FURTHER CHARACTERIZATION OF THE ADENOVIRUS ERYTHROCYTE RECEPTOR-MODIFYING FACTOR*

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Multiplication of certain adenoviruses is known to be accompanied by formation of several factors which possess virus-specific antigens and which are distinct from the infectious particles; these are the complement-fixing antigen or antigens (1), and the cell-detaching factor (2), which is presumably identical with the early cytopathogenicity factor (3, 4), and the C antigen (5). In addition, preliminary evidence (6) has been presented which indicates that infection of tissue cultures with several of the adenoviruses results in formation of a factor demonstrable by its capacity to reduce the agglutinability of human group O erythrocytes by 3 of the 5 adenoviruses which hemagglutinate these cells (7).

The purpose of this report is to present in greater detail the biologic, serologic, and chemical characteristics of the erythrocyte receptor-modifying factor.

Materials and Methods

Tissue Cultures.—HeLa and Hep. 2 tube cultures were obtained from Microbiological Associates, Inc., Bethesda, Maryland. The cultures were handled and maintained as previously described (8). The maintenance medium for HeLa cell cultures consisted of 70 parts medium 199 (9), 25 parts tryptose phosphate broth, and 5 parts chicken serum. The maintenance medium for Hep. 2 cell cultures consisted of 95 parts Eagle's basal medium (10) and 5 parts chicken serum. The chicken serum was heated at 56°C for 30 minutes before use. In addition, the media contained penicillin, streptomycin, and nystatin in a final concentration of 250 units, 250 μg, and 50 units per ml, respectively. Cultures received a fresh fluid change once weekly.

Viruses.—The prototype adenovirus strains of types 1 through 18 (11) were used; type BAR-2 was received from Dr. Leon Rosen (7). For the preparation of adenovirus stocks, tube cultures of HeLa cells were inoculated with 0.15 ml of a virus dilution which produced complete cytopathic effects within 2 to 4 days; 2 days after cytopathic effects were complete, the cultures were frozen, later thawed, and clarified by centrifugation at 1500 RPM for 15 minutes. Fluids were then stored in glass vials and frozen at −20°C until used. Uninoculated HeLa cell cultures were processed in the same manner for use as control fluids.

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Infectivity Titrations.—Titrations for infectivity were done by inoculating 1 ml of serial 10-fold dilutions of virus into each of 2 Hep. 2 cultures from which the maintenance medium had been previously removed. The cultures were incubated at 37°C in a stationary horizontal position and observed as long as uninoculated control cultures remained in satisfactory condition. Cultures developing any definite characteristic cytopathic changes were considered positive; titers were calculated according to the method of Reed and Muench (12).

Erythrocyte Suspensions.—Human group O red cells were obtained from 2 donors. The blood was placed in Alsever's solution, and the erythrocytes were separated and washed by three cycles of centrifugation at 2000 RPM for 5 minutes with resuspension in 3 volumes of saline solution (0.85 per cent NaCl). After the final centrifugation, the packed cells were resuspended in Alsever's solution and stored at 4°C. Before use, the red cells were washed once with 3 volumes of saline. Fresh human cells were collected every 10 days.

Hemagglutination Tests.—Tests were done in 12 × 75 mm tubes; serial 2-fold virus dilutions were prepared in physiologic saline, using a fresh pipette for each dilution. 0.2 ml of a 1 per cent erythrocyte suspension was added to 0.8 ml of virus dilution, and the tubes were thoroughly shaken. Tests with human erythrocytes were held at 37°C for 60 to 90 minutes. The highest virus dilution showing approximately a 50 per cent pattern of sedimentation (13) was the end point of the titration.

