B<sub>x</sub>, A Subtype of B

By Aaron A. Alter and Richard E. Rosenfield

Human erythrocytes that have a weak expression of the B antigenic determinant are rarely observed and have not been subdivided by serologic criteria that are analogous to those used for subtypes of A. Another person, Mrs. Frei, with erythrocytes that have an extremely weak expression of B, has been encountered. The family of Mrs. Frei. did not disclose other examples or direct evidence defining the mode of inheritance. In discussing the findings, the reported examples of subtypes of B will be related with those subtypes of A that appear to be serologically analogous.

Materials and Methods

The source, collection and storage of red blood cells, antisera and saliva, as well as methods of elution, titration and inhibition are described elsewhere. Chromosome studies were done by the method of Hirschhorn and Cooper. Rh and Kell terminologies were defined previously.

Results

The serum of Mrs. Frei. contained anti-A (titer 1:80) but not anti-B, and her red cells were not agglutinated by anti-A, anti-B or anti-A,B sera. These tests were performed at temperatures ranging from 4–37°C and included a variety of saline, enzyme-treated cells and indirect antiglobulin methods. Thus, Mrs. Frei. presented as "type O without anti-B."

Anti-B absorbed by and eluted from Frei. red cells did not agglutinate type B erythrocytes unless acacia was present. Saline and indirect antiglobulin tests, performed at temperatures ranging from 4–37°C, were negative. With acacia, however, type B cells were agglutinated to a titer of 1:2 and the cells of Mrs. Frei. to a titer of 1:1. Furthermore, these eluted agglutinins were completely inhibited by undiluted type B secretor saliva, partially inhibited by the saliva of Mrs. Frei., but unaffected by the saliva of secretors of type A or O. The saliva of Mrs. Frei. had no recognizable effect upon the original serum source of anti-B agglutinins, even at extremely high dilutions of the serum.

Anti-A,B, absorbed by and eluted from erythrocytes of Mrs. Frei., could not be demonstrated by saline and indirect antiglobulin tests, but, with acacia for support, were found to agglutinate type B red cells (1:64), type

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*Mrs. Frei., in normal health and during her second uneventful pregnancy, was brought to our attention by the late Dr. Edward Solomons, Director of Obstetrics and Gynecology, Maimonides Hospital, Brooklyn, N. Y.
Table 1.—Eluates Obtained from Frei. RBC

<table>
<thead>
<tr>
<th>Test RBC</th>
<th>Direct Titer* (reciprocal)</th>
<th>Inhibition of Eluates by Dilutions of Secretor Saliva</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>A1</td>
</tr>
<tr>
<td>Eluate of Anti-B</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type A1</td>
<td>0</td>
<td>+</td>
</tr>
<tr>
<td>Type B</td>
<td>2</td>
<td>+</td>
</tr>
<tr>
<td>Frei.</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Eluate of Anti-A,B</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type A1</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>Type B</td>
<td>64</td>
<td>+</td>
</tr>
<tr>
<td>Type O</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Frei.</td>
<td>2</td>
<td>+</td>
</tr>
</tbody>
</table>

*Done by the acacia method.
†Values given are reciprocals of final dilutions.

A cells (1:16), and the cells of Mrs. Frei. (1:2). Type B secretor salivas completely inhibited all eluted anti-A,B agglutinin activity, whereas type A secretor salivas fully inhibited only the agglutination of type A while partially inhibiting the agglutination of type B cells and the cells of Mrs. Frei. In contrast, the saliva of Mrs. Frei. partially inhibited the agglutination of type B cells and of Frei. cells, but did not appear to inhibit the agglutination of type A cells at all.

These results are summarized in table 1. The study of Mrs. Frei. was repeated at 6 months post-partum with identical results.

The ABO phenotypes of the Frei. family are summarized in table 2.

Some of the other blood types of Mrs. Frei. were: Rh: 1, -2, 3, 4, -5, -6, -7; K: -1, 2, -3; MSMS; P-positive; Fy(a+); Le(a-b+); Jk(a+b+); Lu(a-).

