns and the direct antiglobulin test becomes positive. Often, as the antigen reverts to full strength, the antibody becomes undetectable. AD's RBCs have been repeatedly tested over a period of 3½ years. During this time, his Rh phenotype has remained unchanged and the DAT remains negative.

Unfortunately, we have been unable to perform a comprehensive family study. Only from such studies will we be able to ascertain the possible genetic explanation for AD's Rh phenotype. Further testing will also include DNA and RNA analysis. Since AD remains a committed donor, we will continue to study his RBCs.

Acknowledgments
We thank Dr. Pierre Le Penneck, Dr. George Garratty, Pat Arndt, Joyce Poole, Linda Herris, and Peter Byrne for performing tests included in this study. Also, we thank Peggy McCarthy for providing samples of AD's blood, and Virginia Hare and Marion Reid for their helpful discussion.

References