Erythrocyte Receptor Modification Test.—In the standard test for demonstrating erythrocyte receptor modification 0.25 ml of packed human group O erythrocytes were added to 4 ml of undiluted virus and HeLa control fluids and thoroughly mixed. The mixtures were held at 22°C for 2 hours and the tubes shaken every 15 minutes during the incubation period. The red cells then were separated by centrifugation at 1500 RPM for 5 minutes, washed in 2 saline changes of 7 ml each, and finally resuspended as a 1 per cent suspension in saline. These treated cell suspensions were used to determine hemagglutination titers of adenovirus types 10, 13, and BAR-2 (test viruses). A test was considered positive for erythrocyte receptor modification if there was an 8-fold or greater reduction in titer of test virus hemagglutinin with the virus-treated cells as compared with the titer obtained with HeLa control fluid–treated cells.

Tests for Inhibition of Erythrocyte Receptor–Modifying Activity by Rabbit Antisera.—Rabbit immune sera for adenoviruses and HeLa control fluid were prepared as described by Rowe *et al.* (1). The sera were adsorbed with human group O erythrocytes before use by adding 0.1 ml of packed erythrocytes to 2 ml of undiluted serum. The mixture was kept at 4°C for 18 hours, and the red cells were removed by centrifugation at 1500 RPM for 20 minutes. Before use, a 1:20 dilution of the adsorbed serum was tested for the presence of red cell agglutinins; on occasion, it was necessary to repeat the adsorption procedure.

The inhibition test was done as follows: 0.2 ml of undiluted serum (inactivated at 56°C for 30 minutes) was added to 4 ml of a virus dilution containing 1.5 logs of erythrocyte receptor–modifying activity. Controls for each test consisted of a titration of the erythrocyte receptor–modifying activity of each virus fluid, cell treatment with mixtures of HeLa control fluid and each of the antisera used in the test, and cell treatment with mixtures of each virus and HeLa control fluid rabbit antiserum. After the mixtures were incubated at 37°C for 60 minutes, 0.25 ml of packed erythrocytes were added. Resuspended red cell suspensions were prepared and assayed as described above. Complete inhibition of erythrocyte receptor–modifying activity by serum was considered positive neutralization.

EXPERIMENTS AND RESULTS

General Characteristics of the Erythrocyte Receptor Modification Reaction

Prevalence of Erythrocyte Receptor–Modifying Activity Among Adenoviruses.—Table I presents the results of experiments to determine the prevalence of

erythrocyte receptor–modifying activity among the adenovirus serotypes which lack the capacity to agglutinate human group O erythrocytes. Also, a preparation of type 10, from which the hemagglutinin had been removed by adsorption with human O erythrocytes, was tested. In each experiment, a type 1 or type 15 suspension was included as a positive control, and the results of the repeat assays are given in the table. Only adenovirus type 1, 2, 4, and 15 suspensions

TABLE I

Agglutinability of Human Group O Erythrocytes Pretreated with Adenoviruses

Treating virus	Hemagglutination titer with HCF*-treated erythrocytes / Hemagglutination titer with virus-treated erythrocytes				
	Ad.‡ 8	Ad. 9	Ad. 10	Ad. 13	Ad. BAR-2
Ad. 1	2; 2; 1; 1; 1	1; 2; 2; 2; 1	16; 64; 64; 32; 64	16; 32; 32; 32; 32	16; 16; 32; 16; 32
Ad. 2	2	1	32	32	32
Ad. 3	1	1	1	1	1
Ad. 4	1	1	16	16	8
Ad. 5	2	1	2	1	1
Ad. 6	1	1	1	2	1
Ad. 7	2	1	1	1	1
Ad. 7a	1	2	1	2	1
Ad. 10§	1	1	1	ND‖	1
Ad. 11	1	1	1	1	1/2
Ad. 12	1	1	1	1	1
Ad. 14	1/2	1	1	2	1
Ad. 15	1; 2	1; 1	16; 32	8; 8	16; 32
Ad. 16	1	1	1	1	1
Ad. 17	1	1	1	1	1
Ad. 18	1	1	1	1	1

* HeLa control fluid.

‡ Adenovirus.

§ Suspension adsorbed 5 times with human O erythrocytes to remove hemagglutinin.