Peripheral blood culture of leukocytes revealed a normal female karyotype of 46 chromosomes. There was no evidence of mosaicism.

DISCUSSION

Mrs. Frei. was brought to our attention because her erythrocytes had been typed as O while her serum failed to contain anti-B. Initial studies revealed no evidence for B antigenic determinants on her red cells or in her saliva, although the latter was found to have a normal capacity to inhibit H-anti-H.

The ABO status of Mrs. Frei. was determined only with the combined use of eluates made from Mrs. Frei.'s red cells and acacia to support agglutination. Anti-B or anti-A,B absorbed by and eluted from the red cells of Mrs. Frei. provided a special population of reactive antibody molecules. These antibodies agglutinated the red cells of Mrs. Frei. and the red cells of type B persons, and were sensitive to at least partial inhibition by the saliva of Mrs. Frei. and complete inhibition by the secretor saliva of type B persons.

The weak B antigenic determinants on Frei. red cells and in Frei. saliva could differ structurally from ordinary B antigenic structural groupings or could result from a unique steric hindrance that prevents Frei. B from combining with most anti-B molecules. Isolation of the Frei. B hapten could be
Table 2.—Pedigree of the Frei Family

<table>
<thead>
<tr>
<th>Member</th>
<th>Relationship</th>
<th>Phenotype</th>
<th>Anti-A</th>
<th>Anti-B</th>
<th>Anti-A,B Eluate</th>
</tr>
</thead>
<tbody>
<tr>
<td>I-1</td>
<td>Father of II-1</td>
<td>A₁</td>
<td>640</td>
<td>N</td>
<td>&gt;640 N</td>
</tr>
<tr>
<td>I-2</td>
<td>Mother of II-1</td>
<td>B</td>
<td>N</td>
<td>640</td>
<td>&gt;640 16</td>
</tr>
<tr>
<td>II-1</td>
<td>Propositus</td>
<td>B₁</td>
<td>N (N)</td>
<td>N (2)</td>
<td>N (2) 2</td>
</tr>
<tr>
<td>II-2</td>
<td>Husband of II-1</td>
<td>A₁</td>
<td>1280</td>
<td>N</td>
<td>&gt;640 N</td>
</tr>
<tr>
<td>III-1</td>
<td>Daughter of II-1</td>
<td>A₁</td>
<td>640</td>
<td>N</td>
<td>&gt;640 N</td>
</tr>
<tr>
<td>III-2</td>
<td>Son of II-1</td>
<td>A₂</td>
<td>320</td>
<td>N</td>
<td>640 N</td>
</tr>
</tbody>
</table>

*Tests by acacia method gave identical results unless indicated by parentheses.
†Done by the acacia method.
N = no agglutination observed.

of value in distinguishing the better of these two possibilities. Since Frei B is reactive with some anti-B molecules, by definition it cannot be representative of the hypothetical "C factor" of Wiener⁹ because anti-B (from type A persons) cannot contain anti-C.

If a B* gene exists in the Frei pedigree, it might have been inherited from I-2, who could be BB*. Alternatively, however, I-1 could be A'B*, if the B* gene of such a heterozygote were silent under the methods employed to test the phenotype.

A standard nomenclature for subtypes of B does not exist. The same symbol has been employed for two dissimilar bloods (e.g., the B₁ blood described by Body and Boyd¹⁰ and the B₃₀ blood described by Levine et al.,¹¹ and the terms that have been used for subtypes of B differ irregularly from terms used for serologically analogous subtypes of A. To clarify this area, subtypes of B have been reviewed and reclassified (table 3) in accordance with the generally accepted criteria used for subtypes of A.¹

Mrs. Frei falls into the category of the B₁ phenotype because of the exceedingly weak expression of B on her erythrocytes and in her saliva. B₁, like A₁, can be agglutinated by anti-B and by anti-A,B, but more readily with acacia than with saline and antiglobulin methods. Ordinary tests of Frei saliva to inhibit B-anti-B agglutination were entirely negative as were those of A saliva with A-anti-A agglutination.¹ There are two other reports in the literature which would satisfy these criteria for the B₁ phenotype although both reports employed the term B₃.

