Some Observations on the New Human Blood Factor Di

By Philip Levine and Elizabeth A. Robinson

In June, 1953, blood specimens of an Rh positive mother and her Rh positive, group compatible infant suffering from hemolytic disease were sent from Caracas, Venezuela; they were in excellent condition when received at Raritan.* The clinical course of the disease was typical. Jaundice had appeared after 12 hours and the infant, the propositus, died on the third day with symptoms suggestive of kernicterus.

The infant’s red cells were strongly “coated” but no antibody was demonstrable in the maternal serum when tested with an extensive panel of selected test cells, which, however, did not include the husband’s red cells. A negative result was also obtained in tests with the infant’s serum and with the eluate prepared from his coated red cells. In the absence of ABO incompatibility, the occurrence of a “low-incidence” blood factor with its corresponding antibody was suspected. This was confirmed one month later when on request blood specimens of both man and wife were mailed to Raritan for simultaneous studies. It was found that the maternal serum reacted strongly with her husband’s group compatible red cells, but failed to react with 200 random group O bloods.1 Subsequently, another series of 800 random bloods were tested, but not one positive reaction was observed.2

On the occasion of a visit to New York on October 26, 1953, the father of the propositus agreed with one of us, (P. L.), to the name of the new blood factor as Diego (Di). The corresponding antibody will be referred to as anti-Di. In 1953, it was possible to demonstrate that Di was not identical with 2 other low-incidence blood factors, Mi and Be. In short, the three low-incidence antibodies, each associated with hemolytic disease, when tested with the red cells of the three corresponding husbands, were individually specific. No further tests could be carried out because of difficulties in submitting specimens from members of the rather extensive family tree. When the mother became pregnant again in 1955, Dr. Layrisse was consulted in Caracas and this made possible the further extensive tests both in Venezuela and at Raritan.

The obstetrical history of the mother, who was never transfused, follows:

1. 1949—normal full-term female infant.
2. 1950—miscarriage at 3 months.
3. 1951—full-term male infant. Anemia developed slowly and was very distinct at one month. There was no jaundice and recovery was spontaneous. The history gave no indication that isoinmunization with subsequent hemolytic disease was the cause of the anemia.
4. 1953—full-term male infant—the propositus—who at birth appeared to be normal clinically and hematologically but a bilirubin determination was not carried out. Jaundice which was evident after 12 hours became increasingly severe and the infant expired at 3 days. The direct Coombs test was positive.

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* The specimens were submitted by Dr. Miguel Raga of Caracas, Venezuela.

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5. 1955—normal full-term male infant. There was no jaundice or anemia; the direct Coombs test was negative, and the red cells failed to react with anti-Di\textsuperscript{a} (this blood was studied by Dr. Layrisse).

As will be shown below, the red cells of man and wife were incompatible for the factors E, c, and S but antibodies for these factors could not be demonstrated. The maternal serum, which reacted only in the indirect Coombs test, gave a titer of 1:512 on the husband's red cells and the same titer on all other members of the family whose red cells contained the antigen Di\textsuperscript{a}.

In order to determine a possible identity of the Diego factor with other low-incidence red cell antigens or a possible genetic relationship to high-incidence factors, the husband's red cells were tested with all available appropriate sera. At the same time, anti-Diego was tested with selected red cells, preserved in the frozen state, containing rare or lacking high-incidence blood factors. These tests were carried out with saline suspended red cells followed by the indirect anti-globulin reaction. The findings presented in table 1 and briefly referred to elsewhere\textsuperscript{2} seem to indicate that the Diego antigen represents a new agglutinable blood factor which is genetically independent of four high-incidence factors. Tests

| Table 1. Relationship of Diego Antigen to Low and High Incidence Blood Factors |
|---------------------------------|-------------------------------------------------|-------------------------------------------------|
| Reactions of Di\textsuperscript{a} Red Cells With: | Reactions of Anti-Di\textsuperscript{a} With Test Cells Specific For: |
| Low Incidence Antibodies | Low Incidence Antigens |
| anti-Be\textsuperscript{a} | 0 |
| anti-Becker | 0 |
| anti-By\textsuperscript{a} | 0 |
| anti-Ca | 0 |
| anti-C\textsuperscript{a} | 0 |
| anti-C\textsuperscript{c} | 0 |
| anti-Di\textsuperscript{a} | + |
| anti-E\textsuperscript{a} | 0 |
| anti-Gr | 0 |
| anti-Henshaw | 0 |
| anti-Mi\textsuperscript{a} | 0 |
| anti-Rm | 0 |
| anti-V | 0 |
| anti-Ven | 0 |
| anti-Wr\textsuperscript{a} | 0 |

