Human Blood Group Influence on Reovirus Hemagglutination Titers. (29130)

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Preliminary observations indicated that reovirus hemagglutination (HA) titers varied with the blood group of the erythrocyte suspension used. Although Group O erythrocytes are usually chosen when human cells are to be used in viral HA(1,2,3,4,5,6) or hemagglutination-inhibition (HI)(2,4,7) tests, the need to use O cells exclusively in HA tests has not been established. If the blood group substance on human erythrocytes is the site of the erythrocyte-reovirus linkage in HA tests, as suggested by Lerner, Cherry, and Finland(1), then virus HA titer variations would be expected according to the blood group of the erythrocytes used. This study compares the effect of blood groups A, AB, O, and B on reovirus HA titers.

Materials and methods. The virus strains used were: (a) Reovirus Type 1 "Lang," (b) Reovirus Type 2 "D5 Jones," and Reovirus Type 3 "Abney." Dr. A. B. Sabin supplied the original of these virus materials to the Communicable Disease Center. An additional sample of Reovirus Type 2 supplied by the American Type Culture Collection (ATCC) was passaged here for use in this study and is designated by (ATCC) after the name.

One hundred eighty-seven specimens of oxalated whole human blood were obtained from a large municipal hospital.* Approximately equal numbers of adult males and females, Negro and white, were included. Blood groups were determined in this laboratory. The first 77 samples were tested only with Reovirus Type 2 (ATCC); an additional 110 samples were tested with Reovirus Type 2 (ATCC) and with Reovirus Type 2 and Type 3; and the last 67 samples of the 110 were also tested with Reovirus Type 1.

* We are indebted to Miss Jewell Mitchell of Grady Memorial Hospital, Atlanta, for obtaining blood specimens and patient data for our use.

Each oxalated blood sample was washed 3 times in 8 to 10 volumes of sterile dextrose-gelatin-veronal (DGV) solution(8). A wash consisted of centrifugation at approximately 540 $\times$ *g* for 5 minutes. The supernatant fluid was removed, and the cells were resuspended in fresh DGV solution. The cells were packed on the third wash by centrifugation for 10 minutes as before, after which a 10% suspension (v/v) of the cells in fresh DGV was made for storage. Before use in the HA test a portion of each 10% suspension was recentrifuged for 10 minutes at 540 $\times$ *g*. An 0.8% red cell working suspension was then made in 0.85% NaCl.

The hemagglutination test was essentially the procedure of Rosen(2). Serial 2-fold dilutions of virus in 0.4 ml amounts (or 0.85% NaCl as a cell control) and 0.2 ml of 0.8% human red cell suspension were added in each 12 $\times$ 75 mm tube. Blood samples representing each major blood group were used each time HA tests were performed. The tests were incubated at room temperature for one hour prior to recording titers. Titers are expressed as the reciprocal of the initial dilution of the virus at the hemagglutination endpoint.

Red cell suspensions prepared from Group A and Group AB blood samples are referred to as "A-factor" suspensions, while suspensions of Group O and Group B cells are hereafter referred to as non-A suspensions.

Results. Figure 1 illustrates differences among the blood groups in the hemagglutination titer of each reovirus. A geometric mean titer (GMT) of 78 was obtained when 67 red cell suspensions were tested with Reovirus Type 1. The GMT with A-factor red cell suspensions was 121 while the GMT with non-A bloods was 55.

Although the results are not as pronounced with Reovirus Type 2, differences between A

TABLE I. Frequency Distribution of Hemagglutination Titers of Reovirus Types 1, 2 and 3 with Different Human Blood Groups.

HA titer	Reovirus Type 1 Blood group A	AB	O	B	All
160	15	3	0	0	18
80	9	3	14	5	31
40	0	0	6	10	16
20	0	0	2	0	2
Total	24	6	22	15	67

HA titer	Reovirus Type 2 (ATCC) Blood group A	AB	O	B	All
1280	1	0	1	0	2
640	61	15	28	18	122
320	6	2	39	15	62
160	1	0	0	0	1
Total	69	17	68	33	187

HA titer	Reovirus Type 2 Blood group A	AB	O	B	All
320	4	0	0	0	4
160	32	9	22	11	74
80	8	0	16	8	32
Total	44	9	38	19	110

HA titer	Reovirus Type 3 Blood group A	AB	O	B	All
160 or 320	11	3	6	1	21
80	19	5	16	7	47
40	12	1	9	10	32
20 or less	2	0	7	1	10
Total	44	9	38	19	110

and non-A bloods were observed. Reovirus Type 2 (ATCC) had a GMT of 508 with 187 blood specimens, but the GMT was 595 if only blood samples containing the A factor were considered. Non-A suspensions had a mean of 445.

