Studies in Rh Sensitization

VI. A-B-O Blood Groups and Rh Subtypes in Sensitized and Nonsensitized Rh Negative Pregnant Women

By ALEXANDER S. WIENER, M.D., F.A.C.P., RAFFAELE NAPPI, M.D., AND EVE B. GORDON

The A-B-O Blood Groups

AS LEVINE has pointed out, A-B-O incompatibility is less frequent in families with erythroblastotic children due to Rh sensitization than in the general population. That is, an Rh negative mother is less likely to become sensitized to the Rh factor if her Rh positive fetus possesses either the A or B agglutinogen when the corresponding agglutinogen is absent from her own red cells. Wiener has ascribed this phenomenon to competition between the A-B and Rh antigens. Another interpretation could be that when fetal cells enter the maternal circulation through a break in the placenta they are rapidly destroyed and eliminated if they belong to an incompatible blood group, while if the fetal cells are compatible with the maternal serum they survive and there is greater opportunity for the Rh agglutinogen to exert its antigenic action.* Whatever the explanation may be, all workers who have investigated this problem have been able to confirm Levine’s original observation.

One would surmise that group O, Rh negative women would have less than the average chance of becoming sensitized to the Rh factor during pregnancy because women of group O are more likely to have husbands of an incompatible blood group. On the other hand, group A, Rh negative women would have a somewhat greater chance of becoming sensitized because relatively fewer group A women would have husbands belonging to an incompatible group. Accordingly, one would expect the distribution of the A-B-O groups to show a lower incidence of group O and a higher incidence of group A among sensitized Rh negative mothers than among the nonsensitized Rh negative mothers. In order to test this prediction, we have analyzed the distribution of the A-B-O blood groups and subgroups in four hundred fifty-seven sensitized Rh negative women in

From the Division of Immunohematology of the Jewish Hospital of Brooklyn, Brooklyn, N. Y., the Serological Laboratory of the Office of the Chief Medical Examiner of New York City, New York, N. Y., and the Clinica Ostetrica e Ginecologica, Università di Napoli, Naples, Italy.

Submitted April 25, 1953; accepted for publication June 5, 1953.

* In unpublished experiments by one of us (A. S. W.) it has been found that cross-immunization of group A and group B individuals by daily intravenous injections of 5 cc. of blood failed to stimulate a rise in titer of the isoagglutinins. On the other hand, as little as 1 cc. of Rh positive blood will stimulate a marked rise in antibody titer in pruned individuals. This may be due to the fact that the natural A and B isoagglutinins quickly hemolyze incompatible red cells while univalent Rh antibodies often coat Rh positive cells without destroying them. Apparently, such coated Rh positive cells can survive in the circulation for a long time and thus may have a greater opportunity to stimulate the production of antibodies.
comparison with eight hundred forty-three nonsensitized Rh negative women. In table 1 are presented the results of this analysis.

As expected, the frequency of group O was lower among the sensitized Rh negative mothers. The difference is 3 times the probable error and therefore on the borderline of statistical significance. Also in conformity with expectations, the incidence of group A is significantly higher among the nonsensitized mothers. Strangely, however, this difference applies only to the subgroup A₁ and not to subgroup A₂. The difference in the case of subgroup A₁ is 5 times the probable error, so that this difference is almost surely not accidental. It will be interesting to ascertain whether analysis of additional series by other investigators also shows the same puzzling lack of difference in frequency of subgroup A₂.

As is to be expected, the frequency of group B individuals is lower among the sensitized mothers than among the nonsensitized mothers, but the difference is not statistically significant. The frequency of group AB is the same in both series of mothers, although a priori one would expect a higher frequency among the sensitized mothers.

### Table 1.—Comparison of the A-B-O Blood Group Distribution in Sensitized and Nonsensitized Rh Negative Women

<table>
<thead>
<tr>
<th>Blood group</th>
<th>Nonsensitized</th>
<th>Sensitized</th>
<th>Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number</td>
<td>% ± P.E.*</td>
<td>Number</td>
</tr>
<tr>
<td>0</td>
<td>362</td>
<td>43.98 ± 1.17</td>
<td>175</td>
</tr>
<tr>
<td>A₁</td>
<td>244</td>
<td>29.65 ± 1.06</td>
<td>178</td>
</tr>
<tr>
<td>A₂</td>
<td>72</td>
<td>8.74 ± 0.66</td>
<td>40</td>
</tr>
<tr>
<td>B</td>
<td>109</td>
<td>13.24 ± 0.80</td>
<td>43</td>
</tr>
<tr>
<td>A₁B</td>
<td>31</td>
<td>3.77 ± 0.44</td>
<td>17</td>
</tr>
<tr>
<td>A₂B</td>
<td>5</td>
<td>0.61 ± 0.19</td>
<td>4</td>
</tr>
<tr>
<td>Totals</td>
<td>823</td>
<td>100.0</td>
<td>457</td>
</tr>
</tbody>
</table>

*Per cent ± probable error.