‖ Not done.

demonstrated the property of markedly reducing the agglutinability of erythrocytes for type 10, 13, and BAR-2 hemagglutinins. The reduction in hemagglutinin titer of the test viruses varied from 8- to 64-fold, the greater reduction being produced by type 1 and 2 virus fluids. In no instance were significant decreases observed for types 8 and 9.

To determine if production or lack of production of the erythrocyte receptor–modifying factor was characteristic for the adenovirus serotypes, a number of additional strains of types 1, 2, 3, and 5 were tested. Freshly isolated strains were obtained from Dr. Robert Parrott of the Children's Hospital Research Center, Washington, D. C.; these consisted of 5 strains of type 1, 5 strains of

type 2, 4 strains of type 3, and 4 strains of type 5, all in second or third passage in HeLa cells. All of the type 1 and 2 strains, and none of the type 3 and 5 strains produced modification of erythrocyte receptors. However, all of the type 5 strains resulted in a 2-fold reduction of test virus hemagglutinin.

Different lots of HeLa cultures infected with adenoviruses produced relatively constant titers of erythrocyte receptor-modifying activity for each serotype. The titers of type 1 and 2 suspensions were 1:100 to 1:320, type 4 titers were usually 1:32, and type 15 titers were from 1:10 to 1:32.

Dose-Response Relationship.—The pattern of relationship of dose of treating virus (adenovirus type 1) to the degree of receptor modification is shown in

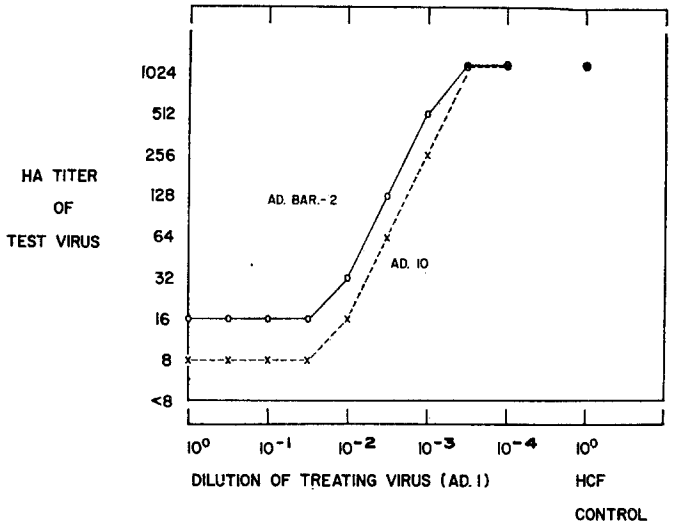

FIG. 1. Relation of dose of treating virus to HA titers obtained with test viruses.

Fig. 1. Through a portion of the dilution range the relation was approximately linear, but a point was reached at which increase in dose of treating virus produced no further decline in agglutinability. This pattern was also seen with the other adenovirus types which produced the erythrocyte receptor-modifying factor; only rarely was agglutination prevented completely. In contrast, treatment of human O cells with the receptor-destroying enzyme (RDE) of *Vibrio cholerae* rendered them inagglutinable by the larger doses of adenovirus types 10, 13, and BAR-2.

The slope of the linear portion of the curve varied with different batches of red cells, but tended to be constant with a given batch; the variation was primarily steeper slopes than those shown in Fig. 1. In general, the various alterations of experimental conditions and treatments of the erythrocyte receptor-modifying factor reported here, did not alter the form of the dose-response relationship.

Effect of Temperature and Time on Erythrocyte Receptor Modification.—Since the initial studies of adenovirus receptor modification were carried out with treatment of red cells at 22°C for 2 hours, this exposure was used as the standard method in subsequent work. To determine the effect of variation in temperature and duration of red cell treatment, experiments were done in which one factor was varied and the other maintained constant as in the standard treatment conditions.

Table II presents the results of an experiment which tested the effect of temperature during a constant incubation period on the erythrocyte receptor-modifying activity of adenovirus type 1 and 15 suspensions.