Another Reovirus Type 2 tested with fewer samples yielded results similar to the ATCC strain. The GMT with 110 red cell suspensions was 134. When the 53 A-factor bloods were considered separately the GMT was 152; however, the GMT was 119 with the 57 non-A red cell suspensions.

Reovirus Type 3 was also titrated with 110 erythrocyte suspensions yielding a mean HA titer of 65. A GMT of 78 was obtained with A-factor suspensions and a mean of 56 with non-A suspensions.

The frequency distribution of HA titers of Reovirus Types 1, 2, and 3 with different blood groups is presented in Table I.

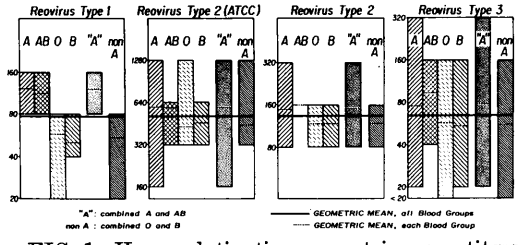

FIG. 1. Hemagglutination geometric mean titers and titer ranges of reovirus types 1, 2, and 3 with human A, AB, O, and B red blood cells.

Discussion. Evidence is presented of small but significant differences in the hemagglutinating reactivity of reoviruses with the 4 human ABO blood groups. The receptor sites on erythrocytes for reovirus attachment are present on cells of all human blood groups, but cells with the group A factor contain either more receptor sites or they are more accessible. The GMT of Reovirus Type 1 demonstrates this: 123 (Group A), 113 (Group AB), 58 (Group O), and 50 (Group B). The GMT of Reovirus Type 2 further illustrates this: 596 (Group A), 590 (Group AB), 434 (Group O), and 467 (Group B). Although the GMT were lower when Reovirus Type 2 (seed) was used, the higher HA titers again occurred with Group A and Group AB red cells. When Reovirus Type 3 is the test organism the GMT is 75 (Group A), 93 (Group AB), 57 (Group O), and 54 (Group B). These titer increases, related to A-factor containing erythrocytes, though small are statistically significant by chi square and analysis of variance tests at the .01 probability level for Reovirus Types 1 and 2. Reovirus Type 3 showed significant titer increase with A-factor bloods at the .05 probability level by analysis of variance and at the .06 level by chi square test.†

† We thank Mrs. Rebecca Plender for statistical analysis.

Differences in cell susceptibility to HA have been observed in other virus-red cell systems among individuals of one donor species. A slight variation was noted when chicken red blood cells were agglutinated by influenza virus(9), but a 4-fold variation occurred with mumps virus(10). These are similar to our findings that individual differences occur when human erythrocytes are agglutinated by the reoviruses. Not only do variations occur within a blood group, but significant differences occur from group to group.

Although reoviruses agglutinate A-factor erythrocytes at a higher virus dilution than non-A erythrocytes, this is not necessarily due to the Group A substance on the red cell, since erythrocytes of other blood groups are agglutinated also. Whether O-α-N-acetyl-D-galactos-aminoyl- (1→3) galactose, the substance most clearly associated with Group A specificity, as determined by inhibition of human A-isoagglutinins(11), would inhibit HA of reovirus remains to be tested. Although neuraminic acid of human red cells participates in myxovirus attachment as do sulfhydryl receptors, these same receptors are not implicated in reovirus attachment in HA tests(1,5); except that neuraminic acid is involved in attachment when Reovirus Type 3 is reacted with ox and sheep erythrocytes(12,13). Sulfhydryl groups on the viruses, rather than on the red cells, influence attachment in reovirus HA tests(1,12,13). Interaction between a glucoprotein on the surface of human erythrocytes and a glucoprotein, containing sulfhydryl groups, on the surface of the reoviruses results in hemagglutination, but the essential bond between virus and erythrocyte may not be through a sulfhydryl linkage(1). The factor or factors involved in higher HA titers when A-factor erythrocytes are reacted with reoviruses remains to be clarified.

Summary. A total of 187 human blood specimens were tested with one or several representative viruses of the reovirus group. The titers obtained with human erythrocytes containing the A grouping factor were slightly but significantly higher than those obtained with Group O or B cells.

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Evaluation of the Fibrinolytic State After Administration of Urokinase. (29131)

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During fibrinolysis, plasminogen(1,2), which is a constituent of human and animal blood, is converted into an active enzyme which lyses fibrin clots. This conversion occurs through the agency of so-called "fibrinolytic activators." A fibrinolytic activator,