Antigenic Variation of Rhinovirus Type 22[1] (34468)

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Rhinoviruses, a subgroup of human picornaviruses, are now considered to be one of the more important groups of viruses causing mild upper respiratory tract disease in adults (1–3). Presently there are 55 distinct serologic types and 1 subtype (4).

Antigenic variants, designated as "prime strains," have been described for certain serotypes of enteroviruses (5–7). By definition prime strains exhibit a broader antigenicity than do prototype strains, and while they are neutralized poorly by immune sera to the prototype strain they yield antisera which neutralize the prototype strain to the same titer, or nearly so, as the prime strain. Antigenic variants which do not meet the criteria of prime strains produce antisera with high titers for the homologous strains but significantly lower titers for the heterologous prime strains. We have recently isolated a number of strains of rhinovirus type 22 which were not neutralized by antisera to the prototype strain; however, antisera prepared against one of these variant strains, the Chase strain, neutralized the prototype strain and also the other variant strains.

The purpose of this report is to describe the isolation and serological characteristics of a number of isolates which were found to be antigenic variants of rhinovirus type 22.

Materials and Methods. Cell cultures. The preparation of diploid cell cultures of human fetal lung (HFDL) and primary rhesus monkey kidney (MK) as well as the media for outgrowth and maintenance of these cells have been described (8).

Clinical specimens. The collection and treatment of specimens and inoculation of cell cultures for virus isolation attempts have been described elsewhere (9).

Isolation and identification of viruses. Viruses were recovered in HFDL cells from nasopharyngeal swabs, and a presumptive identification of rhinovirus was based upon characteristic cytopathic effects and the demonstration of a significant (100-fold) loss of infectivity upon exposure of the isolate to a pH of 3.0 for 2 hr at room temperature. Final identification was based upon neutralization tests utilizing type-specific rhinovirus immune sera.

Neutralization tests. Serum neutralization tests were performed in tube cultures of HFDL cells. Serial 2-fold dilutions of serum, inactivated at 56° for 30 min, were mixed with an equal volume of virus containing 50% tissue culture doses (TCD_{50}) (32–100 doses) of virus and incubated for 2 hr at room temperature prior to inoculation into HFDL cells (0.2 ml per tube). Virus titrations were carried out in each test and the serum neutralization titer was calculated when microscopic observation of cell culture for cytopathic effect indicated the test dose of virus to be between 32 and 320 TCD_{50}.

Plaque method. Plaque assays of rhinoviruses were carried out in HeLa cells according to the method of Conant *et al.* (10).

Immune serum preparation. Rhinovirus immune sera were prepared in guinea pigs. Virus-infected tissue culture fluid was concentrated 10-fold by pelleting overnight at 30,000 rpm in the Model L ultracentrifuge; the pellet was resuspended in serum-free Eagle's medium and emulsified with an equal volume of complete Freund's adjuvant. Each

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TABLE I. Isolation of Antigenic Variants of Rhinovirus Type 22 in HFDL Cells.[a]

Patient no.	Age	Sex	Date of onset	Clinical finding
1	23 yr	M	5/7/62	URI
2	17 mo	F	2/14/65	Pharyngitis
3	3 yr	M	11/17/65	Acute rhinitis
4	3 yr	F	1/10/66	Otitis media exudative pharyngitis
5	42 yr	M	2/16/66	URI
6	20 mo	M	4/22/67	Croup
7	16 mo	M	5/2/67	Asymptomatic
8	9 mo	M	5/5/67	URI
9	12 mo	F	5/7/67	URI
10	8 mo	M	5/7/67	Otitis externa
11	12 mo	M	5/8/67	URI
12	4 yr	M	5/8/67	Asthma, pneumonia
13	4 mo	M	5/10/67	Rhinitis
14	10 mo	F	5/15/67	Asymptomatic
15	12 mo	M	5/24/67	Asymptomatic
16	9 mo	M	5/28/67	Severe bronchiolitis
17	16 mo	M	5/30/67	URI
18	7 mo	F	10/1/67	URI

[a] HFDL = human fetal diploid lung cells.

of eight guinea pigs was given two intramuscular injections of 1.0 ml of the virus-adjuvant mixture 6 weeks apart and bled 2 weeks after the second injection.