Erythrocytes were added to triplicate sets of virus dilutions and undiluted HeLa control fluid, and the sets of mixtures were placed at 2°C, 22°C, and 37°C respectively; test fluids and red cells had been brought to corresponding temperatures before mixing. After incubating for 2 hours, the mixtures were chilled and erythrocyte suspensions were prepared under temperature conditions not exceeding 4°C.

TABLE II

Effect of Temperature on the Degree of Erythrocyte Receptor Modification During a Constant Time Period

Treating virus	Highest dilution of treating virus producing ≥8-fold reduction of hemagglutination titer of test viruses Ad. 10 and Ad. 13					
	Mixtures held at 2°C		Mixtures held at 22°C		Mixtures held at 37°C	
	Ad. 10	Ad. 13	Ad. 10	Ad. 13	Ad. 10	Ad. 13
Ad. 1	$10^{1.5}$	$10^{1.5}$	$10^{2.5}$	$10^{2.5}$	$10^{3.0}$	$10^{3.0}$
Ad. 15	$10^{0.5}$	10^{0}	$10^{1.5}$	$10^{1.0}$	$\geq 10^{1.5}$	$10^{1.5}$

As shown in Table II, erythrocyte receptor-modifying activity increased with temperature of the virus–red cell mixture; the erythrocyte receptor-modifying titers obtained were increased approximately 30-fold over the temperature extremes.

The following experiment was done to determine the effect of length of exposure on the degree of receptor modification at a constant temperature.

Red cells were added to replicate sets of adenovirus type 1 dilutions as well as to undiluted HeLa control fluid and held at 22°C; the materials were at 22°C at the time of admixture. At various time intervals, a set of the treatment mixtures was placed in a chilled centrifuge head and centrifuged at 1500 RPM for 3 minutes in a refrigerated centrifuge; suspensions of washed erythrocytes were then prepared. The results of this experiment are shown in Table III.

After only 1 minute's exposure at 22°C the 10^{-1} dilution of adenovirus type 1 suspension had reduced the agglutinability of the cells by a significant amount.

Contact for 2 hours was necessary for the $10^{-2.5}$ dilution to effect significant receptor modification.

Erythrocyte Receptor–Modifying Activity Following Reaction with Red Cells.—

To determine if the erythrocyte receptor–modifying factor in adenovirus suspensions adsorbed irreversibly to red cell receptor sites, erythrocyte receptor–modifying titers of adenovirus suspensions were determined following successive treatment with fresh erythrocytes. 1 volume of packed red cells was added to 16 volumes of undiluted adenovirus types 1 and 15 and HeLa control fluids, respectively, and the mixtures held at 22°C for 2 hours. Erythrocytes were separated by centrifugation and discarded, and the supernatant fluid was then added to fresh erythrocytes. This procedure was repeated 5 times with adenovirus type 1 and 6 times with type 15 suspensions. Erythrocyte receptor—modifying activity of these suspensions and corresponding suspensions which had not been subjected to cyclic treatment with red cells were assayed in the erythrocyte receptor modification titration test (Table IV).

TABLE III

Rate of Erythrocyte Receptor Modification by Adenovirus Type 1 Suspension at 22°C

Length of treatment	Titer of erythrocyte receptor–modifying activity*
min	
1	$10^{1.0}$
5	$10^{1.5}$
10	$10^{1.5}$
20	$10^{2.0}$
60	$10^{2.0}$
120	$10^{2.5}$

* Highest dilution of the Ad. 1 suspension which produced $\geq$ 8-fold decrease in agglutinability of the erythrocytes by Ad. 10 test virus.