### The Rh Types

Early in the studies on the Rh blood types occasional blood specimens were encountered which gave reactions of weak or intermediate intensity with one or more of the Rh antisera. Thus, it became clear that variants of the Rh-Hr factors exist of which the most important are the Rh₀ variants (Rhₐ). Classification of blood containing factor 9Rh₀ offered difficulties because with most agglutinating anti-Rh₀ sera the reactions are negative, though with certain antisera (anti-9Rh₀*) distinct agglutination is obtained. The problem was clarified when it was found that treatment of the cells by proteolytic enzymes or the use of the antiglobulin technic makes possible the ready demonstration of the 9Rhₐ factor with many of the commonly available Rh₀ antisera. The most extensive and illuminating study on this subject is that of Rosenfield et al.

*Anti-9Rh₀ sera react with both blood factors Rh₀ and 9Rh₀ due to a single cross-reacting antibody. Thus the name anti-D+D⁺, implying the presence of two separable antibodies is misleading, if not incorrect.
STUDIES IN RH SENSITIZATION

It is evident that the earliest reports on the frequencies of types rh, rh', rh", and rh'rh" in the general population were subject to the error that the blood factor \( \text{Rho} \) was not taken into account. Among Negroes, blood containing \( \text{Rho} \) has a considerably higher incidence than among Caucasoids. In the present study, the individuals investigated have all been Caucasoids, among whom the factor \( \text{Rho} \) is relatively rare. When factor \( \text{Rho} \) is found in blood cells from Caucasoids almost invariably either factor rh' or rh" or both are also present. It is evident therefore that the error in the earlier reports on the distribution of the Rh types among Caucasoids pertains to the types rh', rh", and rh'rh" and hardly at all to type rh.

| TABLE 2.—Results of Subtyping Sensitized and Nonsensitized Rh Negative Women |
|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|
|                          | Total number of individuals | Rh negative individuals of type |                         |                         |                         |
|                          |                          | rh | rh' | rh" | rh'rh" | Totals |
| "Control" series         | 4,974                    | 13.3 ± 0.32 | 0.95 ± 0.093 | 0.42 ± 0.062 | 0 | 14.7 |
| Nonsensitized Rh negative women | 5,760* | Number | 766 | 40 | 16 | 1 | 823 |
|                          |                          | Per cent | 13.3* | 0.69 ± 0.74 | 0.28 ± 0.47 | 0.017 | 14.3 |
| Sensitized Rh negative women | 3,240* | Number | 431 | 13 | 12 | 1 | 457 |
|                          |                          | Per cent | 13.3* | 0.41 ± 0.75 | 0.37 ± 0.72 | 0.031 | 14.1 |
| Rosenfield et al. A.R.C. donors | 4,657 | Number | 628 | 36 | 24 | 2 | 690 |
| Corrected for \( \text{Rho} \) | 4,657 | Number | 625 | 22 | 19 | 0 | 666 |
|                          |                          | Per cent | 13.43 | 0.47 | 0.41 | 0 | 14.31 |

* The Rh negative women are part of a population which includes Rh positive as well as Rh negative women. By assuming the frequency of type rh to be 13.3 per cent, as in the control series, the size of the entire population could be estimated and from this the percentages for types rh', rh", and rh'rh" could be calculated.

With very rare exceptions individuals whose bloods contain the \( \text{Rho} \) factor would fail to produce Rh\( \text{b} \) antibodies when injected with ordinary Rh positive blood; thus, if a type rh' individual has Rh\( \text{b} \) antibodies in his serum, it is hardly likely that his blood cells contain blood factor \( \text{Rho} \). On the other hand, if a supposed type rh' individual has no Rh\( \text{b} \) antibodies in his serum this leaves open the possibility that his blood cells may contain the \( \text{Rho} \) factor, so that his type may actually be \( \text{Rho} \) instead of rh'. Therefore, by comparing the distribution of the Rh blood types among sensitized and nonsensitized Rh negative individuals one may be able to estimate indirectly the incidence of the \( \text{Rho} \) factor.

