ANTI-A HAEMOLYSIN IN GROUP O BLOOD DONORS

AN EAST ANGLIAN SURVEY

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It was shown by Ervin and Young (1950) and by Ervin, Christian, and Young (1950) that haemolytic transfusion reactions might be produced by the transfusion of group O blood to patients of group A. They concluded that the reactions were associated with the presence in the donor's serum of an antibody which displayed "immune" characteristics, and they further showed that the serum of 12 out of 100 donors of group O, picked at random, displayed immune characteristics.

"Immune" antibodies have been said to differ from "naturally occurring" ones in various respects, but Crawford, Cutbush, Falconer, and Mollison (1952) state that of the various immune characteristics of an anti-A serum the ability to haemolyse group A cells in the presence of complement is the easiest to detect, and they suggest that the *in vitro* haemolysin titre of a serum gives a good indication of its ability to destroy A cells *in vivo*. Grove-Rasmussen, Soutter, and Marceau (1951) reported an extensive study in which it was found that, though there seems to be a relationship between the titre of saline-reacting anti-A iso-agglutinins and the presence of immune anti-A antibodies, no exact correlation exists. Accordingly Grove-Rasmussen, Shaw, and Marceau (1953) described a screen test by which group O blood with titres of immune anti-A higher than 1/16 could be excluded from use as "universal donor" blood. This titre was chosen because it was thought to be the highest that could be expected to be rendered insignificant by dilution in the recipient's blood stream. The test involves partial absorption of the sera with group-specific substance and then an indirect anti-human-globulin (Coombs) test.

Test for Anti-A Haemolysin

Mollison (1951) described a test for the detection of anti-A haemolysin, and Gardner and Tovey (1954) adapted this as a routine method for the investigation of group O donated blood. McDermott and Muschel (1956) screen their donors by a saline agglutinin and a haemolysin technique using human complement. It appears to have been tacitly accepted that the acquisition of the power of producing harmful reactions in group A recipients is a consequence of the immunization of the donor to the A antigen, but there seems to be a lack of experimental evidence to support this.

In fact, Ervin *et al.* (1950) are careful to say: "All that can be claimed is that the anti-A antibodies in the serum of these group O donors behave *in vitro* like those found in the serum of donors known to be actively immunized by pregnancy, transfusion, or the injection of soluble A factor." Crosby and Akeroyd (1954) say: "Following large transfusions of low-titre group O

blood into patients of groups A, B, and AB it was not possible to demonstrate foreign haemolysins or incomplete antibodies in the serum of recipients. . . . In most of these patients there was evidence of a selective destruction of recipient red cells after the transfusion of O blood. . . . It is suggested that there may exist forms of antibody that cannot be demonstrated by laboratory methods. These antibodies manifest themselves only by causing destruction of red cells *in vivo*." Crosby and Howard (1954), reporting on patients in the Korean campaign who had had massive transfusions of as much as 30 pints (17 litres) of group O blood in less than six hours, said that the patient's plasma sometimes contained antibodies against the red cells of his hereditary blood group, and that, though gradual haemolysis of the native red cells was observed, this was not a clinical haemolytic reaction, and did not appear to be detrimental to the patient. They recommended that after transfusions of the universal donor blood had begun, no change should be made to blood of another group until at least two weeks had elapsed.

Classification

McDuffie and Kabat (1956) brought forward evidence that anti-A produced by immunization with various blood-group substances and by heterospecific pregnancy could be divided into two groups, one showing enhancement of the titre in the anti-human-globulin test, and the other failing to show such enhancement, from which it was concluded that no proper distinction could be made by these methods between such antibodies on the basis of their being "natural" or "immune." Filitti-Wurmser, Jacquot-Armand, and Théoleyre (1958) have described a thermodynamic method enabling distinctive characters to be identified between "natural" iso-agglutinins anti-A and anti-A₁, on the one hand, and serum from a group B donor who had been immunized by an injection of group A substance prepared from hog gastric mucin on the other, and Konugres and Coombs (1958) have shown that the antibody in the so-called "immune" anti-A human sera, which is recognized by the Witebsky partial neutralization reaction, and also the anti-A haemolysin, has a specificity against an antigen found in pig cells and also certain cattle and sheep cells, and known as A^p, a specificity which is absent in "natural" human anti-A sera.

However this may be, it appears to be clear that anti-A sera can be classified into those which produce haemolysis *in vitro* in the presence of complement, and those which do not, and that the latter do not seem to be harmful to group A cells *in vivo*, whereas in at least some instances the former do.