TABLE IV

Erythrocyte Receptor–Modifying Activity of Adenovirus Type 1 and 15 Suspensions Following Successive Reactions with Human Erythrocytes

Erythrocyte treatment	Titers of erythrocyte receptor–modifying activity as determined with three test viruses		
	Ad. 10	Ad. 13	Ad. BAR-2
Ad. 1, initial suspension	$10^{2.5}$	$10^{2.5}$	$10^{2.5}$
Ad. 1, suspension after 5 cycles of reaction with erythrocytes	$10^{2.5}$	$10^{2.5}$	$10^{2.5}$
Ad. 15, initial suspension	$10^{1.5}$	$10^{1.5}$	$10^{1.5}$
Ad. 15, after 6 cycles of reaction with erythrocytes	$10^{1.5}$	$10^{1.5}$	$10^{1.5}$

The adenovirus erythrocyte receptor–modifying factor was not irreversibly attached to the erythrocytes, since the virus fluids after successive exposures with red cells had activity equal to the initial suspensions.

Adsorption of Test Virus to Erythrocyte Receptor–Modifying Factor–Treated Cells.—To investigate whether inagglutinability of virus-treated erythrocytes was due to the modification of receptors with consequent lack of adsorption of hemagglutinin or due to failure of red cells with attached virus to form characteristic agglutination patterns, experiments were done to determine if test virus hemagglutinin adsorbed to the treated cells.

1 ml of packed group 0 erythrocytes was added to tubes containing 16 ml of saline, undiluted HeLa control fluid, and adenovirus type 1, respectively. After incubation at 22°C for 2 hours, red cells were washed twice with saline. 2 ml of undiluted adenovirus type 10 was then added to each tube containing 0.5 ml of packed treated red cells. These mixtures were held at 22°C for 30 minutes, and then centrifuged at 1500 RPM for 5 minutes. The supernatant fluids were assayed for unadsorbed type 10 by determining the hemagglutinin titers against fresh untreated erythrocytes. The results of this experiment are shown in Table V.

TABLE V

Hemagglutination Titer of Adenovirus Type 10 Suspension Following Adsorption with Treated Erythrocytes

Treatment of Ad. 10 suspension	Hemagglutination titer* of adsorbed Ad. 10 suspensions
Untreated	128
Adsorbed with saline-treated RBC‡	8
Adsorbed with HCF-treated RBC	8
Adsorbed with Ad. 1–treated RBC	64

* Reciprocal of titer obtained with untreated erythrocytes.

‡ Red blood cells.

The type 10 virus hemagglutinin adsorbed far less efficiently to the adenovirus-treated cells than to either the saline or HeLa control fluid–treated cells.

Effect of the Erythrocyte Receptor–Modifying Factor on Red Cell Antigens and Human A and AB Erythrocytes.—Group O Rh-positive erythrocytes which were pretreated in the standard manner with physiologic saline, HeLa control fluid, and an adenovirus type 1 suspension were tested for phenotype characteristics by Dr. W. Bronson from the National Institutes of Health, Clinical Center Blood Bank. The 3 samples gave a positive reaction for M, N, V, P, D, c, e, Kp^b, k, JK^a, and Le^b antigens, and were negative for S, C^w, C, E, K, Kp^a, Fy^a, JK^b, V, and Le^a antigens. All negative results were checked microscopically. It was noted, however, that adenovirus type 1–treated cells exhibited a stronger reaction than the saline and HeLa control fluid–treated cells with c and e antisera.

Human erythrocytes from donors with blood group typings A and AB treated with an adenovirus type 1 suspension exhibited the same decrease in agglutinability against the test viruses as did group O erythrocytes.

Physicochemical Studies of the Erythrocyte Receptor–Modifying Factor

Dialysis.—Suspensions of adenovirus types 1, 2, and 15 were dialyzed against several changes of continuously stirred 1 M tris buffer solution or distilled water at 4°C for 18 hours. The erythrocyte receptor–modifying factor of the adenovirus suspensions was always retained in the non-dialyzable portion.

Heat.—Erythrocyte receptor–modifying activity of adenovirus type 1 and 2 suspensions was not affected by heating in sealed and submerged ampules at 70°C for 30 minutes. Heating at 80°C for 30 minutes, however, reduced the erythrocyte receptor–modifying titer by 0.5 $\log_{10}$, and heating at 90°C for 30 minutes or 100°C for 10 minutes completely destroyed the erythrocyte receptor–modifying activity.

