Certain Immunological Relationships between Human Erythrocytes and Selected Mouse Cells. (21154)

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In the course of an immunological study of cells obtained from the mouse, it was observed that antiserums prepared against the mouse cells were capable of agglutinating human erythrocytes. Landsteiner(1) had previously observed the agglutination of mouse erythrocytes by antihuman B serum and later Gorer(2) was able to differentiate at least 2 antigenic types of mouse corpuscles by means of normal anti-human A serum. Because our anti-mouse cell serums were capable of agglutinating A, B, and O human erythrocytes, it seemed of interest to investigate the reactions of these anti-mouse cell serums with human red blood cells.

Materials and Methods. Erythrocytes of humans and of C3H and Webster mice were obtained from citrated whole blood. These cells were washed 3 times with veronal buffered saline* and the contaminating leucocytes were removed, in so far as possible, by pipetting, following each centrifugation.

Leucocytes were obtained from Webster mice following intraperitoneal injection of 5 ml of a 1% suspension of Aleuronat in Locke's solution. After a period of 27 hours, the mice were sacrificed and 5 ml of saline was injected intraperitoneally. The abdomen was massaged, the peritoneal cavity opened by a mid-line incision, and the suspended leucocytes were removed with a capillary pipette. The cells so obtained were placed in a tube containing sufficient 10% sodium citrate to give a final concentration of 3%. The cell suspension was mixed and allowed to stand at 25°C for 30 minutes. The cells which remained suspended were removed and washed 3 times in veronal buffered saline. After each centrifugation during the washing procedure the contaminating erythrocytes, which are aggregated by centrifugation, were removed by a capillary pipette.

The L-strain of mouse fibroblasts was isolated by Earle(3) from the C3H mouse and later was developed into a pure cell strain(4). These cells were grown in 3-ounce prescription bottles in a medium containing 50% Hanks's balanced salt solution, 10% chick embryo extract, and 40% horse serum or human ascitic fluid. The cells were harvested by shaking them free from the glass surface. After being centrifuged and resuspended in veronal buffered saline the cells were allowed to stand at 25°C for 30 minutes; the suspended cells were removed and washed 3 times in veronal buffered saline.

All the above cells were used in serological tests on the same day they were obtained.

To standardize the erythrocyte suspensions the cells in a 1 ml aliquot were lysed by the addition of distilled water to a volume of 10 ml following which readings were made on the lysed suspension in the Klett-Summerson colorimeter. Direct turbidimetric readings were made on the L-strain cells and the leucocytes. Calibration curves were prepared for each of the cell types by plotting %-cell concentration (from packed cell volume) against Klett readings. Fairly reproducible cell suspensions could be prepared from these curves.

Antiserums for the 3 types of mouse cells were prepared in rabbits by giving a series of 3 weekly injections of 1 ml of a 5 to 10% suspension of the cells for a period of 3 weeks; intraperitoneal, subcutaneous, and intravenous injections were given alternately. In certain instances 1 to 3 additional booster injections were given. The animals were bled 10 days after the final injection.

Anti-human A and B serums were obtained

* 85.0 g NaCl, 5.75 g 5,5-diethyl barbituric acid, 3.75 g sodium 5,5-diethyl barbiturate. Dissolve the acid in 500 ml hot water, add to the solution of the other components, cool and make up to 200 ml with water. Each day dilute accurately 1 part up to 5 with water; the pH of the diluted buffer should be 7.3 to 7.4.

from individuals of type A and type B blood groups.

Agglutination tests were carried out in 10 x 75 mm tubes in which 0.1 ml of the antigen suspension (0.5% for erythrocytes and 0.75% for the leucocytes and the L-strain cells) was added to 0.2 ml of the serum dilution. The tests were incubated at 37°C for 1 hour, then centrifuged at 1000 rpm for 2 minutes. The tubes then were shaken vigorously and the agglutination was read with the aid of a microscope lamp. The tests were checked again after standing at 25°C for a period of 2 to 3 hours. There was usually no change in titer during this second incubation period; however, there was a tendency for the weak reactions to become stronger. The highest dilution of the serum showing discrete clumping of the cells, as compared to the control, was taken as the end point.

Antiserums were absorbed 3 times, with a 5% absorbing dose of cells, for 30 minutes each time, at 25°C. Such treatment was sufficient to remove all the antibody from a 1:10 dilution of the homologous antiserum.

The absorption procedure was as follows: A sufficient amount of washed cells to give a 5% cell concentration in a volume of 1.5 ml was added to a McNaught sedimentation tube. This tube was centrifuged for 15 minutes at 2300 rpm in a Clay-Adams Safety-Head centrifuge and the supernate was carefully removed and discarded. One and one-half ml of an appropriate dilution of the antiserum to be absorbed was added and the cells were resuspended. After a 30-minute incubation period at 25°C the suspension was centrifuged and the antiserum was removed and absorbed twice more in a similar manner. It was found that a volume correction for the saline contained in the absorbing mass was not necessary.

