Antibody to Rhinovirus in Human Sera. II. Heterotypic Responses* (32725)

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Rhinoviruses have been shown to be the most common of the known etiologic agents of upper respiratory illness in adults, and an important cause of upper and lower respiratory illness in infants and children (1–4). Because of the large number of apparently distinct serotypes, it has been suggested that vaccine development is impractical (5). Recently, the findings of low level heterotypic responses in hyperimmunized cattle and of anamnestic responses to heterologous rhinoviruses in cattle and guinea pigs have suggested the possibility of antigenic groups among rhinoviruses (6). If this is true in man and a sufficiently small number of groups can be identified, then vaccine development might be practical.

The present study was undertaken to explore the possibility of heterotypic responses to rhinovirus infection in humans, by studying sera obtained from four groups of volunteers each inoculated with a different serotype of rhinovirus. The results indicate that heterotypic antibody develops in response to infection with certain types of rhinovirus and suggest that these types may be useful in vaccine development.

Materials and Methods. Volunteers. Twenty-three healthy male volunteers who were between the ages of 21 and 35 years, and who lacked detectable amounts or had low titers of neutralizing antibody to one of four rhinoviruses were inoculated by the nasopharyngeal route with prototype strains of that rhinovirus. The viruses used were Type 13 (strain NIH 353), Type 14 (strain NIH 1059), Type 15 (strain NIH 1734), and Type 16 (strain NIH 11757) (7). Each virus type had been passaged twice in human embryonic fibroblast tissue cultures (WI-26) and Type 14 had been passaged once in human embryonic kidney tissue cultures prior to passage in WI-26 cultures. Preparation and safety-testing of each virus inoculum was performed as described previously (8). In the safety-testing procedure undiluted inoculum was shown to be free of any detectable quantity of other viruses. In addition, each inoculum was diluted 50-fold prior to administering to volunteers. All volunteers were infected with the virus type they received as determined by subsequent repeated isolation of that type from the nasopharynx. Detailed analysis of illness and virus shedding has been reported (9).

Serum. Serum was obtained before inoculation and 3–4 weeks after inoculation, except for two volunteers who had serum collected 5 and 6 weeks after inoculation.

Neutralization tests. Each serum was tested against each of the four inoculum strains and against four other rhinovirus serotypes: Type 1A (strain 2060), Type 2 (strain HGP), Type 17 (strain NIH 33342), and strain Oliver. Strain Oliver is the designation given a strain isolated in a previous volunteer study (10). In cross-neutralization tests with hyperimmune guinea pig sera, Oliver strain does not react with the seven other rhinovirus types used in this study.

Neutralization tests were performed by reported methods using 3.2–10 50% tissue culture infectious doses of virus ($TCID_{50}$).[4]

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TABLE I. Neutralizing Antibody Titers to Eight Rhinoviruses of Paired Sera from Volunteers Infected with One of Four Rhinoviruses.

Infecting virus type	Volunteer no.	Antibody titer to indicate rhinovirus serotype							
		13	14	15	16	1A	2	17	Oliver
13	1	**<2**[a,c]	4	16	2	16	32	≥64	≥64
		32[b,c]	2	≥256	2	256	64	≥256	16
13	2	**<2**[c]	32	16	256	8	16	≥64	16
		16	64	64	256	32	128	128	16
13	3	**4**	4	8	<2	16	<2	≥64	64
		32	16	16	<2	64	4	128	32
13	4	**<2**	32	4	256	8	≥64	512	2
		128	16	8	256	8	32	1024	32
13	5	**<2**	4	32	8	32	16	≥64	8
		8	8	128	8	8	64	128	16
13	6	**<2**	64	<2	8	128	≥64	16	4
		32	128	4	64	<128	64	16	8
13	7	**<4**	2	≥16	32	4	32	16	16
		64	<4	32	128	8	16	16	**32**
13	8	**4**	16	128	≥1024	32	128	4	4
		8	32	256	512	32	256	8	8
14	9	2	**<2**	256	≥64	≥64	32	ND[d]	16
		<2	**128**	256	64	128	64		16
14	10	128	**<2**	64	32	≥64	≥64	≥64	<2
		512	**64**	<128	128	128	64	128	<2
14	11	8	**8**	32	32	32	≥64	32	≥64
		2	**128**	128	16	64	128	16	16
14	12	<2	**2**	8	2	32	≥64	<2	<2
		<2	**256**	8	2	32	64	<2	<2
14	13	4	**<2**	≥64	64	≥64	4	≥64	32
		4	**16**	128	32	64	4	128	16
15	14	4	4	**<2**	8	512	4	≥64	≥64
		4	8	**≥256**	4	1024	<2	64	64
15	15	8	<2	**<2**	2	32	≥64	2	≥64
		4	<2	**<2**	<2	64	64	<2	16
15	16	8	8	**<2**	32	4	≥64	16	4
		4	4	**64**	16	16	128	16	4
15	17	4	<2	**<2**	<2	≥64	2	8	<2
		2	<2	**≥256**	<2	64	8	16	<2
15	18	2	2	**<2**	<2	≥64	16	2	≥64
		4	2	**8**	<2	32	8	4	128
16	19	<2	<2	<2	**2**	≥64	≥64	≥64	ND
		<2	<2	<2	**8**	32	32	32	
16	20	2	256	512	**<2**	16	16	≥64	2
		2	128	256	**32**	128	8	32	8
16	21	2	4	8	**2**	16	2	16	ND
		<2	4	4	**32**	8	4	≥256	