Absorption of serum. The absorption of rhinovirus animal immune sera with human liver powder was carried out according to the procedure described by Conant and Hamparian (11).

Results. Isolation of antigenic variants of rhinovirus type 22. The patients' age, sex, clinical findings, and date of onset of illness are shown in Table I.

Viruses were isolated from 15 patients with clinically evident respiratory disease and from 3 patients without respiratory symptoms. Specimens from asymptomatic patients were collected as part of a study requiring routine home visits. The patients were predominantly young children whose ages ranged from 4 months to 4 years and all viruses were recovered only in HFDL cells.

The virus strains isolated from the patients listed in Table I were recognized as possible "prime strains" of rhinovirus type 22 because they were neutralized poorly or not at all by approximately 20 antibody units of immune serum to the prototype strain (127-CV22) of rhinovirus type 22, but were neutralized by immune serum to Chase virus, the first variant virus isolated.

Reciprocal serum neutralization test with antigenic variants of rhinovirus type 22. The Chase virus strain, after three successive passages by the terminal-dilution method, was used for the preparation of immune serum in guinea pigs. Table II shows the results of neutralization tests with this serum and immune serum to the prototype strain (127-CV22) against the viruses indicated. Immune serum against the prototype virus with a homologous titer of 1:128 neutralized only one of the isolates (Spicer), while immune serum prepared against the Chase virus strain neutralized the prototype virus and all of the isolates to a titer not significantly different from the homologous titer.

The possibility was considered that the reactivity of the prototype immune serum used might be unique to the particular lot (1247-8) employed. Therefore, a second lot

TABLE II. Neutralization of Antigenic Variants of Rhinovirus Type 22.

Virus strain	Serum neutralization titers[a]	
	Immune serum to the prototype strain	Immune serum to the Chase strain
Prototype (127-CV22)	128	128
Spicer	64	64
Chase	<16	256
Magoffin	<16	256
Jones	<16	256
Viennes	<16	128
Conner	<16	512
Gorspe	<16	256
Higgins	<16	256
Royce	<16	128
Goff	<16	128
Cepeda	<16	128
Apple	<16	128
Ashurst	<16	512
Gallant	<16	256
DeVose	<16	512
Chadwick	<16	128
Dillion	<16	128 or >
Neal	<16	128 or >

[a] Reciprocal of serum dilution.

TABLE III. Reciprocal Neutralization Tests with the Prototype Strain of Rhinovirus Type 22 and an Antigenic Variant (Chase).

Rhinovirus type 22	Tube neutralization test			Plaque reduction test[a]	
	Prototype serum		Variant serum	Prototype serum	Variant serum
	Lot 1247-8	Lot 1772-4	Lot 1607-1	Lot 1772-4	Lot 1607-1
Prototype strain	1:256	≥1:2048	1:256	1:8,114	1:12,800
Variant strain (Chase)	<1:16	<1:16	1:128	1:968	1:18,688

[a] Antibody titer based upon an 80% reduction of plaques.

(1772-4) was prepared and reciprocal neutralization tests were performed in tube cultures of HFDL cells. The results presented in Table III show that both lots of prototype immune sera (1247-8 and 1772-4) behaved similarly in that they neutralized the prototype strain but did not neutralize the variant Chase strain even at a 1:16 dilution (the lowest tested).

Prototype immune serum (1772-4) was also compared with immune serum to the variant virus in a reciprocal plaque-reduction neutralization test. The results, also shown in Table III, confirm the antigenic difference observed between the two strains in tube neutralization tests. However, because of the greater sensitivity of the plaque-reduction test, neutralization of the variant virus by the prototype serum was observed but the titer of the prototype serum for the variant strain was more than 8-fold lower than that for the homologous prototype strain. The heterologous and the homologous neutralizing antibody titers of immune serum to the variant Chase virus were not significantly different.

Absorption of immune sera with human liver powder. Conant and Hamparian (12) have clearly demonstrated that certain heterologous neutralizing reactions observed with rhinovirus animal immune sera are attributable to cytotoxic factors or nonspecific inhibitors present in the sera, and that multiple absorptions of the sera with human liver powder serves to remove all, or nearly all, of the nonspecific reactivity without significantly altering the specific antibody titer.