TABLE I. Agglutinating Titers of Anti-Mouse Cell Serums with Human RBC's.

Antiserum	Antigens A	B	0 pos.	0 neg.
Webster-RBC	32	32	8	8
C3H-RBC	8	32	8	4
Webster-WBC	16	16	8	4
L-strain	4	8	2	0
Nor. S.	0	0	0	0

TABLE II. Agglutinating Titers of Anti-Mouse Cell Serums with Human RBC's after Absorption with Group A Rh Pos. Human RBC's.

Antiserum	Antigens A	B	0 pos.	0 neg.
Webster-RBC	0/32*	32/32	0/8	0/8
C3H-RBC	0/8	32/32	0/4	0/2
Webster-WBC	0/16	16/16	0/8	0/4
L-strain	0/2	8/8	0/0	0/0

* Numerator = Titer of absorbed serum. Denominator = Titer of unabsorbed serum.

Unabsorbed serums were tested at the same time as the absorbed serums to eliminate the variation in titer due to differences in serological reactivity of the cell suspensions.

Experimental. Table I gives the results of agglutination of human erythrocytes with anti-mouse cell serums. It may be noted that group A,B,O Rh positive and O Rh negative human erythrocytes were agglutinated by each of the antiserums (with the exception of L-strain antiserum and O Rh negative cells), but not to the same titer. Serum from 3 nonimmunized rabbits did not agglutinate the human cells tested.

TABLE III. Agglutinating Titers of Anti-Mouse Cell Serums with Human RBC's after Absorption with Group B Rh Pos. Human RBC's.

Antiserum	Antigens A	B	0 pos.	0 neg.
Webster-RBC	32/32*	0/32	0/8	0/8
C3H-RBC	8/8	0/32	0/8	0/4
Webster-WBC	16/16	0/16	0/8	0/4
L-strain	2/2	0/8	0/0	0/0

* Numerator = Titer of absorbed serum. Denominator = Titer of unabsorbed serum.

It was of interest to determine whether more than one antibody was responsible for the agglutination of the human erythrocytes. This was done by absorption of the anti-mouse cell serums with human erythrocytes of the different blood groups. The results are given in Tables II to IV.

Table II indicates that anti-mouse cell serums absorbed with group A human erythrocytes failed to react with Group A, group O Rh positive, and group O Rh negative erythrocytes, but were still able to react

TABLE IV. Agglutinating Titers of Anti-Mouse Cell Serums with Human RBC's after Absorption with Group B Rh Neg. Human RBC's.

Antiserum	Antigens A	B	0 pos.	0 neg.
Webster-RBC	32/32*	0/32	0/8	0/8
C3H-RBC	8/8	0/32	0/8	0/2
Webster-WBC	16/16	0/16	0/8	0/8
L-strain	2/2	0/8	0/0	0/0

* Numerator = Titer of absorbed serum. Denominator = Titer of unabsorbed serum.

with group B cells. Similarly (Table III), absorption with group B cells removed antibodies reactive with group B and group O cells leaving antibodies reactive with group A cells. Table IV indicates that absorption with Rh negative cells removed antibodies against group O Rh positive as well as group O Rh negative cells.

To substantiate the relationships between the mouse cells and the human erythrocytes, cross-reaction tests between the mouse cells and isoantibodies were made. The results (Table V) indicate that each of the 4 types of mouse cells were agglutinated by both the anti-A and anti-B serums.

Discussion. It was found that antiserums prepared against selected mouse cells were capable of agglutinating human erythrocytes of the various blood groups indicating an antigenic relationship between human erythrocytes and the mouse cells.

TABLE V. Agglutination Titers of Mouse Cells with Human Iso-Anti-A and Iso-Anti-B Serums.

Anti-serum	Antigens Webster RBC	C3H RBC	Webster WBC	L-strain	Human RBC Gr. A	Gr. B
Iso-A	8	4	4	16	32	—
Iso-B	16	4	8	8	—	8

From the results of absorption with human erythrocytes, it was concluded that the anti-mouse cell serums contain antibodies specific for the group A and group B substances. Further evidence of the presence of both of the group substances in the individual mouse cell types was obtained from the aggutination of the mouse cells by either A or B isoantibody.

There was evidence of a further relationship between human erythrocytes and mouse cells independent of group A, group B, or Rh antigens. This relationship was indicated when antibodies against the group O cells were absorbed by either A or B cells regardless of whether they were Rh positive or negative.

Summary. 1. It was found that erythrocytes and leucocytes from Webster mice and erythrocytes and L-strain fibroblasts from C3H mice contain antigens related to A and B human blood group substances. 2. There was an indication of the presence of further immunological relationships between human erythrocytes and mouse cells independent of group A and B substances and in the Rh factor.

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