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Neutralization Kinetics Study of Selected Reoviruses[1]

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Neutralization kinetic rates were compared between five avian and three human reoviruses and their specific antiserums. Antigenic similarities were not noted between the human and avian reoviruses studied. Antigenic similarities were found between a reovirus isolated from a chicken with Marek's disease, and the Fahey-Crawley virus, avian arthritis virus, and a turkey reovirus isolate designated as BC-3. The Fahey-Crawley virus was found to be antigenically similar to BC-3.

It is becoming more and more evident that isolations of reoviruses from various organs of chickens and turkeys are commonplace (6, 9, 15, 16, 18). Reoviruses have been isolated from both sick and apparently normal chickens and turkeys (6, 10, 15, 16, 18). This study was initiated to attempt to determine whether or not certain selected avian reoviruses could be antigenically categorized and to determine their relationship to human reovirus strains. Neutralization kinetics was used to study the antigenic similarities between the reoviruses. Neutralization kinetics has been used to differentiate closely related bacteriophage strains (1, 8, 11) and between strains of animal (7) and human viruses (3, 4, 7, 12).

MATERIALS AND METHODS

Cell cultures and media. Vero cells (North American Biologicals, Rockville, Md.) and chicken kidney cells, obtained from 1- to 7-day-old chickens, were propagated by standard procedures (5). Viral dilutions were prepared in Hanks balanced salt solution (Grand Island Biologicals Co., Grand Island, N.Y.) supplemented with 0.25% lactalbumin hydrolysate (Nutritional Biochemical Corp., Cleveland, Ohio) and 0.04% sodium bicarbonate (J. T. Baker Chemical Co., Phillipsburg, N.Y.). Serum dilutions were prepared in phosphate-buffered saline (5). Chicken kidney cells were propagated in Hanks balanced salt solution supplemented with 0.25% lactalbumin hydrolysate, 0.04% sodium bicarbonate, and 10% fetal calf serum (Grand Island Biologicals Co.). Vero cells were propagated in Eagle minimum essential medium supplemented with 0.2% sodium bicarbonate, 10% fetal calf serum, and 10% tryptose phosphate broth (Difco Laboratories, Detroit, Mich.). Both cell types were maintained with Eagle minimum essential medium supplemented with 0.2% sodium bicarbonate, 2% fetal calf serum, and 10% tryptose phosphate broth. One percent purified agar (Difco Laboratories) was added to medium when agar overlays were used for plaque formation. Penicillin (Calbiochem, Los Angeles, Calif.), streptomycin (Philadelphia Laboratories, Philadelphia, Pa.), and fungizone (E. R. Squibb and Son, N.Y.) in the concentrations of 100 U/ml, 100 μg/ml, and 2 μg/ml, respectively, were included in all media.

Plaque detection. After a 6-day incubation period, 3 ml of a 1:4,000 dilution of neutral red (Difco Laboratories) was added to the overlay medium and incubated at 37 C in an atmosphere of 3 to 5% carbon dioxide (CO_2). At the end of 1 h, the neutral red was removed and the monolayers were examined for the presence of virus-induced plaques.

Virus sources. Human reoviruses type 1 (lot 033), type 2 (lot 108), and type 3 (lot 030) were obtained from Grand Island Biologicals Co. (Berkeley, Calif.) and propagated in Vero cells. The avian arthritis-producing reovirus and Fahey-Crawley reovirus were obtained from Stanley H. Kleven, University of Georgia. The infectious bursal agent (IBA) was obtained from Phil D. Lukert, University of Georgia. BC-3 reovirus was isolated by Richard E. Wooley (University of Georgia) from a turkey flock in northern Georgia that had undergone a severe epornitic of infectious enteritis (18). A reovirus was isolated by Phil D. Lukert from chicken bursal cells, while studying Marek's disease. This strain is referred to as MA reovirus (personal communication). Avian reoviruses were propagated in chicken kidney cell cultures.

Antigen preparation. Each virus was inoculated on appropriate cell cultures and incubated at 37 C in an atmosphere of 3 to 5% CO_2. After development of cytopathic effects, cells and cell culture fluids were harvested, freeze-thawed three times, and quantitated by plaque enumeration to determine the dilution that would give 30 to 100 plaques per 0.2 ml. Approximately one-half of the antigen prepared was divided into samples and frozen at −60 C. The

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remaining virus was clarified in a Sorvall RC 2-B superspeed automatic refrigerated centrifuge at 7,700 $\times g$ for 30 min and pelleted in a Beckman model L2-65B preparative ultracentrifuge at 92,000 $\times g$ for 90 min. The resulting pellet was resuspended in Hanks balanced salt solution to one-tenth the original volume.

Preparation of hyperimmune serum. Hyperimmune serum was produced in adult chickens raised under filtered-air, positive-pressure conditions (2). Preinoculation serum from each chicken was tested for the presence of antibody against the viruses to be studied. Three adult chickens were inoculated intravenously with the concentrated antigen, twice a week for 1 month. The chickens were bled by cardiac puncture, and serum was recovered, pooled, and stored at −20 C. Antisera were titered to determine the dilution which would produce a 1.5- to 2-log decrease in titer of the homologous virus in 30 min at room temperature. Sera were heat inactivated at 56 C for 30 min.

Neutralization kinetics. The method of Dulbecco et al. (7) was used to study the speed of virus neutralization. Samples of virus antiserum mixtures were taken at 0, 1, 5, 10, 15, and 25 min. Samples were diluted and inoculated on cell monolayers for virus titration.

The virus-antiserum mixtures were allowed to adsorb for 90 min at 37 C in an atmosphere of 3 to 5% CO_2. A 5-ml amount of overlay medium was added to the monolayers and incubated for 6 days. On the 6th day the monolayers were stained with neutral red, and plaques of appropriate dilutions were counted and recorded.