<table>
<thead>
<tr>
<th>High Incidence Antibodies</th>
<th>High Incidence Antigens</th>
</tr>
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<tbody>
<tr>
<td>anti-H</td>
<td>+</td>
</tr>
<tr>
<td>anti-Tj\textsuperscript{e}</td>
<td>+</td>
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<tr>
<td>anti-Vel</td>
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<tr>
<td>anti-U</td>
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The tests with anti-C\textsuperscript{c} and anti-Rm were carried out with a Diego positive blood from another family sent by Dr. Layrisse to Drs. Race and Sanger. With eight other sera there was agreement both in Raritan and at the Lister Institute.

References to these blood factors and their antibodies are given by Race and Sanger,\textsuperscript{3} Kabat,\textsuperscript{4} and Hart et al.\textsuperscript{5} It was recently found that V\textsuperscript{e} is related to M\textsuperscript{e}.

The authors are indebted to numerous workers who generously supplied some of these rare reagents.
Inheritance of the Diego Factor $D_i^a$ in the Original Family

Members of the Diego family were then studied in order to trace the genetic transmission of this new blood factor. As shown in figure 1, the factor was present in 10 of the 33 members of 4 generations whose bloods were available. The factor was derived from the maternal side in generations I and II. Except for 7 individuals (I-1, I-2, II-1, II-4, II-5, II-6, and IV-10), whose bloods were studied by Layrisse and Arends in Caracas, all others were tested for the Diego factor in both laboratories and the findings were in agreement. The factor was present in four siblings of generation III, and among those of generation IV tested, it was transmitted to the propositus (IV-9) and his two older siblings (IV-7 and IV-8). As was to be expected the infant born in 1955 (IV-10) was $D_i^a$ negative and remained free from symptoms. From the data in figure 1, it is evident that at least 4 of the 7 $D_i^a$ positive individuals who had offspring were heterozygous.

The results of an antigenic study of selected members of the family tree are shown in table 2.

The analysis of the data in table 2 shows no apparent correlation or linkage of the Diego factor with sex or any of the blood group systems studied, but more exhaustive tests are indicated.

As mentioned below, 1000 random group compatible bloods were tested at the Ortho laboratories for the Diego factor, 200 in 1953 and 800 in 1955. Almost all were derived from Caucasoids; a small unspecified number of Negroids constituted the remainder. Not one of these was Diego positive. These tests were carried out with the serum diluted 1:4 which thus contained 64 activity units. In each daily run, the tests were controlled by including a Diego positive blood.

The physical features in some members of the family indicated admixture with native Indians and this observation led to studies by Layrisse and his colleagues.
on the distribution of the Diego blood factor in other populations. These findings, along with those on Brazilian Indians,7 are referred to elsewhere7 and all findings to date including Chinese and Japanese are summarized by Layrisse and Arends.8,9 Apparently the Diego factor may be Mongoloid in origin as suspected by Layrisse and Arends8 and Lewis et al.10

In 1955 Levine et al.11 identified a second example of anti-Di* with resulting hemolytic disease in a Caucasian family living near Buffalo, New York. The specimens were submitted by Witebsky and Rosamilia who recognized that they were dealing with isoimmunization by a low-incidence blood factor. The occurrence of Di* in the red cells of the husband who was Polish may be significant in view of the apparent Mongoloid distribution of the blood factor.8,9,10

In the second example of anti-Di*, the affected infant’s red cells were coated. The maternal serum was negative when tested with a panel of carefully selected bloods but an antibody was demonstrated when the husband’s group compatible red cells were included. The antibody was identified as anti-Di* by virtue of its reaction in the indirect Coombs method with known Di* positive red cells from the Venezuelan family and its failure to react with red cells containing other low-incidence factors. Furthermore, the husband’s red cells reacted with the original Venezuelan anti-Di*.

