

NOTES ON A SERUM CONTAINING ANTI-P IN HIGH TITRE

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In 1927 Landsteiner and Levine reported the discovery in human blood of the antigen P. Since this time, the corresponding antibody has been detected frequently, but, in most of the sera described, is weak both in titre and avidity, and requires a special technique in order to demonstrate its presence. For this reason the unexpected finding of a powerful naturally occurring antibody, anti-P, in the serum of a patient is of interest. The following is a description of the reactions of the antibody which we found during routine work.

Observations on the Case

On March 4, 1954, S. W. W., a boy aged 12 years, was listed for operation because of a hydatid cyst of the lung. He was found to be blood group A Rh positive, and his serum was then cross-typed with the pilot cells of four bottles of group A Rh-positive blood in the usual way in order to have blood available for the operation. It was found that the cells of all four bottles of blood were agglutinated, the agglutination becoming more obvious after the cell suspension had been taken out of the incubator for examination.

A fresh specimen of blood (20 ml.) was obtained from the patient immediately in order to investigate the reactions of the serum. The blood group was checked and found to be group A (subgroup A₁) Rh positive. On checking the blood groups of the four bottles of blood, three were found to be A₁ Rh positive and one A₂ Rh positive. The patient's serum was then found to agglutinate the cells of 51 out of 68 bottles of blood of groups A Rh positive, A Rh negative, O Rh positive, and O Rh negative against which it was tested, but sufficient compatible group A Rh-positive blood was obtained for the operation, which took place at the appointed time.

It was noted that the agglutination became more obvious at 8° C. than at 37° C. Nevertheless, the undiluted serum gave a very strong agglutination in the incubator as well as at room temperature. The

titre of the antibody, using cells suspended in serum albumin solution and in Rous Turner solution for a period of one hour, was as follows :

Temperature of Test (° C.)	Cells in Rous Turner Solution	Cells in Albumin
37	4	32
8	64	256

At room temperature the diluted serum caused agglutination of cells suspended in Rous Turner solution in the following times.

Titre	Time
1	30 sec.
2	60 "
4	2 min.
8	5 "
16	8 "
32	15 "
64	75 "

Gene Frequency of Antigen.—The serum of S. W. W. was tested against 92 samples of group A Rh-positive blood chosen at random. Of these, 22 were found to be compatible and 70 were not compatible. The gene frequency corresponding to the antigen which was reacting with the antibody in S. W. W.'s serum and the gene frequency of its presumed allelomorph was worked out using the method described in M.R.C. Memorandum No. 27 (Mollison, Mourant, and Race, 1952). These worked out as 0.51 and 0.49 respectively. The panel of blood used is too small for accurate determination of the gene frequencies, but, as the gene frequencies of P and p are 0.4901 and 0.5099 respectively, the results suggested that we might be dealing with the P blood group and that the antibody was anti-P.

Tests Against Panel of Known Blood Groups.—The serum of S. W. W. was tested against the red cells of 19 individuals in whom the ABO, MNS, P, Lewis, Rh, Duffy, and Kell blood groups were known. Fourteen were P positive, and the cells of these agglutinated with the serum of S. W. W. Four of the panel were P negative and the serum did not agglutinate the cells of these individuals. The red cells of

one of the donors on the panel, A. D., who had been notified as "P negative," were agglutinated by the serum of S. W. W. Accordingly, the blood of A. D. was sent to the Commonwealth Serum Laboratories, Melbourne, so that the cells could be retyped by Mr. R. T. Simmons, who confirmed that A. D. did in fact belong to blood group P: the initial error in grouping the cells was most probably due to low potency anti-P sera. The serum of S. W. W. was then retested against known P-positive and P-negative blood in the Commonwealth Serum Laboratories. The serum agglutinated all "P-positive" cells and failed to agglutinate all "P-negative" cells.

Family Studies.—The family of S. W. W. were interested in the peculiarity of S. W. W.'s blood and were cooperative during the investigations. There are eight members in the family, including S. W. W., his father, mother, two brothers, and three sisters. S. W. W., born in 1941, was the fourth child in the family. He had been immunized against diphtheria in 1942. In 1949 he had penicillin and streptomycin injections for complications of his lung condition, and in 1952 had both active and passive anti-tetanus immunization. He had never had a blood transfusion or injection.

Only the mother of S. W. W. was found to be P positive (by testing the cells with the serum of S. W. W.). All the other members of the family are P negative (including S. W. W. himself); their cells do not agglutinate with the serum of S. W. W.

The serum of each member of the family was tested against known P-positive and P-negative cells. In no case did agglutination occur, indicating that no member of the family other than S. W. W. had produced the antibody, anti-P, even though they were all (with the exception of S. W. W.'s mother) P negative.

Source of the Antibody.—As S. W. W. had not been transfused previously, it was thought that he might have been immunized by the inoculations he had received. Accordingly, serial dilutions of the serum of S. W. W. were made from 1 to 64, and to each serial dilution was added an equal quantity of diphtheria prophylactic, formalized tetanus toxoid, and tetanus antitoxin. After allowing one hour to elapse during which time any reaction could be expected to have taken place, a drop of P-positive cell suspension was dropped into the mixture and the tube noted in which agglutination took place. It was found that in each case agglutination took place in the same tube as the saline control, suggesting that there was no P antigen in the three materials tested and that S. W. W. had not been stimulated to produce anti-P antibody as a result of previous inoculation.

Absorption and Elution.—Using the method of Mollison (1951), the serum of S. W. W. was absorbed with the cells of one of the hospital staff, A. W., who was group O Rh positive, P positive. The absorbed serum was found to have a much weaker agglutinating titre than unabsorbed serum.

The cells of A. W. with the absorbed antibodies were then shaken in saline and the antibody was eluted in the manner described by Mollison. The eluate agglutinated two specimens of P-positive cells, whereas the absorbed serum failed to agglutinate the same cells. It is possible, therefore, to obtain this antibody in a pure form.

Discussion

Approximately 75% of white people are P positive, belonging to the genotype PP or Pp. The remainder are P negative and belong to the genotype pp. The gene p is allelomorphic to P. Due to the fact that transfusion is so widely practised, and as anti-P serum is not available in adequate quantity for the routine grouping of donors and recipients, many P-negative people are transfused with P-positive blood. As a result these recipients could develop anti-P. Transfusion reactions from this cause have indeed been reported. However, most anti-P sera are of low titre and the reactions are generally mild.

The present case indicates that the antibody anti-P may on rare occasions be present in sufficient titre to be a cause of a severe transfusion reaction. Recently Kölbl and Speiser (1953) have reported a case in which the maternal anti-P antibody had a titre of 1 in 16 in human serum at body temperature. The case history of her infant suggests that it suffered from erythroblastosis foetalis due to the presence of anti-P in the maternal blood as a result of maternal immunization.

The need for careful routine cross-typing of donor cells against patient's serum is borne out by this case. Atypical isohaemoglutins occur rarely, but they make their appearance with dramatic suddenness at inconvenient times and it is therefore essential that a rigid technique should always be followed of grouping and cross-typing each patient's serum against each blood bottle issued to him before notifying it as compatible.

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