There is no clear evidence for a B₂ phenotype at the present time. Moullec¹⁴ and Jacobowicz¹⁵ reported bloods which are suggestive of B₃ that would be analogous to A₉, and the report of Levine et al.¹¹ fulfills the same criteria even better by describing weak mixed agglutination of the erythrocytes with unselected anti-B sera and the presence of B inhibiting activity in the saliva.

The reported cases of Armstrong¹⁶ and Liotta¹⁷ fulfill the criteria for the B₃ subtype with indetectible B antigenic determinants on the red cells but an abundance of B substance in the saliva.

A blood from a patient with leukemia was reported¹⁸ which would fulfill the criteria for the B₃ classification.
Table 3.—Usual Serologic Reactions of Subtypes of B

<table>
<thead>
<tr>
<th>Phenotypes</th>
<th>Anti-A</th>
<th>Anti-B</th>
<th>Anti-A-B</th>
<th>Anti-A</th>
<th>Anti-B</th>
</tr>
</thead>
<tbody>
<tr>
<td>B₁</td>
<td>N</td>
<td>S</td>
<td>S</td>
<td>N</td>
<td>S</td>
</tr>
<tr>
<td>B₂</td>
<td>N</td>
<td>M</td>
<td>M</td>
<td>N</td>
<td>W</td>
</tr>
<tr>
<td>B₃</td>
<td>N</td>
<td>VW</td>
<td>W</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>B₄</td>
<td>N</td>
<td>VW to W</td>
<td>W</td>
<td>N</td>
<td>S</td>
</tr>
<tr>
<td>B₅</td>
<td>N</td>
<td>N</td>
<td>VW</td>
<td>N</td>
<td>S</td>
</tr>
<tr>
<td>AB₁</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>W</td>
</tr>
<tr>
<td>AB₂</td>
<td>S</td>
<td>M</td>
<td>S</td>
<td>S</td>
<td>W</td>
</tr>
<tr>
<td>AB₃*</td>
<td>S</td>
<td>VW</td>
<td>S</td>
<td>S</td>
<td>N</td>
</tr>
</tbody>
</table>

N = no agglutination or inhibition; S = strong agglutination or inhibition; W = weak agglutination or inhibition; M = mixed weak agglutination with large number of unagglutinated cells; VW = very weak agglutination.

*Not encountered. Reactions given are predicted.

**SUMMARY**

A subtype of B, termed B₃, was found to have serologic characteristics analogous to those described for subtype A. The B determinants of the erythrocytes and salivary secretions of the B₃ person could be detected when anti-B was absorbed by and eluted from her red cells. These particularly reactive antibody molecules were best demonstrated by using acacia to support agglutination. Other described subtypes of B have been reviewed and reclassified in accordance with analogous criteria employed for subtypes of A. Evidence was found for two other examples of B₃, as well as for B₁, B₅, and B₇, but not for B₂.

**SUMMARIO IN INTERLINGUA**

Esseva constatate que un subtypo de B, hic designate como B₃, habeva caracteristicas serologic analoge a illos describite pro subtipo A. Le determinantes B del erythrocytos e del secretiones salivari del subjecto B₃ poteva esser detegite quando anti-B esseva absorbite per e eluite ab su erythrocytos. Iste particularmente reactive molecules anticorporee esseva demonstrate le melio quando acacia esseva usate in supporto del agglutination. Altere subtypos de B que se trova describite in le litteratura es revistate e reclassificate de acordo con criterios analoge a illos emplemente pro subtypos de A. Esseva trovate evidientia pro duo exemplos additional de B₃, e etiam pro exemplos de B₁, B₅, e B₇, sed non B₂.

**REFERENCES**


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