TABLE I continued

Infecting virus type	Volunteer no.	Antibody titer to indicate rhinovirus serotype							
		13	14	15	16	1A	2	17	Oliver
16	22	16	512	256	**8**	32	≥64	≥64	16
		64	2048	512	**≥256**	512	64	128	16
16	23	2	32	4	**8**	16	≥64	2	2
		4	64	8	**64**	16	64	8	2

[a] Reciprocal neutralizing antibody titer prior to inoculation.
[b] Reciprocal neutralizing antibody titer 3–6 weeks after inoculation.
[c] Homotypic antibody titers are in boldface type.
[d] ND = Not done.

Each rhinovirus serotype was tested simultaneously against all sera. Those tests in which an endpoint was not determined were repeated when sufficient serum was available.

Results. Neutralizing antibody titers. Neutralizing antibody titers to the rhinovirus type administered and to seven other types are presented in Table I for each of the volunteers. As shown, preinoculation homotypic antibody titers ranged between <1:2 and 1:8. Twenty-one of 23 volunteers developed eightfold or greater rises in homotypic antibody following infection. In one, Volunteer no. 8, a twofold rise in homotypic antibody was detected, and in another, no. 15, no titer change was detected.

As shown in Table I preinoculation heterotypic antibody titers ranged between <1:2 and ≥1:1024, and detectable heterotypic antibody to each rhinovirus type was present in 83–100% of preinoculation sera. Because of an indefinite preinoculation neutralizing titer (usually ≥1:64), 35 of the 158 tests were omitted from subsequent analysis. Three other tests were omitted because of an indefinite postinoculation titer. The number of serum pairs in each category of Table I that were used for heterotypic antibody analyses are shown in Table II, along with total numbers and geometric mean antibody titers. The distribution of titers was such that only two of the 23 volunteers had more than one or two tests omitted from the analyses. Although almost twice as many tests were included from volunteers infected with rhinovirus 13, the mean preinoculation titers for each row (infecting virus) were similar. Similar numbers of neutralization tests, 12-17, remained in each column (virus serotype in neutralization test) with a narrow range of preinoculation geometric mean titers (1:4 to 1:19). Thus, the opportunity to detect heterotypic antibody against each rhinovirus serotype and heterotypic antibody rise for each infecting virus appeared similar.

For the purpose of analysis, titers were ex-

TABLE II. Number of Serum Pairs Used for Heterotypic Antibody Analyses.

Infecting virus type	Number of serum pairs used in heterotypic antibody analyses for indicated rhinovirus serotype								Total no.	Mean preinoculation antibody
	13	14	15	16	1A	2	17	Oliver		
13	—	7	7	7	7	6	4	7	45	1:14
14	5	—	3	4	2	2	2	4	22	1:11
15	5	5	—	5	3	3	4	2	27	1:5
16	5	5	5	—	4	2	2	3	26	1:10
Total no.	15	17	15	16	16	13	12	16	120	
Mean preinoculation antibody	1:4	1:18	1:18	1:10	1:19	1:10	1:7	1:5		

TABLE III. Changes in Heterotypic Antibody in Relation to Virus Type Administered to Volunteers.

Infecting virus type	No. of tests with indicated change (log_2) in antibody titer							Mean change (log_2)
	−2	−1	0	1	2	3	4	
13	0	4	10	16	9	2	3	1.09
16	0	6	8	5	4	1	2	.69
14	1	5	11	2	3	0	0	.05
15	1	9	9	6	2	0	0	−.04

Comparison of means by F test

	F	p
Entire group	6.20	<.001
15 vs 13 14 vs 16	18.60	<.001
15 vs 14	.05	>.20
13 vs 16	1.71	>.20

pressed as logarithms in the base 2, and the differences between preinoculation and postinoculation log_2 titers were calculated.