Both Mollison and Gardner insist on the test being done within 24 hours of collection, or, alternatively, with the addition of fresh group O serum which is known to be free from haemolysin, as a source of complement. This involves preliminary investigations to identify group O subjects whose serum is free from haemolysin, and who are available to be bled at suitable times so that a supply of fresh complement may be forthcoming. Crawford *et al.* (1952) confirmed the observation of Davidsohn (1938) that in people receiving injections of horse serum there might be a considerable increase in the anti-A and anti-B titres of the recipient's serum, and reported a series of 12 persons attending the casualty department of Hammersmith Hospital to receive a prophylactic injection of antitetanic serum who also

attended nine days later for a second blood sample to be taken. Six were of group O and two of group B. Of these eight, only one had anti-A haemolysin before the serum injection, and all eight had anti-A haemolysin after the injection. Seven of the eight also showed a rise in the agglutinin titre.

The authors also described the cases of 14 R.A.M.C. officers of group O who were about to have an initial T.A.B. vaccine injection. The serum of four haemolysed A cells before immunization. In samples taken 18 days later, one of the four showed a considerable increase in haemolysin titre, and, of the 10 officers who had no haemolysin at all initially, six developed a substantial amount after immunization. They suggested that, since it is very troublesome to screen all group O donors for haemolysin, it would be a wise precaution to refuse as donors all persons who had received an injection of any vaccine or animal serum during the previous three months, or, alternatively, bottles of blood obtained from such people might be marked to indicate that they should be given only to group O recipients.

Gardner and Tovey (1954) pointed out that, because of the regular inoculations given to members of the armed Services, the organization of visits of blood-collecting units to Service stations would be gravely jeopardized if this recommendation were adopted. They went on to report that of 70 Service donors whose serum contained strong haemolysins when examined by them 17 (nearly 25%) had received inoculation more than three months before they gave their blood.

In an attempt to overcome the difficulties involved in the Gardner technique, we have introduced a one-tube haemolysin test which uses animal serum as a source of complement. The method was described by Dennis (1954), and this report summarizes the results of the screening of 25,541 consecutive group O donations. Sheep, ox, horse, rabbit, guinea-pig, and mouse sera were tried. It was found that the removal of anti-human-species lysis by human group O cells was more effective with horse and rabbit sera than with the others. Horse serum was finally selected because the anti-human lysis could always be completely absorbed out by two absorptions, whereas in the case of rabbit sera the lysis varied considerably, and in some cases numerous absorptions were necessary. Further, horse serum is easy to obtain in large volumes. The absorbed horse serum was titrated against a unit volume of a 5% saline suspension of pooled human A₁ red cells in the presence of an equal volume of a high-titre human anti-A haemolytic serum which had been inactivated by heat (56° C. for 30 minutes). The titre is never very high and is frequently 1:2. It is adjusted with saline to a titre of one volume of complement giving partial haemolysis in the presence of equal volumes of 5% pooled A₁ cells suspension and anti-A haemolytic serum.

Activity of Complement

Preservation of complement activity was tried by (a) Richardson's boric acid, sorbitol, borax, and sodium azide method; (b) freezing at -20° C.; and (c) spin freeze-drying. It was found that either of the latter two methods was suitable, but that chemical preservatives interfered with the haemolytic reaction. This was interpreted as support for the view that the requirements of complement by anti-A haemolysins differ from those of sheep-cell lysis in a Wassermann test. One tube is required for each serum to be

examined and also three tubes for control, and they should be set up as shown in Table I.

The tubes are agitated to mix the contents, and are then incubated in a water-bath at 37° C. for one hour. They are then centrifuged or, alternatively, allowed to stand at room temperature for a further settling period of at least 90 minutes, and then examined for the presence of unlysed cells. In reading the tests, no attempt is made to assess degrees of haemolysis. The end-point is read as: complete haemolysis (positive) versus partial or no haemolysis (negative).

Table II shows the result when 35 and 34 group O bloods (69 in all) from two sessions were examined by Gardner's method and also by the added horse

TABLE I.—*Haemolysin Test and Controls*

	Test Proper	Volumes		
		Complement Positive	Complement Negative	Cell
Serum under test	1	—	—	—
Complement	2	2	2	—
Pooled A ₁ red cells	1	1	1	1
Anti-A haemolysin	—	1	—	—
Saline 0.85%	—	—	1	3

TABLE II.—*Added Horse Complement Method Compared with Gardner's Method*

Gardner's Method		Added Horse Complement	
Degree of Haemolysis		Positive Complete Haemolysis	Negative Partial or No
None	} "weak" { 55 ..	1	54
Trace			
25%	} "strong" { 9 ..	4	5
50%			
75%			
Complete			
	69 ..	9	60

complement method. The results were in good agreement except for one specimen which gave 75% haemolysis by Gardner's method and none by added horse complement, and another which gave complete haemolysis by added horse complement and none by Gardner's method.