Ultraviolet Irradiation.—Tests for sensitivity to ultraviolet irradiation were done by Dr. Samuel Baron of the Division of Biologic Standards of the National Institutes of Health. Undiluted type 1 and 2 adenovirus suspensions and HeLa control fluid in 10 ml aliquots were placed in 90 mm diameter Petri dishes and placed on a rotary shaker below a 15 watt General Electric germicidal lamp for 30 minutes. The radiation intensity was 120 μw per cm^2 as measured with an Archer radiation meter. (Archer-Daniels-Midland Co., Cleveland). The erythrocyte receptor modification titers of the irradiated virus suspensions were identical to the non-irradiated virus suspensions.

Ether.—An 18 hour exposure at 4°C and extraction of adenovirus type 1 and 2 suspensions with 50 per cent diethyl ether did not affect the erythrocyte receptor—modifying activity of these suspensions.

Enzymes.—Crystalline ribonuclease (RNAase) and desoxyribonuclease (DNAase) were received through the courtesy of Dr. Hilton B. Levy from the National Institutes of Health. Enzyme solutions containing 1 mg/ml were prepared with 1 M tris buffer, pH 7.8. 1 volume of enzyme solution was added to 10 volumes of undiluted HeLa control fluid and adenovirus type 1 and 15 fluids. Control preparations consisted of fluids treated with buffer solution. Prior to the addition of RNAase, test materials were dialyzed overnight in the cold and adjusted to pH 7.8. The materials used for DNAase treatment were adjusted to pH 7.8 and contained in addition to the enzyme, 0.03 M $MgCl_2$. Following incubation at 37°C for 60 minutes, the materials were kept at 4°C until assayed. The erythrocyte receptor modification titers of the virus suspensions treated with enzymes were identical to those of the untreated suspensions.

Type 1 and 2 adenovirus suspensions and HeLa control fluid which were prepared in HeLa cell cultures containing protein-free medium 199 were treated with trypsin (Armour and Co., Chicago) and chymotrypsin (Worthington Biochemicals Corp., Harrison, New Jersey). A 1 per cent enzyme solution was prepared in 0.02 M phosphate buffer, pH 7.8. 1 volume of enzyme solution was added to 3 volumes of HeLa control fluid and virus fluids, which had been adjusted to pH 7.8. Virus and HeLa control fluids mixed with buffer were used as controls. After incubation of the mixtures at 37°C for 60 minutes, 1 volume of a 1 per cent soybean trypsin inhibitor (Nutritional Biochemicals Corp., Cleveland) prepared in phosphate buffer was added to the enzyme-treated and control fluids. When assayed, the virus suspensions treated with trypsin and chymotrypsin gave erythrocyte receptor modification titers comparable to those of the non–enzyme-treated virus fluids.

Serum Inhibition

Inhibition of the erythrocyte receptor–modifying factor of adenovirus suspensions by rabbit antiserum was demonstrated in neutralization tests done as described under Materials and Methods. The results of representative tests are shown in Table VI. Type-specific neutralization was observed since there

was no reduction in test virus hemagglutinin titers in tests of erythrocytes which were pretreated with homologous virus-serum mixtures. In contrast, test virus

TABLE VI

Effect of Rabbit Antisera on Erythrocyte Receptor Modification by Adenoviruses

Erythrocytes treated with mixture of	Hemagglutination titer* of test virus (type 10) against treated erythrocytes				
Treating virus / Rabbit antiserum (1:1)	HeLa control fluid	Ad. 1	Ad. 2	Ad. 4	Ad. 15
Normal rabbit serum	256	16	16	8	16
HeLa control fluid–immunized	256	16	16	8	16
Ad. 1–immunized	256	256	16	8	16
Ad. 2–immunized	256	8	256	8	16
Ad. 4–immunized	256	16	8	256	16
Ad. 15–immunized	256	16	16	8	256

* Reciprocal of dilution.