Changes in heterotypic antibody in relation to virus type administered to volunteers. As indicated in Table III and shown graphically in Fig. 1, volunteers infected with rhinovirus types 14 or 15 showed negligible changes in mean titers of heterotypic antibody. The number and magnitude of changes for these viruses is similar to that previously seen between duplicate serum samples[4]. In contrast, volunteers infected with rhinovirus types 13 and 16 developed significant increases in mean heterotypic antibody titer (F test, $p<.001$).

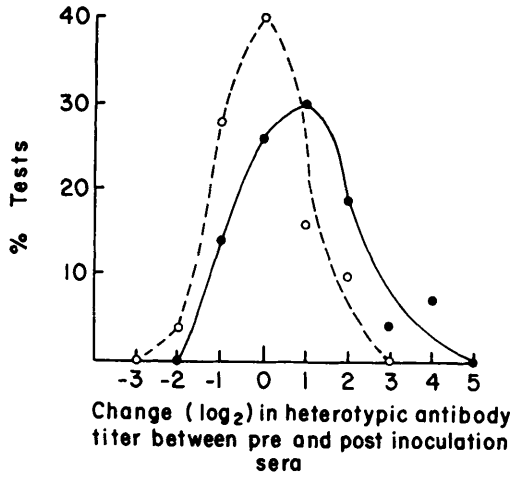

FIG. 1. Distribution of log_2 changes in heterotypic antibody titers between pre- and postinoculation sera from men inoculated with rhinovirus types 13 or 16 (●) compared to changes in titers in sera from men inoculated with rhinovirus types 14 or 15 (○).

Type of heterotypic antibody elicited. To determine if the heterotypic antibody responses were directed towards a particular rhinovirus serotype, the log_2 changes were analyzed according to type of heterotypic antibody elicited. As shown in Table IV, there was a wide variation in mean changes in heterotypic antibody to the seven rhinoviruses tested. Greater mean rises in heterotypic antibody to rhinovirus types 1A and 15 were observed than to the other rhinoviruses, but the differences noted were of doubtful significance (F test, $p = 0.085$).

Relatedness of rhinovirus antigens. The antigenic relationships between the four rhinovirus serotypes used as inocula can be investigated by calculating relatedness values (see footnote *b*, Table V). The relatedness of a pair of identical serotypes tends to be zero whereas increasing antigenic differences between pairs of serotypes are usually associated with increasingly negative relatedness values. There is some suggestion in Table V that rhinovirus types 13 and 15, and rhinovirus types 13 and 16 are more closely related than the remaining pairs, however, overall there are

TABLE IV. Changes in Heterotypic Antibody Following Inoculation of Volunteers with One of Four Rhinovirus Serotypes.[a]

Antibody vs	No. of tests with indicated change (log_2) in antibody titer							Mean change (log_2)
	−2	−1	0	1	2	3	4	
1A	0	1	4	4	3	1	2	1.33
15	0	2	3	5	4	0	1	1.00
17	0	2	4	4	1	0	1	.67
2	1	3	1	4	3	1	0	.62
Oliver	0	2	8	4	1	0	1	.50
14	0	4	5	6	2	0	0	.35
16	0	5	8	0	2	1	0	.13
13	1	5	5	2	2	0	0	−.07

[a] Comparison of means by F test, $F = 1.90$, $p = 0.085$.

marked antigenic differences between all pairs of serotypes.

Effect of magnitude of pre-inoculation heterotypic antibody. The mean preinoculation heterotypic antibody titers (Table II) according to virus type used in testing were compared with the mean changes in corresponding antibody following rhinovirus infection (Table IV). This comparison suggested positive correlation (Spearman rank correlation, $r = 0.814$, $p = 0.06$). A similar comparison of data for those volunteers infected with rhinovirus 13 or 16 revealed similar results ($r = .539$, $p = 0.09$).

TABLE V. Mean Change (log_2) in Serum Antibody Following Inoculation of Volunteers with One of Four Rhinovirus Serotypes.

Serotype of inoculum	Serotype of antibody			
	13	14	15	16
13	**4.25**[a]	0.63	1.88	0.75
14	−0.20	**5.60**	0.80	0
15	−0.40	0	**4.80**	−0.60
16	0.40	0.40	0	**3.80**

	Relatedness value[b]		
	13	14	15
14	−9.42		
15	−7.57	−9.60	
16	−6.90	−9.00	−9.20

[a] Homotypic responses are in boldface type.