Chaplin, Wallace, and Chang (1956) discuss the difficulties in reading and interpreting the significance of partial degrees of haemolysis, and emphasize the importance of keeping the cell suspension weak and complement in excess. They also mention the considerable variation in the activity of (human) complement among sera from normal persons, leading to undesirable variations in tests that are widely used as a means of screening donors. In the six samples which were judged to give "strong" (50% or 75%) haemolysis by Gardner's method, and read as "partial haemolysis" (negative) with added horse serum, the apparent discrepancy may be reflecting the difficulty of assessing the extent of partial degrees of haemolysis, or of ensuring that an adequate dose of human complement was available to react with the 5% suspension of cells. If "over 50%" versus "50% or less" is taken as the end-point in Gardner's method, the results are closely comparable.

Results of Test

Tables III and IV show the results of the test on 25,541 consecutive group O donations to the East

TABLE III.—*Haemoglobin Frequencies in East Anglia Blood Donors*

	Civilians		H.M. Forces		Totals			
	M	F	M	F	M	F	Civ.	H.M.F.
No. examined	11,089	6,830	7,241	381	18,330	7,211	17,919	7,622
No. with anti-haem }	1,113 10%	523 7.7%	421 5.8%	21 5.5%	1,534 8.4%	544 7.5%	1,636 9.1%	442 5.8%

TABLE IV.—*Age Distribution*

Years:	20	20-29	30-39	40-49	50+
No. examined	2,179	9,588	5,231	4,699	3,844
No. with anti-haem }	114 5.2%	570 5.9%	498 9.5%	472 10.1%	424 11.0%

Anglian Blood Transfusion Service. It will be seen that 2,078 (8.1%, S.D. 0.16) gave a positive result. There was a general tendency among males and females, civilians and Service personnel, for the frequency of haemolysins to increase with advancing years, and in all age groups the proportion of males showing haemolysins was higher than that of females; but in this series the difference was not significant at the 3 S.D. level. Out of 18,330 males, 1,534 were positive (8.4%, S.D. 0.205) against 544 females out of 7,211 (7.5%, S.D. 0.31).

Among 17,919 civilians, aged 18-65, 1,636 (9.1%, S.D. 0.215) were positive, but only 442 out of 7,622 members of H.M. Forces (5.8%, S.D. 0.275), and this is decidedly significant at the 3 S.D. level. We are unable to find evidence that the effect of the routine inoculations received by members of the armed Services has been to raise the frequency of anti-A haemolysins among group O Services donors to an appreciable extent as compared with the frequency in the civilian population who attend at civilian or industrial donor sessions; and since the detection of haemolysins *in vitro* is so easy, we do not support the suggestion of Crawford and her associates that all such donors should be refused without investigation. We think that any effect of inoculations in raising the frequency of anti-A haemolysins in Services donors is more than offset by the predominance of younger persons in these populations, and, as we have seen, the frequency is low at the younger ages. We think that the problem raised by these donors, whether occurring in H.M. Services establishments or elsewhere, is a small one, and can be adequately met by the routine screening of all group O donors for anti-A haemolysin, and the labelling of the donations in which it is observed, so that these donations will be used only for group O patients, after satisfactory cross-matching.

Summary

A test suitable as a routine screening test for the detection of anti-A haemolysin in group O donors is described, using the serum sample collected for the routine grouping and Wassermann testing of the donation, and using horse serum as a source of complement.

In a series of 25,541 consecutive group O donations there was a tendency for the frequency to increase with advancing years. The effect of vaccines and similar injections to members of the armed Services was not sufficient for the frequency among them to increase to a significant figure. In our series of nearly 18,000 civilians and over 7,600 members of H.M. Forces it was actually lower in the Services than among civilians.

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We think, however, that this merely reflects the preponderance of young donors at the Services sessions.

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ON THE IMMUNOLOGICAL REACTIVITY OF TUBERCULOUS PATIENTS

PRELIMINARY REPORT

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Greenwood, Smellie, Barr, and Cunliffe (1958) published the results of a study concerning the immunization of sarcoidosis patients against tetanus. Their object had been to ascertain how far these patients reacted "normally" to the administration of an antigen. Their choice fell on the tetanus antigen (fluid tetanus toxoid) because on practical grounds (sporadic occurrence of tetanus) these patients might be assumed not to have come into contact with this antigen before, just like non-immunized healthy controls; in other words, they might be expected not to have acquired a potential immunity for this antigen.

Their sarcoidosis patients and non-immunized controls received two intramuscular injections of fluid tetanus toxoid at an interval of six weeks. In both groups the reaction to this immunization was ascertained by determining the antitoxin in the blood serum a fortnight after the second antigen injection.

During this primary immunization the sarcoidosis patients formed much less antitoxin than the normal controls. Sarcoidosis patients who had been immunized against tetanus some years before, and with whom the tetanus toxoid administered during the experiment therefore acted as a "booster injection," reacted to it like healthy persons.

We have been engaged for a considerable time in an investigation of the reactions of tuberculous patients to immunization. The results recorded by Greenwood