It was therefore of interest to determine what effect absorption with human liver powder might have on the broad antigenic reactivity of immune serum to the variant virus. Antiserum to the variant virus strain was absorbed twice with human liver powder and tested in this laboratory, and in addition identical lots of serum were sent to Drs. Conant and Hamparian who very kindly absorbed and tested them for neutralizing antibodies. The neutralizing antibody assays were performed in HFDL cells in this laboratory and in HeLa cells by Drs. Conant and Hamparian. The results obtained in both laboratories are presented in Table IV and clearly indicate that the absorption had no significant effect on either the homologous or heterologous antibody titers of the immune serum to the variant virus. (The lower antibody titer obtained after the fourth absorption is not unexpected as a certain amount of dilution occurs after repeated treatment). These results would indicate that the Chase virus represents a prime strain of rhinovirus type 22.

Discussion. Intratypic variation among the rhinoviruses has been described for types 1A and 1B (13, 14). In one report (12) the heterologous serum titers were approximately 15-fold or more lower than the corresponding homologous titers. Other workers reported (14) the antigenic relationship of the JH strain of type 1A rhinovirus to type 1B virus to be reciprocal, while the cross between the 2060 strain and type 1B appeared to be only one way. Thus the nature of the serologic relationship between these two viruses is not clear at present.

Antigenic relationships between other prototype rhinoviruses have also been reported (15, 16). However, further serologic characterization of all prototype rhinovirus strains

TABLE IV. Effect of Absorption with Human Liver Powder on the Homologous and Heterologous Neutralizing Activity of Chase Immune Serum.

	Neutralizing antibody titer of immune serum to:		
	Variant virus (Chase strain)		Rhinovirus type 22 (prototype strain)
Rhinovirus type 22	Unabsorbed	Absorbed	Unabsorbed
Chase strain	1:256	1st 1:256 2nd 1:128	<1:16
Prototype strain	1:256	1st 1:128 2nd 1:256	1:256
Chase strain[a]	1:1024	1st 1:1536 2nd 1:1536 3rd 1:768 4th 1:384	<1:5
Prototype strain[a]	1:1024	1st 1:1536 2nd 1:1536 3rd 1:768 4th 1:384	1:960

[a] Results obtained by Drs. R. M. Conant and V. V. Hamparian, Ohio State University, Columbus, Ohio.

by Conant and Hamparian (12) disclosed that with 10 viruses and 9 antisera 14 reproducible one-way crosses were obtained when unabsorbed antisera were used in the neutralization test. After absorption with liver powder the heterologous reactivity of the sera was completely removed or reduced to an insignificant level. Multiple absorptions of the Chase immune serum with human liver powder, as reported herein, did not significantly alter either the heterologous or homologous neutralizing antibody titer. This further confirms that the intratypic antigenic variation demonstrated for Chase virus is most probably a "prime strain" relationship.

Seventeen of the eighteen rhinovirus type 22 strains isolated in this laboratory were more closely related to the variant (Chase) virus strain than to the prototype strain. Only one isolate, the Spicer strain, exhibited a serologic reactivity similar to or identical with the prototype virus strain.

Although the experience of this laboratory may reflect the exception to the rule, the fact that most of the rhinovirus type 22 viruses isolated reacted poorly or not at all with the prototype immune serum stresses the importance for awareness of possible antigenic variation. This is especially important when viruses are being considered as candidates for prototype strains of new immunotypes. The necessity of selection of prototype strains with broad antigenic reactivity is obvious.

Summary. Seventeen of eighteen type 22 rhinoviruses isolated in this laboratory were shown to be antigenic variants of the prototype strain (127-CV22). The variant viruses were neutralized poorly or not at all by immune serum to the prototype strain. However, immune serum prepared to one of the variant viruses (Chase) neutralized both the prototype and homologous strains to similar titer and also neutralized all of the other isolates. The neutralizing reactivity of the Chase virus immune serum for the prototype and homologous strains was found to be essentially unaltered by multiple absorptions with human liver powder, a procedure capable of removing cytoxic factors or nonspecific inhibitors found in certain rhinovirus antisera. The Chase virus thus appears to represent a "prime strain" of rhinovirus type 22.

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