In table 2, we have compared the distribution of the Rh types in the series of four hundred fifty-seven Rh negative mothers with that in the series of eight hundred twenty-three nonsensitized Rh negative mothers. As shown in table 2, among four thousand nine hundred seventy-four Caucasoids of both sexes, taken
from the general population, the incidence of the Rh negative types were: type rh, 13.3 per cent; type rh', 0.95 per cent; and type rh", 0.42 per cent. The Rh negative mothers studied by us were, of course, part of a larger population which included Rh positive as well as Rh negative individuals. To calculate the frequencies of the Rh types it is necessary to know the size of the entire population. We have, therefore, assumed that the incidence of type rh among our mothers was the same as our control series, namely, 13.3 per cent. On this basis the eight hundred twenty-three nonsensitized Rh negative women represent a larger population of five thousand seven hundred sixty mothers while the four hundred fifty-seven sensitized Rh negative women are estimated to be part of a population of three thousand two hundred forty mothers. Having estimated the size of the entire population, the frequencies of the types rh', rh", and rh'rh" were then readily calculated.

As shown in table 2, while the frequency of type rh' in the control series was 0.95 per cent, among the sensitized Rh negative women it was only 0.41 per cent, and the difference, 0.54 ± 0.12, is statistically significant. This result indicates that about half the supposed type rh' individuals in the control series actually belong to type Rh0. Among the nonsensitized Rh negative women the calculated frequency of type rh' was 0.69 per cent. That this frequency was lower than in the control series may be attributed to the use of the sensitive slide technic of Rh testing by which Rh0 blood frequently, but not always, reacts positively. With regard to type rh" the frequencies in the three series showed no statistically significant difference, indicating that relatively fewer of the type rh" bloods of the control series actually belong to type Rh2.

It is of interest to compare our results with those of Rosenfield et al. (cf. table 2). Among four thousand six hundred seventy-five consecutive Caucasoids who presented themselves as donors at the American Red Cross Blood Bank, there were 0.77 per cent type rh' and 0.52 per cent type rh". When tests were carried out for factor Rh0 the frequency of type rh' was reduced to 0.47 per cent, and type rh" to 0.41 per cent. The parallelism between our findings and those of Rosenfield et al. is indeed striking.

SUMMARY

The distribution of the A-B-0 blood groups among four hundred fifty-seven sensitized Rh negative mothers was compared with the distribution among eight hundred twenty-three nonsensitized Rh negative mothers. As expected, the frequencies of groups O and B were lower while that of group A was higher in the series of sensitized women. The difference in frequency of group A was 5 times its probable error and therefore statistically significant. Surprisingly, however, the difference proved to be due entirely to subgroup A1, while the frequency of subgroup A2 was the same in both series.

In a random series of four thousand nine hundred seventy-four Caucasoid individuals in New York City, the frequency of type rh' was found to be 0.95 per cent. Statistical analysis of the Rh types of four hundred fifty-seven sensitized Rh negative mothers indicates, on the other hand, that the frequency of type rh' is actually 0.41 per cent. The discrepancy is explained by postulating that in the control series, about half of the supposed type rh' individuals were
STUDIES IN Rh sensitization
really type $Rh_0$, since no special test had been made for Rh$^a$ variants. In the
series of sensitized Rh negative mothers, individuals of type $Rh_1$ are automatically
screened out since when factor $Rh_0$ is present sensitization to the Rh$^a$ factor is
hardly likely to occur.

REFERENCES
1 Levine, P.: Serological factors as possible causes in spontaneous abortions. J. Hered. 24: 71-81, 1943.
5 Wiener, A. S. and Sonn-Gordon, E. B.: Simple method of preparing anti-Rh serum in
9 — and Brancato, G. J.: Problems in the management of erythroblastosis fetalis, with
five examples exhibiting unusual serological findings. J. Lab. & Clin. Med. 40: 27-38,
1952.
14: 171-184, 1948.
11 Rosenfield, R., Vogel, P., Miller, E. B., and Haber, G.: Weakly reacting Rh-positive
Studies in Rh Sensitization: VI. A-B-O Blood Groups and Rh Subtypes in Sensitized and Nonsensitized Rh Negative Pregnant Women

ALEXANDER S. WIENER, RAFFAELE NAPPI and EVE B. GORDON