**DISCUSSION**

The findings reported indicate that a blood factor characterized as of the low-incidence variety in one population group may have a considerably higher incidence in other population groups. The question arises whether or not this obsen-
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vation with the D_{i}^{*} factor holds also for the low-incidence factors listed in table 1. Such studies have not yet been carried out because there are insufficient supplies of the appropriate antibodies. The striking differences with anti-D_{i}^{*}, however, are not surprising because a similar situation, perhaps less pronounced, applies also to other blood factors, such as the well-established differences in incidence of type Rh in Caucasians and African Negroes. Another example is the high incidence in Negroes of a type of blood failing to react with both anti-Fy^{a} and anti-Fy^{b} and its complete absence in Caucasoids.

In spite of these findings, it is not necessary to discard the term, "low-incidence" because it describes the characteristics of the blood factor in the Caucasian population in which it was first observed. Thus, the term "Indian" for the Diego blood property is not appropriate because this name was suggested at a time when Chinese and Japanese were not yet studied.

With regard to the findings of Layrisse and Arends, and Junqueira et al., it may be remarked that in populations almost exclusively Rh positive and group O, blood factors other than Rh or A and B may be responsible for almost all hemolytic disease, assuming comparable degrees of antigenicity. It is hoped that future studies will establish the relative importance of hemolytic disease due to anti-D_{i}^{*} in relationship to that caused by antibodies for other blood factors among Venezuelan Indians and other populations with varying incidences of the Diego factor as summarized by Layrisse and Arends.

Further investigations are required to determine whether or not the genetically related D_{i}^{b} and anti-D_{i}^{b} exist. Theoretically, the antibody could be produced by individuals of genotype D_{i}^{a}D_{i}^{a} who are not so rare among Caribe or Kaingangues Indians of Venezuela and Brazil, respectively.

The large number of low-incidence blood factors described mainly among Caucasoids of Europe and the United States makes it necessary to establish some sort of central agency, perhaps under the supervision of the World Health Organization of the United Nations, for correlative studies in order to establish independence or duplication of these rare blood factors. At present such studies could be carried out only after much patience and perseverance, because continuous effort is required to obtain the appropriate sera and the rare test cells.

The extreme difficulty in studying the specificities of antibodies such as anti-D_{i}^{*} is illustrated in table 1 which presents evidence for the independence of D_{i}^{*} from 15 other low-incidence blood factors if one includes the tests with antigen Chr^{a}. Duplication in the naming of blood factors has already occurred as shown by the observation of Levine, et al., that the V^{a} factor of Hart, Bosman, and Von Loghem is another example of the M_{i}^{a} factor.

The question arises as to the total number of independent low-incidence blood factors capable of inducing is immunization, or demonstrable by antibodies occurring in the absence of known antigenic stimulation. So far as hemolytic disease is concerned, many more probably exist because frequently the husband's blood is not taken into account and comparative studies of the maternal serum with the rare essential test cells are, as a rule, not carried out. Furthermore, such antibodies more active at 18 C than at 37 will escape detection because routine studies are generally carried out at 37 C. In at least two instances of low-incidence antibodies, anti-Gr and anti-Chr^{a}, not associated with hemolytic disease, the observation depended entirely on including in the panel of test cells bloods which by chance contained the particular antigen.
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With an increasing number of low-incidence blood factors, it will not be surprising if comprehensive studies will reveal that some of them will be found to be genetically related as alleles to or linked with other well-known blood group systems. This view was anticipated by Race and Sanger who suggest that a low-incidence factor may result from mutations in one or more of the established blood group systems as was shown to occur for V\textsuperscript{w} and MNSs systems,\textsuperscript{5} and still more recently for Ti\textsuperscript{w} and P.\textsuperscript{14}

In general, the tendency has been to underestimate the practical and theoretical importance of so-called low-incidence blood factors. The unexpected finding with Diego indicates the potential significance of all other low-incidence and, by the same token, high-incidence blood factors in comprehensive studies of all racial groups.

**Summary**

Evidence is presented that the Diego antigen is independent of 15 other low-incidence blood factors and is not genetically related to 4 high-incidence factors. In all cases of low incidence antigens, similar comprehensive studies with a panel of essential test cells are required to exclude possible serologic or genetic relationship to other blood factors.

**Summary in Interlingua**

Es presentate datos in supporto del thes que le antigeno Diego es independente de 15 altare factores sanguine de basse incidentia e que illo es non geneticamente relationate con 4 factores sanguine de alte incidentia. Le hereditate del factor Diego es traciate in 10 ex 33 membros de 4 generationes de un familia venezuelan con admixitura americano-indian.

**References**

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