Observations on the New Rh Agglutinin Anti-f

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Rosenfield, Vogel, Gibbel, Sanger, and Race recently described the antibodies found in the serum of a hemophiliac who had received numerous blood transfusions. In addition to anti-N, anti-S, and anti-K, the serum contained an antibody which reacted with bloods of certain Rh types, only blood cells from persons bearing at least one chromosome of type cde or cDe being agglutinated by this antibody. It was postulated that these reactions were due to a new antigen f. It was further suggested that the inheritance of this antigen was determined by a pair of allelic genes F and f tightly linked to the familiar Cc, Dd and Ee pairs.

In the data of Rosenfield et al., as well as in those of Race and Sanger, individuals who did not have a chromosome bearing the gene combination cc failed to have the f antigen on their cells. Typical examples of the Rh genotypes of persons who lack the f antigen are CDe/CDe, cDE/cDE and CDe/cDE. Persons of the genetic categories CDe/CDe and cDE/cDE are capable of producing anti-c and anti-e respectively. Since such persons lack the f antigen, they are potentially capable of developing anti-f in addition to anti-c or anti-e when immunized with cells from persons with a chromosome of type cDe or cde. The serum of such a person was available in this laboratory and opportunity was taken to test this hypothesis.

MATERIALS AND METHODS

Dr. Kr., whose blood was of type cDE/cDE, had been deliberately immunized with repeated injections of Rh negative (cde) blood during a five year period. His serum agglutinated saline-suspended Rh negative cells to an end-point of 1:128. It agglutinated all cells containing the antigen c and failed to agglutinate cells lacking this antigen.

In order to remove the anti-c, the Kr. serum was subjected to four separate absorptions with equal volumes of washed, packed CDe/CDe cells, each absorption being carried out at 37 C. for 2 hours. To ensure completeness of absorption two further absorptions were made with cells of type CDe/cDE. This serum, when tested against CDe/CDe cells and against CDe/cDE cells by saline, albumin, and indirect Coombs technics, gave negative results but was shown to contain a residual antibody which agglutinated saline-suspended Rh negative cells to a titer of 1:32.

In all the reactions described below, 0.05 ml. of the absorbed Kr. serum was mixed with 0.05 ml. of a 2 per cent saline suspension of the cells to be tested. The mixture was incubated for 45 minutes, then centrifuged for 2 minutes at 1000 r.p.m. and the presence or absence of agglutination was determined by gently shaking the tube.
All reactions were read blind. In the family studies the tubes were randomly numbered by one of us and read by another. The results of the reactions of all other bloods were read as follows: in a series of bloods the results obtained with the absorbed Kr. serum were read first, followed by the results given by anti-C, anti-D, anti-E, anti-e, and anti-e. Although the reader knew which antiserum was being used he did not know which blood was being tested.

**Results**

1. **Fully Typed Bloods**

Tests of the absorbed Kr. serum against a selected panel of eleven fully typed bloods failed to disclose any association between the Kr. antibody and the following blood group systems: ABO, MNS, P, K, Le, Lu, Fy, and Jk. However, these tests suggested an association between the Kr. antibody and the cde chromosome. Since the donor was c-positive the serum could not contain anti-c. Furthermore, careful tests with CDe CDe blood by saline, albumin, and indirect Coombs' techniques had shown that the serum no longer contained anti-e. The residual antibody, if it were indeed associated with the Rh factor, could therefore be only anti-f or anti-d.

2. **Kr. Serum and the Rh Chromosome**

*Family studies.* Five of the several families studied provided critical evidence showing that the antibody in the absorbed Kr. serum was not anti-d. Two of the five families involved matings of CDe cDe fathers with cde cde mothers and the remaining three families involved matings of CDe CDe fathers with cde cde mothers. One example of each of these matings is shown in figure 1.

The family studies are of value insofar as they enable an exact determination to be made of the genotype of certain critical bloods. Thus in family 1 the father (member 1) is undoubtedly homozygous DD and therefore does not have d. Nevertheless his cells were agglutinated by the Kr. serum. In family 2 the genotype of member 4 must be CDe cdE and therefore heterozygous Dd. Despite the presence of the d antigen, the cells of this individual were not agglutinated by the Kr. serum. The reactions with these critical bloods enabled us to exclude the possibility that the unknown antibody was anti-d.

