PROOF OF EXISTENCE OF THE HUMAN Le(c+) BLOOD GROUP OBTAINED WITH GOAT IMMUNE SERUM

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Diagnostic anti-Le^c sera with incomplete antibodies in titers of 1:8-1:16 have been prepared from the serum of a goat immunized over a period of 18 months with 210 ml of boiled saliva of the OLe($a-b-$) nonsecretor group. The sera have been tested by Race, Sanger, and Tippett in London.

KEY WORDS: blood groups.

After the writer [1] had discovered the Le(d+) human blood group, on the basis of analysis of the Grubb–Ceppellini concept, it was postulated [1] that erythrocytes of nonsecretors of the Le($a-b-$) group would also contain a specific factor.

The first evidence of the existence of this hypothetical factor, described as Le^c, was obtained 2 years later [3] by means of an accidentally discovered human serum. Meanwhile, the writer's efforts over a period of 3 years to prepare a heteroimmune anti-Le^c serum were crowned with success only in 1972.

The object of this investigation was to prepare a heteroimmune anti-Le^c serum in order to study the antigenic activity of the Le^c factor and the possibility of preparing diagnostic sera.

EXPERIMENTAL METHOD

Considering the special features of the Lewis system [2], boiled saliva from two persons of group OLe($a-b-$)se was used as the antigen. Five rabbits, 1 sheep, and 3 goats (in succession) were immunized for a long period with negative results. Anti-Le^c antibodies were produced in goat No. 4, which received 3 cycles of immunization in the course of 18 months. Each cycle consisted of 6-8 injections of antigen (2 ml saliva intramuscularly, 8 ml intravenously) at intervals of 1-2 days. Altogether 210 ml saliva was injected. During the third cycle, the goat frequently developed shock. The immune serum was not heated but was kept for 3 months at 4°C. After dilution 15 times with physiological saline, it was absorbed repeatedly (4-6 times) with trypsinized group O erythrocytes, with control of all the serological characteristics before each absorption. The serum of batch 1 was absorbed by group Le($a+$) erythrocytes, that of batch 2 by erythrocytes of group Le(d+), and that of batch 3 by erythrocytes of group Le(b+). For 1 volume of serum, 0.5-0.64 volume of erythrocyte residue was used. The time taken to prepare the sera was 24-66 days. When the sera had reached specificity for group OLe(d+), side-antibodies against Le^a and Le^d antigens were not removed because of the low titer of anti-Le^c antibodies in the resulting sera (1:8-1:16). An anti-Le^c serum with complete antibodies (titer 1:4) was also successfully prepared from the serum of goat No. 4 after its absorption with native erythrocytes of groups Le($a+$), Le(b+), and Le(d+).

EXPERIMENTAL RESULTS

The anti-Le^c sera of all batches, used in the trypsin test [1], differentiated human group O blood within the limits of group Le($a-b-$): Group Le(c+) erythrocytes were agglutinated but group Le(d+) erythrocytes

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were not. Activity of the sera of batches 1 and 2 was completely preserved after 11 months, but that of the sera of batch 3 disappeared almost completely.

In March, 1973 the sera of all three batches were sent to the MRC Blood Group Research Unit in London. They were tested by Race, Sanger, and Tippett in the papain test parallel with an anti-Le^c serum found by Gunson and Latham [3] and the results coincided completely: Group Le($a-b-$) of nonsecretors were agglutinated, and erythrocytes of group Le($a-b-$) secretors of H substance were not agglutinated. With the present writer's agreement, these results were published by Race and Sanger in their book [5].

Double proof of the existence of the Le^c antigen (with the aid of human and goat immune sera) confirms the validity of the scheme of the Lewis system [1, 2, 4]. The results give promise of the systematic preparations of anti-Le^c sera, whereas the chance of discovery of such human sera has been estimated by the writer to be less than 1:100,000. Anti-Le^c isoantibodies can evidently be the cause of immunological conflicts (blood transfusion, hemolytic disease of the newborn).

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