TABLE VII

Separation of Erythrocyte Receptor–Modifying Factor from Infectious Virus in Adenovirus Suspensions by Ultracentrifugation

Material used to treat human erythrocytes	Titer of erythrocyte receptor–modifying activity as determined with three test viruses			Infectivity titer*
	Ad. 10	Ad. 13	Ad. BAR-2	
				TCD_{50}/ml
Ad. 1, initial suspension	$10^{3.0}$	$10^{2.5}$	$10^{3.0}$	$10^{7.0}$
Ad. 1, supernate fraction, S-3	$10^{2.5}$	$10^{2.5}$	$10^{2.5}$	$10^{2.5}$
Ad. 1, pellet fraction, P-3	$<10^{0.5}$	$<10^{0.5}$	$<10^{0.5}$	$10^{4.0}$
Ad. 2, initial suspension	$10^{2.5}$	$10^{2.5}$	$10^{2.5}$	$10^{8.0}$
Ad. 2, supernate fraction, S-3	$10^{2.0}$	$10^{2.5}$	$10^{2.5}$	$10^{2.5}$
Ad. 2, pellet fraction, P-3	$<10^{0.5}$	$<10^{0.5}$	$<10^{0.5}$	$10^{5.8}$
Ad. 15, initial suspension	$10^{1.0}$	$10^{1.0}$	$10^{0.5}$	$10^{4.5}$
Ad. 15, supernate fraction, S-3	$10^{1.0}$	$10^{0.5}$	10^{0}	$10^{1.5}$
Ad. 15, pellet fraction, P-3	$<10^{0}$	$<10^{0}$	$<10^{0}$	$10^{2.5}$

* Titer calculated after 25 days in Hep. 2 cultures.

hemagglutinin titers were significantly reduced (8-fold or greater) in tests against erythrocytes pretreated with heterologous virus-serum mixtures.

Separation of Erythrocyte Receptor–Modifying Factor from Infectious Virus

In a previous report (6) evidence was presented which indicated that erythrocyte receptor–modifying activity of an adenovirus suspension was not a function of the infectious virus particle, in that the bulk of the infectivity of a type

15 adenovirus suspension could be removed by adsorption with rat erythrocytes, without decreasing the erythrocyte receptor–modifying titer. To further establish the distinctness of the two factors, separation by ultracentrifugation was attempted.

Three tubes containing 11.6 ml of infectious tissue culture fluid were placed in a Spinco No. 40 rotor head and centrifuged in a Spinco model L ultracentrifuge at 40,000 RPM (average 105,400 *g*) for 90 minutes. Following centrifugation, the uppermost 8 ml of fluid was removed from each tube and pooled, the remaining fluid discarded, and the pellets resuspended in 11.6 ml of saline and pooled. This procedure was repeated through three cycles of centrifugation; the final pooled supernatant fluids were designated as S-3 and the final pooled resuspended pellets as P-3. Adenovirus type 1, 2, and 15 fractions were prepared in this manner. These fractions and initial virus fluids were assayed for infectivity and erythrocyte receptor–modifying titers. The results of these experiments are shown in Table VII.

Erythrocyte receptor–modifying activity was found only in S-3 fractions, while the pellet fractions contained the bulk of the recovered virus. Thus, the centrifugation studies confirmed that the erythrocyte receptor–modifying factor was separable from infectious virus and also indicated that it was smaller than the virus particle.

DISCUSSION

The experiments presented here demonstrated that adenovirus type 1, 2, 4, and 15 infectious tissue culture fluids contained a non-dialyzable factor separable from and smaller than the virus particle which was capable of modifying human erythrocyte receptors for type 10, 13, and BAR-2 adenovirus hemagglutinins. It was somewhat puzzling that the erythrocyte–modifying factor was found only with these types, and not with other adenovirus types closely related in many biological attributes to the types producing the factor. It is possible that the failure to demonstrate the activity in the other adenovirus suspensions is due to differences in the experimental conditions necessary for demonstrating the activity, or to differences in the ease of extraction of the factor from infected tissue culture cells.