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**Fig. 1.—Examples of two families providing critical bloods which enable anti-d to be excluded in the serum studied.**
TABLE 1.—The Reactions of the Absorbed Kr. Serum with Blood of Various Rh Types. The Relationship between the Antigen and the Gene Combination ce is Demonstrated

<table>
<thead>
<tr>
<th>Gene combination ce present</th>
<th>Probable genotype</th>
<th>Reactions with absorbed Kr. serum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>cde/cde</td>
<td>31</td>
<td>0</td>
</tr>
<tr>
<td>Cde/cde</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>edE/cde</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>cDe/cde</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>CDe/cde</td>
<td>32</td>
<td>3*</td>
</tr>
<tr>
<td>cDE/cde</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>CDE/cde†</td>
<td>2</td>
<td>0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Gene combination ce not present</th>
<th>Probable genotype</th>
<th>Reactions with absorbed Kr. serum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>CDe/CDe</td>
<td>0</td>
<td>20</td>
</tr>
<tr>
<td>CDe/cDE</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>cDE/cDE</td>
<td>0</td>
<td>3</td>
</tr>
</tbody>
</table>

* See text for a discussion of these exceptions.
† This genotype is given as the most likely to fit in with a positive Kr. reaction.

Bloods of unrelated individuals. The bloods of 121 unrelated (but not randomly selected) white and colored persons were tested with the absorbed Kr. serum. At the same time the Rh phenotypes of these bloods were determined with the five usual anti-Rh sera. The results are presented in table 1.

The data confirm that the absorbed Kr. serum does not detect any of the known Rh antigens. They show also that only bloods derived from persons having at least one chromosome with the gene combination ce are agglutinated by this serum. Attention is called to three exceptional instances in which CDe/cde bloods were not agglutinated by the absorbed Kr. serum; this will be discussed below.

It is apparent from the foregoing data that the antibody which remains in the Kr. serum after complete removal of the anti-c closely resembles the anti-f described by Rosenfield et al.

3. Presence of Anti-f in Other Antisera

It has already been suggested that donors of anti-c and anti-e sera are likely candidates for the production of anti-f since they can be shown to lack this antigen. Acting on this assumption we tested for the presence of anti-f in the anti-c and anti-e sera routinely used in this laboratory.

The anti-c serum was absorbed twice with CDE/CDE cells and the anti-e serum was absorbed three times with CDE/CDE cells. After this treatment for removal of anti-c and anti-e, both sera failed to react with cells of the type used for absorption but they continued to react with cells from individuals in whom the ce chromosome was present in homozygous or heterozygous condition and they showed other characteristics typical of anti-f. It is perhaps pertinent to note in passing how remarkable it is that the existence of a second antibody in such sera has not previously been detected in view of the wide range of reactivity shown by most examples of anti-c.
We also tested the serum from a CDe/CDe donor who had produced anti-c plus anti-E as a result of isoimmunization of pregnancy by a fetus of type CDe/cDE. Since the antigenic cells in this case were of a type in which f has not so far been found, the serum was not expected to contain anti-f. Absorption with cDE/cDE cells removed all activity from the serum thus strengthening the hypothesis that anti-f was not present. Had anti-f been present some residual activity against cde/cde cells would have been expected.

DISCUSSION

Rosenfield et al. showed that the new antigen f was associated with the Rh system. After examining various possibilities these authors concluded that the new antibody anti-f detected an antigen, the inheritance of which was determined by a fourth pair of allelic genes in the Rh complex. They named the new locus Ff with identification of the F antigen awaiting the discovery of a serum containing the anti-F antibody. They recognized that the proof of this hypothesis requires the existence of chromosomes such as CDeF, cDEf and cdeF.

In our data f was detected only in bloods derived from persons with the gene combination cc on one or both chromosomes, thus confirming the observations of Rosenfield et al. It will be recalled (table 1) that three out of thirty-five bloods of type CDe/cde were not agglutinated by the absorbed K.r. serum; these bloods therefore lacked the f antigen. Hence, the presence of f in blood from persons with a cc chromosome cannot be said invariably to obtain.

In our series, f was not found in association with chromosomes such as CDe, cDE, cdE when at the same time the opposite chromosome did not carry cde or cDe. However, the fact that we have demonstrated the presence of anti-f in our routinely used anti-c and anti-e sera precluded the finding of such exceptions. For example, consider the hypothetic case of an individual whose genotype was CDe/CDe but whose cells were f positive. The blood of this person would be agglutinated by our anti-c serum because of the fact that this serum contained anti-f. Therefore the blood would be classified mistakenly as CDe/cde. In order to detect the presence of f in bloods of type CDe/CDe or CDe/Cde it is necessary to employ an anti-c which does not contain anti-f. We have shown that such a serum may be obtained from a susceptible individual who has been immunized with CDe/cDE cells rather than with cde/cde or cDe/cde cells.