[b] Difference between sum of heterotypic mean changes and sum of homotypic mean changes, e.g., relatedness of serotypes 13 and 16 is $-6.90 = (0.75 + 0.40) - (4.25 + 3.80)$. The relatedness value is equal to ½ of the logarithm (base 2) of the "antigenic ratio" described by Chu, Andrewes and Gledhill (11) and, like the antigenic ratio, is not influenced by certain types of nonspecific effects.

Effect of magnitude of homotypic response. To test the possibility that heterotypic antibody responses (Table III) are correlated with magnitude of homotypic response (Table V), the mean changes in each were compared according to infecting virus. Rhinovirus types 13 and 16, which evoked significant heterotypic responses, did not evoke greater homotypic responses than rhinovirus types 14 and 15. In addition, comparison of the rise in homotypic antibody with the mean rise in heterotypic antibody among volunteers who were infected with rhinovirus types 13 or 16 showed no significant correlation ($r = 0.15$, $p > .20$).

Discussion. This study demonstrated the occurrence of heterotypic antibody responses to rhinovirus infection in humans. Infection was induced in normal volunteers with one of four rhinovirus types, 13–16, to which they either lacked or possessed low levels of detectable antibody. Paired sera from these men were tested against 8 rhinovirus types including the infecting type. Significant homotypic antibody responses occurred to all four infecting types. In contrast, significant heterotypic antibody responses occurred only in volunteers infected with rhinovirus types 13 and 16, and the responses to these two types were similar. Since virus shedding, illness, and homotypic antibody responses were similar for

all infecting types, it is suggested that rhinovirus types 13 and 16 are more capable of broadening the rhinovirus antibody response of humans than are types 14 and 15 (9).

There were no significant differences in the heterotypic antibody response among the eight rhinovirus types tested either as a group or for types 13 and 16 alone. However, the differences that were noted tended to be related to the level of preexisting antibody to that particular type; i.e., the higher the mean preexisting heterotypic antibody, the higher the mean heterotypic antibody rise. This suggests that, in addition to immunizing type, prior experience with rhinovirus infection may be important in determining heterologous antibody responses. It is of interest in this regard that when the preselected titers were omitted (usually <2), 83–100% of the volunteers possessed demonstrable preexisting antibody to each of the eight serotypes used in this study, presumably as a result of prior natural infections. This relative frequency is somewhat higher than previously reported (3, 12), and may be explained in part by the higher mean age of the volunteers included in the present study and in part by the sensitivity of the neutralization test procedure.[4] In another previous study preexisting antibody to a variety of rhinoviruses was much less frequent in persons aged 1–4 years than in those aged 18–34 years (13). In addition, essentially no change was noted in heterotypic antibody titers following rhinovirus infection in nine individuals aged 1.5 months to 4 years or seven of nine individuals aged 18–34 years. However, the remaining two older individuals who were infected with rhinovirus type 31 exhibited a change in heterotypic antibody pattern similar to that seen in the present study with rhinovirus types 13 and 16. These results combined with those in the present study suggest that the age range (21–35 years) encompassed by the volunteers in the present study is nearly ideal for determining heterologous antibody responses to rhinoviruses in humans. With such subjects it may be possible to identify serologic groups and superior immunizing serotypes for man.

In a detailed study using cattle and intramuscular injections of adjuvant-virus suspensions, a suggestion of serologic groups of rhinovirus was noted (6). In this study, bovine antisera were prepared to all four infecting rhinovirus types used in the present study and were tested against seven of the eight types. Comparisons to present results in man are complicated by the numerous toxic responses in tests with cattle serum; however, no indication of the suggested relationship noted in the present study between rhinovirus types 13 and 16, and 13 and 15, or the superior immunizing ability of types 13 and 16 were noted in cattle.

Early results with a small number of rhinoviruses (1A, 1B, 2) were interpreted as suggesting that vaccine development was practical (16). However, with the subsequent identification of a large number of distinct serotypes of rhinovirus development of vaccines has been termed impractical (5). The present study does not resolve this difference, but suggests that further exploration of responses in man should be pursued and that if a suitable number of antigenic groups and serotypes capable of inducing broad heterotypic antibody responses in man can be identified, it may be possible to develop an effective vaccine. In this regard, the present study suggests that rhinovirus types 13 and 16 might be useful for this purpose.

