ANTI-M AGGLUTININ IN A HUMAN SERUM

BY

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The occurrence of anti-M agglutinin in human serum was until comparatively recently considered to be an event of great rarity. The antibody was found in its naturally occurring form, active at low temperatures only, by Moureau and Lambert (1939) in the serum of a blood donor during routine testing. Examination of antenatal sera yielded further examples, described by Paterson *et al.* (1942) and by McFarlane (1945); and Unger *et al.* (1946) detected a case during the cross-matching of blood prior to transfusion. Since the end of the second world war the enormous increase in the use of blood transfusion has resulted in many more cases having been found (Race and Sanger, 1954).

The following case is of interest because the antibody, which was detected during routine pretransfusion cross-matching, was at first thought to be anti-D, and the patient narrowly escaped a possible haemolytic reaction.

Case Report

The patient, a married woman aged 61, was admitted to hospital at night as an emergency, with a bleeding duodenal ulcer. The pathologist on duty was asked to cross-match a bottle of blood urgently. The information provided on the laboratory request form was that the patient had had one previous pregnancy and no transfusions. Her blood group was found to be A Rh(D)-negative. Since no Rh-negative blood was immediately available it was decided, in view of the obstetric history and the patient's condition, to cross-match Rh-positive blood without further delay. The cross-matching was carried out in albumin at 37° C. and in saline at room temperature. After one hour's incubation it was found that the blood was incompatible, agglutination having occurred in both tubes, but more strongly in saline. The donor's cells were confirmed as being of group A, and the provisional assumption was made that the antibody was probably anti-D, and that the obstetric and transfusion history given on the request form was incorrect owing to the difficulties attendant upon the questioning of a seriously ill patient. Accordingly a bottle of Rh-negative blood was obtained, and as some considerable time had now elapsed this blood was cross-matched rapidly, in albumin at 37° C., and appeared to be compatible after 10 minutes' incubation followed by centrifugation. This blood, however, was not transfused, as the patient's condition improved following surgery.

On the following day it was decided to confirm the presence of anti-D in the serum by the indirect Coombs test, but no antibody could be demonstrated. The serum was then tested against a small panel of cells of group A or O, and of varying Rh group, and was found to agglutinate some of the cell samples but not others, irrespective of their Rh group, in saline at room temperature. As there was a suspicion that the antibody might be anti-M, the cells of the panel were grouped accordingly, and the agglutinated cells were all found to possess the M antigen, and the remainder to lack it. The patient's cells were at the same time found to be of group N. The serum was then tested against a total of 83 cell samples, and agglutinated only those cells possessing the M antigen, which comprised 60 of the 83. Perhaps needless caution was exercised in testing so large a panel, for subsequently the odds against these results being due to some antibody other than anti-M were calculated and found to be $2,058 \times 10^{16}$ to 1.

The tests were carried out by incubating equal volumes of serum and 5% cell suspensions at 4° C. for 30 minutes,

followed by centrifugation and resuspension of the cells by tapping the tubes. Marked agglutination visible with the naked eye occurred with the M cells. Of the cell samples belonging to group M, 30 were also tested with the serum in albumin and in saline at 37° C. Agglutination after 30 minutes' incubation was moderate in albumin and weak in saline. At 4° C. the serum agglutinated homozygous M cells to a dilution of 1/64. Absorption of the serum with M cells at 4° C. led to a complete disappearance of activity at either 4° C. or 37° C., indicating that a single antibody with a wide temperature range was present rather than two distinct antibodies, a naturally occurring and an immune type.

As soon as the identity of the antibody was reasonably certain the bottle of "compatible" blood was examined and found to be of group M, and it was confirmed that, whereas the cells of this bottle were agglutinated in albumin at 37° C. by the patient's serum after 30 minutes' incubation, 10 minutes was inadequate. In saline at 4° C., however, agglutination occurred within 10 minutes.

On being interviewed the patient confirmed that she had not previously received a blood transfusion, and that she had had only one child. This child was born when the patient was 36 years of age, and had been healthy up to the age of 3 years, when it had died of meningitis. The husband's blood group was found to be A Rh(D)-positive M.

Discussion

It is still widely believed that antibodies of the specific cold agglutinin type—for example, anti-M, anti-P, anti-Le^a—are not dangerous to recipients of blood transfusions, particularly when the blood is warmed to 37° C. before administration. Recent work has tended to cast doubt upon this belief. Mollison and Cutbush (1955), investigating the survival of transfused red cells which had been labelled with radioactive isotopes, found an example of anti-P which was strongly active at room temperature and only weakly active at 37° C. The patient in whose serum the antibody was detected was injected with labelled P-positive cells, and 50% of the injected cells disappeared from the circulation within the first 10 minutes; furthermore, the rate of elimination was found to be the same whether the cells were given cold or warmed to 39° C. Clearly an antibody demonstrating such *in vivo* behaviour would be potentially dangerous in a patient receiving a massive transfusion of cells containing the reciprocal antigen. The fact that in the present case the antibody showed some degree of activity at 37° C. is significant. There is no doubt that the specific cold agglutinins can extend their thermal amplitude and titre as a result of antigenic stimuli.

Broman (1944) reported the appearance of a form of immune anti-M active at 37° C. in a patient with aplastic anaemia who had received eight blood transfusions, and Wiener (1950) unintentionally produced immune anti-M agglutinin and haemolysin in a young woman whose serum already contained anti-D, to whom he had given several injections of Rh-positive blood over a period of three years, in order to maintain a high titre so that her serum might be used for Rh-grouping purposes. Tovey (1955) mentions four cases within his own experience where similar changes in the nature of antibodies of the type under discussion have occurred following transfusion or pregnancy, or both.