When considering the genetics of the f antigen the attractiveness of a theory involving the postulation of a fourth pair of allelic genes must not allow other hypotheses to be overlooked. In the present state of evidence, equal validity may be claimed for a hypothesis which would consider the antigen to be the result of a "position effect" similar to that shown clearly in drosophila. This hypothesis would state that when the genes c and e are together on the same chromosome the antigen f is present in the red cells. When the genes c and e are on opposite chromosomes (as in the genotype CDe/cDE) the f antigen is absent. The occasional absence of f when c and e are on the same chromosome admits of several possible explanations, one of which would postulate the existence of alleles of c and e which fail to interact to produce f. There is evidence to indicate that alleles of c do exist.

For those who favor the multiple allele theory of the inheritance of the Rh
antigen the simplest assumption appears to be that the antigen f is determined by the genes $r$ and $R^0$ as part of the usual antigen mosaic which these genes produce. The exceptions described by us would require the postulation of a new allele of $r$.

If anti-f does describe a new locus in the Rh system the array of possible chromosomes would be doubled and the number of genotypes multiplied by an amount equal to $(4n + 2)/(n + 1)$ where $n$ equals the original number of chromosomes. Thus if we consider only the common pairs of alleles at each of the three loci $(Cc, Dd, Ee)$, $n$ equals 8 and the actual number of possible new genotypes would be 136, compared with 36 when the $Ff$ locus is not recognized.

If the new antigen proves to be the result of a “position effect” due to the simultaneous presence of certain alleles of $c$ and $e$ on the same chromosome, the effect of f on the Rh system would be to add two new chromosomes $cde(f-)$ and $cDe(f-)$. The number of possible genotypes would be multiplied by an amount equal to $(n + 2)(n + 3)/n(n + 1)$. If $n$ equals 8 the possible number of genotypes would be increased from 36 to 55.

There is evidence to suggest that many if not all antisera commonly used to test for the presence of $c$ and $e$ (especially those produced by immunization with $cde/cde$ cells) contain anti-f. If f never occurs with C or E, the presence of anti-f

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**Fig. 2.**—Frequency with which $CDe/CDe$ would be typed as $CDe/cde$ as a function of the frequency with which $f$ is associated with the $CDe$ chromosome.
in the sera used to test for c or e is of no consequence. However, if f does occur with C and E, errors in typing may well be introduced. As an example of this, figure 2 illustrates the frequency with which bloods of CDe/CDe would be misclassified as CDe/cde (because of the presence of anti-f in the anti-c sera) in relation to the frequency of f in chromosomes containing CDe.*

Calculations based on data quoted by Race and Sanger⁶ indicate that f is rarely if ever associated with C or E, provided that the assumption that anti-f was present in the anti-c and anti-e sera used for these typings is correct.

It is difficult to envisage, at this stage of our knowledge, a place for a pure and potent anti-f in the general clinical laboratory. For the immediate future, its primary use will be as an aid in the analysis of theoretic problems. Geneticists will appreciate that if f does in fact represent a fourth locus the chances of finding a cross-over within the Rh complex are somewhat increased. The significance of the antigen in population studies is obvious. The laboratory engaged in immunohematologic work will find anti-f serum useful in differentiating two types of blood both of which contain the antigens C, D, E, c, e. If blood of this phenotype is f negative the most probable genotypes are obviously CDe/cDE, CDe/cdE or cDE/Cde, but if it is f positive the genotypes would probably be CDE/cde, or less commonly, CDE/cDE. In the field of paternity testing it now becomes necessary to consider the possible effect of the presence of the antigen f in results which depend upon the use of anti-c or anti-e sera which have not been tested for the presence of anti-f.

**Summary**

1. An example of the new anti-Rh serum, anti-f, is described.
2. It is shown that anti-c and anti-e sera may contain anti-f.
3. The significance of the antigen f is discussed in terms of its genetics and practical importance.

**Summario in Interlingua**

1. Es descritite un exemplo del nove factor anti-f in le systema Rh.
2. Es demonstrate que anti-c e anti-e pote continer anti-f.
3. Le signification del antigeno f es discutite in relation a su genetica e su importantia practic.

**References**

2 Race, R. R. and Sanger, R.: Personal communication.

* Appendices to this paper, containing the calculations of gene frequencies, and the derivation of the equation for the curve of figure 2, have been omitted for economy of space. Copies of these appendices may be obtained by writing to the authors.
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