Summary. Serum specimens were obtained from 23 male volunteers prior to and after inoculation with one of four rhinovirus serotypes and were tested for the presence of heterotypic antibody responses to seven distinct rhinovirus serotypes as well as for homotypic responses. Twenty-one of 23 volunteers developed eightfold or greater rises in homotypic antibody following infection. Heterotypic antibody to each rhinovirus type was present in 83–100% of preinoculation sera. Volunteers infected with rhinovirus types 13 and 16 developed significant increases in mean heterotypic antibody titers, whereas volunteers infected with rhinovirus types 14 or 15 showed negligible changes in mean titers of heterotypic antibody. There was a tendency for the heterotypic antibody rises to occur for those serotypes with the highest preexisting antibody. In addition, it was shown that the heterotypic antibody response was not related to the magnitude of homotypic antibody response.

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1. Bloom, H. H., Forsyth, B. R., Johnson, K. M., and Chanock, R. M., J. Am. Med. Assoc. 186, 38 (1963).
2. Hilleman, M. R., Hamparian, V. V., Ketler, A., Reilly, C. M., McClelland, L., Cornfeld, D., and Stokes, J., Jr., J. Am. Med. Assoc. 180, 101 (1962).
3. Gwaltney, J. M. and Jordan, W. S., Bacteriol. Rev. 28, 409 (1964).
4. Cherry, J. D., Diddams, J. A., and Dick, E. C., Arch. Environ. Health 14, 390 (1967).
5. Hilleman, M. R., Am. J. Med. 38, 751 (1965).
6. Fenters, J. D., Gillum, S. S., Holper, J C., and Marquis, G. S., Am. J. Epidemiol. 84, 10 (1966).
7. Kapikian, A. Z., Nature 213, 761 (1967).
8. Knight, V., Progr. Med. Virol. 6, 1 (1964).
9. Fleet, W. F., Couch, R. B., Cate, T. R., and Knight, V., Am. J. Epidemiol. 82, 185 (1965).
10. Cate, T. R., Couch, R. B., and Johnson, K. M., J. Clin. Invest. 43, 56 (1964).
11. Chu, C. M., Andrews, C. H., and Gledhill, A. W., Bull. World Health Organ. 3, 187 (1950).
12. Johnson, K. M. and Rosen, L., Am. J. Hyg. 77, 15 (1963).
13. Hamparian, V. V., Leagus, M. B., Hilleman, M. R., and Stokes, J., Jr., Proc. Soc. Exptl. Biol. Med. 117, 469 (1964).
14. Hamre, D., Conelly, A. P., Jr., and Procknow, J. J., J. Lab. Clin. Med. 64, 450 (1964).
15. Mufson, M. A., Kawane, R., James, H. D., Jr., Gould, L. W., Bloom, H. H., and Chanock, R. M., Am. J. Epidemiol. 81, 32 (1965).
16. Mogabgab, W. J., Am. Rev. Respirat. Diseases 88, part 2, 246 (1963).

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Effect of Adenosine, Inosine Triphosphate, Uridine Triphosphate, and Adenosine Triphosphate Upon the Plasma Volume of Intact and Adrenalectomized Dogs* (32726)

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This study is concerned with the marked increase in the plasma volume (PV) of intact and adrenalectomized dogs induced by iv injections of 100 mg of adenosine triphosphate (ATP), compared with the noneffect of similar doses of adenosine, inosine triphosphate (ITP) and uridine triphosphate (UTP).

Methods. Long-term, adrenalectomized, male dogs were used. They had been operated approximately 3 years previously and maintained in normal health by adequate substitution therapy during those periods when the animals were not in actual use as test objects for studies of adrenal insufficiency, stress-induced circulatory failure, or effect of vasoactive drugs. The methods employed for measuring alterations in the blood constituents have been cited in earlier publications from this laboratory (1). The PV was determined according to recommendations of Gregersen and Rawson (2) and Chien and Gregersen (3) using the dye T 1824 and the dye-tinged plasma read at 620 mμ in 10-mm cuvettes on a Beckman DB Spectrophotometer. The test substances were administered iv in 4 ml of water and injected slowly over 7 min; no untoward reactions were observed. The dogs were fasted 18–24 hours before sampling but water was allowed *ad libitum* during this interval. They were trained to lie quietly during the various manipulations required for injecting and withdrawing blood samples. Fifty min elapsed between injection of the compounds tested and sampling via the jugular vein.

Results. Essential data detailing the results obtained from adenosine injections are recorded in Table I A and B. Adenosine had no PV-raising action on either the long-term adrenalectomized or intact animal, instead a decline in PV followed its administration to dogs lacking adrenals. Table I sections C–F record data showing that inosine triphosphate

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