In our own case it is possible that the patient's one pregnancy had resulted in her naturally occurring anti-M becoming weakly active at 37° C. Had she been transfused with the bottle of blood intended for her she would probably have had some degree of haemolytic reaction, possibly severe. This risk would have been obviated had the cross-matching been carried out at a low temperature as well as at 37° C. Cross-matching at 4° C., in addition to detecting specific cold agglutinins potentially capable of causing haemolytic reactions, will also detect those too weak to cause a reaction, but capable of becoming potentially dangerous upon receiving the appropriate antigenic stimuli. The use of blood transfusion in hospital practice is still on

the increase, and when it is remembered that the sera of some 16% of the population contain anti-P it will be realized that it is a matter of great importance that those whose serum contains antibody of this type should not be transfused with blood that will lead to their developing potentially dangerous immune antibody.

Summary

A case of naturally occurring anti-M agglutinin in the serum of an Rh-negative woman is described.

The antibody was erroneously assumed to be anti-D, and a bottle of group M Rh-negative blood, cross-matched by a rapid technique at 37° C., was supposed to be compatible. The blood was not transfused.

The potentially dangerous nature of antibodies of this type and their relationship to cross-matching techniques are discussed.

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NON-BANCROFTIAN ELEPHANTIASIS IN TANGANYIKA

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A filarial survey of Tanganyika has recently been completed. During this work night blood samples were taken from several thousand Africans in order to determine the distribution of *Wuchereria bancrofti* infection. In addition to taking blood slides the incidence of elephantiasis was noted in the different areas visited, and, generally speaking, more cases of elephantiasis were seen in those areas where bancroftian microfilariæ was common than in those areas in which it was rare.

In one locality, however, in which night blood slides were all negative for microfilariæ of *W. bancrofti*, elephantiasis indistinguishable from bancroftian elephantiasis appeared to be not uncommon.

The area involved lies south-west of Lake Victoria, and is between 4,000 and 6,000 ft. (1,220 and 1,830 metres) above sea-level. In this area night blood samples were obtained from 475 adults in four villages in Bukoba, Biharamulo, Ngara, and Kibondo Districts. All were found to be negative for microfilariæ of *W. bancrofti*, though 12 of the 475 persons had elephantiasis of one or other legs. From other villages in Tanganyika which were found to be free of bancroftian infection—or in which the incidence of microfilariæ was less than 5%—6,866 night blood specimens were obtained and only eight cases of elephantiasis were seen.

The higher incidence of elephantiasis in the four districts mentioned above suggests that the disease in this part of Tanganyika is caused by something other than *W. bancrofti*.

One of us (M. H. T.) revisited the area and found 62 further cases of elephantiasis. This figure excludes any person who had travelled to a known bancroftian area and cases for which any obvious cause for elephantiasis could be found—that is, leprosy, tropical ulcer, and oedema due to other conditions. A total of 74 persons were thus seen in this area. Onchocerciasis does not occur in the area.

Clinical Manifestations

During the filarial surveys throughout the territory, 259 cases of elephantiasis were seen in areas in which bancroftian infection was endemic, and in Table I certain clinical manifestations observed in this series have been compared with those in the non-bancroftian series. The age incidence of the two series is shown in Table II, and it will be seen that the disease occurs at a slightly earlier age in the non-bancroftian series.

TABLE I.—Clinical Manifestations of Bancroftian and Non-Bancroftian Elephantiasis

	No. in Series	Unilateral Disease	Inguinal Adenitis	Hydrocele Present
Bancroftian	259	55%	60%	40% of 147
Non-bancroftian	74	20%	39%	6% „ 35

TABLE II.—Age Distribution of Bancroftian and Non-Bancroftian Elephantiasis

	20 and Under	21–30	31–40	41–50	51–60	61 and Over
Bancroftian	6%	12%	26%	28.5%	19.5%	8%
Non-bancroftian	6.9%	29.8%	25.8%	15.9%	10.8%	10.8%

Histories obtained from persons with non-bancroftian elephantiasis suggest that the condition usually started with pains in the groins and frequently fever, but the pain does not appear to have the centrifugal distribution sometimes seen in cases of filarial elephantiasis. In the established case recurrent attacks of pain in the groin were reported.

Discussion

The high incidence of bilateral disease and the low incidence of inguinal adenitis in the non-bancroftian series suggest that if lymphatic blockage is responsible for the elephantiasis it may be higher in the lymphatic system than in the inguinal glands. This would place the possible obstruction in the iliac glands or in the para-aortic nodes. It is unlikely that obstruction occurs at the latter site, since numerous anastomosing lymphatic vessels connect up these glands; and obstruction, sufficient to cause lymph stasis in the legs, would probably lead to other evidence of blockage—namely, elephantiasis of the scrotum and lymphocele, and this was present in only 2 out of 35 males.

Although *Acanthocheilonema persians* is not generally accorded a pathogenic role, it is worthy of note that infection with it is very common in the area in which this series of cases was seen (145 of the 475 bloods had microfilariæ of *A. persians*). The adult worm lives in the peritoneal cavity of the host, and it is not unreasonable to postulate that on occasion the adult—or embryos—may inadvertently enter abdominal lymph nodes, and, being in an abnormal site, initiate tissue changes leading to lymph blockage. Enzer (1928) found microfilariæ of *A. persians* in gland punctures in persons with enlarged femoral glands, and Sharp (1928) was unable to satisfy himself that the worm was not responsible for cases of elephantiasis.

Other reports of non-bancroftian elephantiasis elsewhere in East Africa describe the condition in the Kampala area of Uganda (Loewenthal, 1934). No cause could be found