The neutralization rate (K) was calculated from the formula: $K = D/t\ 2.3 \log_{10} V_0/V_t$, where t equals time, V_0 equals virus concentration at time 0, V_t equals virus concentration at time t, and D equals 1/C or the serum dilution. If C equals the antibody concentration, K becomes an expression of serum titer based upon the initial rate at which a great excess of antiserum neutralizes the virus. K values of different antisera cannot be compared due to the difficulty of determining absolute antibody concentration. This limitation is overcome by assigning a value of 100 (normalizing K or NK) to the K values of homologous systems and expressing the heterologous K values as proportions of the homologous systems and expressing the heterologous K values as proportions of the homologous. Each virus was tested against each antiserum and the results were expressed as $\log_{10} V_0/V_t + 1$, where t equals 25 min. The value of one was added to avoid manipulations with negative logarithms (17). Reciprocal NK values of 90 to 100 indicated that the viruses were homologous, those of 60 to 89 indicated antigenic similarity, and those of less than 60 indicated antigenic distinction.

RESULTS

Comparison of the normalized K values (NK) indicate that the MA reovirus (100) is antigenically homologous to the avian arthritis reovirus (123.0) and the Fahey-Crawley virus (109.5), and similar to reovirus BC-3 (63.3). Fahey-Crawley reovirus (100) is similar antigenically to reovirus BC-3 (68.8). None of the five avian reoviruses were antigenically similar to the three human reovirus strains. K and NK values are shown in Table 1.

DISCUSSION

Ashe and Scherp (3) considered viruses giving reciprocal NK values of 90 or more to be serologically homologous. During a study of polioviruses, Nakano et al. (13) considered strains with NK values of 80 to 100 to be vaccine like, 65 to 79 to be intermediate, and less than 65 to be non-vaccine like. For this study viruses with reciprocal NK values of 90 to 100 were considered homologous, those with NK values of 60 to 89 were considered antigenically similar, and those with NK values of less than 60 were antigenically distinct.

Deshmukh et al. (6) found similarity between three avian reoviruses, isolated from 1-week-old chickens with cloacal pasting, using hemagglutination inhibition, complement fixation, agar gel diffusion, and reciprocal neutralization tests. However, in this study no cross-neutrali-

TABLE 1. *Comparison of reciprocal neutralization rates of selected reoviruses by K and normalized K values*

Virus	Arthritis[a]		IBA[a]		Crawley[a]		BC 3[a]		Marek's reovirus[a]		Reovirus 1[a]		Reovirus 2[a]		Reovirus 3[a]	
	K[b]	NK	K[b]	NK	K[b]	NK	K[b]	NK	K[b]	NK	K[b]	NK	K[b]	NK	K[b]	NK
Arthritis	12.88	100	0.57	4.2	1.76	28.0	0.26	13.9	7.69	123.0	2.22	38.5	0.83	30.3	0.94	39.1
IBA	5.33	41.4	13.56	100	2.68	42.7	0.29	15.5	3.34	53.5	0.72	12.3	0.57	20.8	0.78	32.5
Crawley	1.95	15.1	0.15	1.1	6.28	100	0.38	20.3	6.83	109.5	1.12	19.4	1.35	49.3	0.63	26.2
BC 3	1.78	13.8	1.68	12.4	4.32	68.8	1.87	100	3.95	63.3	1.77	30.7	0.52	19.0	0.69	28.8
MA reovirus	3.93	30.5	1.72	12.7	3.00	47.7	0.34	18.2	6.24	100	0.83	14.4	0.58	21.2	0.92	38.3
Reovirus 1	1.87	14.5	2.07	15.3	3.31	52.7	0.54	28.8	2.07	33.2	5.77	100	2.15	78.5	3.69	153
Reovirus 2	1.48	11.5	1.28	9.5	0.98	15.5	0.32	17.4	1.80	28.8	1.42	24.7	2.74	100	1.37	57.3
Reovirus 3	0.78	6.1	1.32	9.7	1.35	21.5	0.25	13.3	1.04	16.7	0.95	16.5	0.77	28.1	2.40	100

[a] Antisera.
[b] $K = 2.3\ D/t \log_{10} V_0/V_t$.

zation tests were done between the avian reoviruses studied and human reoviruses.

A recent study by Olson and Weiss (14) indicated by reciprocal neutralization that avian arthritis reovirus and Fahey-Crawley virus were the same serotype. This was not found to be the case with the strains of the viruses used in this study. This contradiction is explicable due to the greater sensitivity of neutralization kinetics (12). Anti-arthritis reovirus serum did not significantly neutralize any of the heterologous viruses nor did IBA or BC-3 virus antiserum. Based on the NK value, antiserum produced to the Fahey-Crawley virus significantly neutralized BC-3 virus. However, both Fahey-Crawley and avian arthritis viruses, as well as BC-3, were neutralized significantly by anti-MD reovirus serum. IBA was the most antigenically distinct of all the viruses studied. This may be significant in that IBA has not definitely been proven to be a reovirus (10). All of the avian reoviruses studied except IBA appear to be similar to MA reovirus, the only reovirus studied which was not associated with disease.

There seems to be little correlation of serological properties with the illnesses produced by the avian reoviruses. IBA, which causes an infection of the bursa of Fabricius, in chickens, is the most antigenically distinct. The arthritis-producing virus of chickens, BC-3 virus, which produces enteritis in turkeys, and Fahey-Crawley virus, isolated from chronic respiratory disease in chickens, all appear similar to the nonpathogenic MA reovirus. The similarity noted between Fahey-Crawley, a respiratory virus, and BC-3 and enteric virus reinforces this observation.

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