A rabbit anti-a2 macroglobulin serum, acting as anti-C', has been studied. Its specificity to C' or C' fractions, and some peculiar features of this type of reactions are discussed. Furthermore analogies and differences between this type of anti-C' serum and the conglutinin system, natural and immune, are presented.

The Use of Complement in the Serological Diagnosis of Hemolytic Disease of the Newborn

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Dacie, in 1950, called attention to the part played by fresh serum in red cell absorption of cold auto-antibodies. He noted that sensitization did not occur when heat inactivated serum was used and that absorption was inhibited by heparin, oxalate and citrate. The similarity of this property of fresh serum to complement is obvious and recent efforts by many workers have left little doubt that complement or one or more of its components is in fact essential for the action of certain blood group antibodies. Dacie also found that cells coated with cold auto-antibodies could be agglutinated by some, but not all, rabbit antiglobulin sera. In a later paper he showed that when agglutination did take place, the rabbit antibody appeared to be combining with complement on the cell rather than antibody. Mollison and Cutbush stressed the need for a Coombs serum containing anti-beta-globulin in addition to anti-gamma-globulin when testing for sensitization of cells with Lewis and P antibodies in the presence of fresh serum. This recommendation can now be extended to include the following: anti-A, -B, -Tja, -H, the Donath-Landsteiner antibody and the antibody of acquired hemolytic anemia of the cold variety. These antibodies with the addition of the three previously mentioned (normal cold, Lewis and P) can be said to be potentially hemolytic requiring complement for their action. Some anti-Fya and anti-Jka can also be included.

In the course of work on the amounts of complement taken up by these antibodies we found that cells sensitized in vitro with inactivated antiserum and then exposed to fresh serum were able to remove far greater amounts of complement than the same cells to which antiserum and complement were added simultaneously. Sera containing the following antibodies were incubated with cells carrying the appropriate antigen with the simultaneous addition of fresh serum: anti-D, -Lea, -Leb, -Fya, -Jka and immune anti-A. Parallel tests were set up in which the complement was added after antigen-antibody combination had taken place. Heat
inactivated antisera were used throughout. Following incubation all tubes were centrifuged and the supernates removed and retained for two-stage testing.

1. A scoring method was used to measure the total complement in the original fresh serum and in the supernates, using a sheep anti-sheep cell hemolytic system.

2. Residual antibody values were obtained by titrating the supernates in fresh serum and comparing their sensitizing ability with the original unabsorbed antisera.

Table I

Antibody-Complement Uptake Values Typifying the Respective Antisera Studied

From table I it can be seen that when Lea + cells are incubated with anti-Lea, the amount of antibody absorbed from the serum is very small. The low residual complement score indicates that a large amount of complement is capable of being attached to the weakly sensitized cell surface.

By contrast, all samples of anti-D showed considerable reduction of antibody content with little or no effect on the serum complement. Antibodies in the Duffy and Kidd systems varied with individual sera, some following the Lewis pattern, others behaving in a similar manner to anti-D. Immune anti-A seems to hold an intermediate position. These results provide a possible explanation of the inability of anti-human serum containing only anti-gamma-components to detect anti-Lea on the cell, for the minute amounts of attached antibody seem to be necessary only to adsorb serum complement which is then recognized by the nongamma-antibodies in the antiglobulin sera. Greater uptake of complement by a cell sensitized prior to the addition of fresh serum was a consistent finding.

The problem of weakly positive or negative direct Coombs tests on cord cells from babies with clinical signs of hemolytic disease, presented itself for investigation. A study of these cases was made using the traditional direct Coombs test in parallel with a complement requiring two-stage direct Coombs procedure. All cord-bloods examined in our series had been submitted to the Consultation Service of the Ortho Research Foundation and gave negative results in the direct Coombs tests by routine methods. Three healthy non-immunized males were chosen as complement donors, primarily on the basis of ABH and Lewis secretor status.

Donor I was group A, Le(a-b-), a secretor of A substance and a nonsecretor of Lea or Leb substance. Donor II was group A4B, Le(a+b~), a nonsecretor of A and the Serological Diagnosis of Hemolytic Disease of the Newborn 521
B substance, but a secretor of Lea substance. Donor III was group A\(\text{~j}\)B, Le(a-b +), a secretor of A, B and Lewis substances. Freshly drawn blood was allowed to clot at room temperature for one hour and the serum removed from the clot. The serum was used immediately or stored frozen at -70 C. The anti-human serum used throughout this phase of our study had been standardized to react optimally with cells sensitized with anti-D. After neutralization with gamma-globulin, it still showed undiminished activity against red cells sensitized in the presence of fresh serum with the so-called non-gamma-globulin type antibodies.

Cord cells were throughly washed with saline until free from hemoglobin, serum or traces of anticoagulant which would be anticomplementary. Each sample was then made up to a 4 % suspension in physiological saline containing 0.1 % magnesium sulphate. Two drops of these cell suspensions were added to each of four tubes, the first three containing two drops of fresh (or freshly frozen) serum from complement donors I, II, and III, respectively. The fourth tube containing pooled, inactivated human serum served as a control. After mixing, all tubes were incubated for 15 minutes at 37 C. Each tube was then carried through the normal procedure of the direct Coombs test. The following table shows some typical results obtained.

Table II
Cord Cell Study Using the Traditional Direct Coombs Test and the Two Stage Direct Coombs Test

A-B incompatibility could be incriminated in several of the cases in which the traditional direct Coombs test remained negative, but the more sensitive two-stage procedure produced frankly positive results. A study of the maternal sera in three cases revealed the presence of a very weak anti-D. Perhaps the most surprising feature of this study was the finding that a number of cord specimens showed positive two-stage direct Coombs tests although blood group antibodies could not be demonstrated in the maternal sera when tested against panels of known cells which included (wherever feasible) the fathers cells. The possibility of an antibody directed against fetal hemoglobin was also considered and for this reason the maternal sera were examined by various methods against cord-cell hemoglobins and crystalline hemoglobin F. The Ouchterlony double-gel diffusion plates4 and the tanned cell hemagglutination technique of Boy den3 were consistently negative. However, in one case a reaction specific for hemoglobin F was obtained by the interfacial ring test. The infants in each case showed clinical manifestations of hemolytic disease, some requiring replacement or supplementary transfusions. We have as yet failed to clarify the nature of this antibody, other than establishing its existence.
It was not possible to substitute guinea pig complement for human complement in the two-stage direct Coombs test contrary to our findings in the complement consumption studies in which the two were interchangeable.

References


High Molecular Weight Antibodies

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It has long been known that normal human serum contains small amounts of a high molecular weight substance with sedimentation rate of about 19 Svedberg units. Simultaneous separation on the same starch block of a normal human serum and of the heavy molecular weight component of the same serum, obtained by repeated cycling in the preparative ultracentrifuge, discloses that the heavy component is comprised of two peaks of different electrophoretic mobility, and also discloses that the 19 S gamma-globulin has an electrophoretic mobility slightly faster than that of the bulk of the gamma-globulin. Recently it has become apparent that 5-10 per cent of the total gamma-globulin of normal serum consists of this 19S component (molecular weight around one million). The other 90-95 per cent consist of material with sedimentation of 7 Svedberg units, implying a molecular weight of approximately 160,000. Gamma-globulin intermediate in molecular...