Both ultracentrifugation and red cell adsorption studies indicated that the erythrocyte receptor–modifying factor was distinct from the infectious virus particle, but differentiation from or identification with other non-infectious adenovirus antigens is not yet possible. The type specificity of its antigenic structure as indicated by the serum inhibition tests, and the fact that the factor was produced by only a few adenovirus types, makes it quite unlikely that the erythrocyte receptor modifying–factor is the soluble complement–fixing antigen. Also, the resistance of the erythrocyte receptor–modifying activity to trypsin treatment serves effectively to differentiate it from the cell-detaching factor, early cytopathogenicity factor, or B antigen (5). The C antigen (5) is both trypsin resistant and type-specific; however, it is produced in large

amounts by adenovirus type 5, whereas, the erythrocyte receptor–modifying factor is not. It is possible that the erythrocyte receptor–modifying factor is the C antigen, but in the case of type 5, devoid of the biologic activity.

Attempts to identify the chemical nature of the erythrocyte receptor–modifying factor by demonstrating inactivation by specific chemical and physical means were unsuccessful since the activity remained intact after exposure to desoxyribonuclease, ribonuclease, trypsin, chymotrypsin, ether, and ultraviolet irradiation. Perhaps enzyme inactivation tests with highly purified erythrocyte receptor–modifying factor, free of potential inhibitors, should such become available, would give positive results. Since the factor is a macromolecular, heat-labile substance which reacts with immune serum, and which behaves in some respects like an enzyme, it is suggested that it is a protein which is resistant to trypsin and chymotrypsin digestion.

The mechanism of action of the erythrocyte receptor–modifying factor is not known, but certain biologic data suggest that the reaction may be of an enzymatic nature. The degree of receptor destruction was a function of temperature and time; also, the erythrocyte receptor–modifying activity of an adenovirus suspension remained functionally unchanged following repeated reactions with red cells, whereas the erythrocytes were rendered inagglutinable for test virus hemagglutinin. However, definitive proof of an enzyme-substrate reaction will require further evidence, such as the demonstration of reaction products formed as the result of the reaction.

Although erythrocyte receptors for the adenovirus hemagglutinins were abolished by treatment with the RDE of *Vibrio cholerae* and markedly reduced after treatment with influenza types A and B (6), the mechanism of action of the adenovirus factor did not appear to be identical to that of neuraminidase. This lack of similarity is indicated by the findings that the adenovirus factor did not alter the agglutination capacity of erythrocytes for myxovirus hemagglutinins, and that adenovirus type 1 and 2 suspensions were devoid of neuraminidase activity (14) in assays done by the method of Warren (15). A further difference from the activity of RDE is the fact that *Vibrio cholerae* and influenza enzymes destroyed the receptors of human red cells for adenovirus types 8 and 9, whereas, the adenovirus erythrocyte–modifying factor did not affect the receptors for types 8 and 9.

The functional significance, if any, of the erythrocyte receptor modifying–factor in adenovirus infection needs further definition.

SUMMARY

Agglutinability of human erythrocytes for 3 hemagglutinating adenoviruses was markedly reduced by pretreatment of red cells with a factor present in tissue cultures which had been infected with adenovirus types 1, 2, 4, or 15.

The factor responsible for erythrocyte receptor modification was non-

dialyzable and unaffected by the action of ribonuclease, desoxyribonuclease, trypsin, chymotrypsin, or ether. The factor was smaller, more thermostable, and separable from the infectious virus.

Erythrocyte receptor modification was found to be a function of time and temperature. Titers of erythrocyte receptor–modifying activity were not diminished by successive exposures to fresh erythrocytes. Erythrocytes treated with erythrocyte receptor–modifying factor suspensions failed to significantly adsorb test virus hemagglutinin.

Inhibition of erythrocyte receptor modifying–activity of the adenovirus suspensions by rabbit antiserum was type-specific.

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