Characterization of Four New Rhinovirus Serotypes[1] (34914)

C. ALAN PHILLIPS, JOSEPH L. MELNICK, AND LINDA SULLIVAN

Department of Medicine, University of Vermont, Burlington, Vermont 05401; and WHO International Reference Centre for Enteroviruses, Baylor College of Medicine, Houston, Texas 77025

Since the rhinoviruses were first recognized as a subgroup of the picornaviruses (1, 2), 89 district serotypes have been recognized (3–5), including 34 recently added in an extension of the numbering system by the Collaborative Rhinovirus Program of the National Institute of Allergy and Infectious Diseases (NIAID) Vaccine Development Branch (VDB) and the World Health Organization (WHO) (6). Included in the latter 34 serotypes are 3 rhinoviruses isolated during a study of acute respiratory tract illness in students (7). One, rhinovirus Baylor 7, has been designated the prototype strain of rhinovirus (RV) 83. Rhinovirus Baylor 5 is similar to CH82 (8), designated the prototype strain of RV56 and rhinovirus Baylor 6 is similar to 6258–CV44 (9), designated the prototype strain of RV64. This report will describe the properties of rhinoviruses Baylor 5, 6, and 7 together with those of Baylor 8, a rhinovirus distinct antigenically from the 89 numbered serotypes. Baylor 8 will be submitted to the Collaborative Rhinovirus Program as a candidate for the third phase of the program.

Materials and Methods. Tissue culture. Methods used in the growth and maintenance of tissue cultures have been described (10, 11). The aorta cells used in the isolation of rhinoviruses Baylor 5 and Baylor 6 were derived from human aorta containing atheromatous plaques and have proved very useful for the isolation and propagation of rhinoviruses (11).

Preparation of antisera. Antisera for rhinoviruses Baylor 5, 6, and 7 were prepared in baboons (*Papio doguera*) using the method of Ocampo and Melnick (12). Antiserum for rhinovirus Baylor 8 was prepared in guinea pigs. Guinea pigs were given 2 intramuscular inoculations of a mixture of 2 ml of purified antigen plus 2 ml of Freund's complete adjuvant 6 weeks apart. They were bled out 1 week following the second inoculation. Antigens were purified by 3 terminal end point dilutions in WI-38 or aorta cells.

Acid lability, ether sensitivity, nucleic acid determination, neutralization tests, and mouse pathogenicity tests. Methods have previously been described (10). Rhinovirus antisera for reciprocal neutralization tests not made in our laboratory were supplied by Dr. Vincent Hamparian, Children's Hospital, Columbus, Ohio. Enterovirus antisera were obtained from the stocks of the WHO International Reference Centre for Enteroviruses. All neutralization tests were performed in tube cultures of WI-38 cells rolled at 33°.

Estimation of size. Rhinovirus Baylor 5 was measured from electron micrographs (13). The sizes of rhinoviruses Baylor 6, 7, and 8 were estimated by filtration methods. Purified rhinovirus preparations were mixed with herpes simplex virus and filtered through a 50-mμ Gelman membrane filter. Filtrates were then titered and identified.

Hemagglutination. The method of Kern and Rosen (14) described for enteroviruses was used. Human group O, Rh positive, pigeon, guinea pig, and chicken red blood cells were all employed.

Results. Virus isolations. All isolations were made from naso-pharyngeal swabs obtained from students in Houston with common colds. Baylor 5 isolates were recovered in cell cultures of aorta and WI-38 in September

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TABLE I. Preparation of Antisera for Baylor Rhinoviruses.

Strain	Passage history	Animal	Reciprocal of neutralization end point	$TCID_{50}$ in test
Baylor 5[a]	WI 8	Baboon	10,000	250
Baylor 6[a]	$A_4 WI_6$	Baboon	4800	50
Baylor 7[a]	$HEK_2 WI_7 A_3$	Baboon	200	100
Baylor 8[a]	$WI_4 A_1 WI_{16}$	Guinea pig	400	500

[a] All viruses used in production of antisera were purified by 3 terminal dilution passages.

and October of 1964, Baylor 6 isolates were recovered in cell cultures of aorta and WI-38 in November and December of 1964 and January of 1965, Baylor 7 was isolated in cell cultures of human embryonic kidney (HEK) in October of 1964 and Baylor 8 was isolated in cell cultures of WI-38 in October of 1964. All strains could be recovered from original naso-pharyngeal swabs after 6 months of storage at —30°.

Properties of the viruses. All viruses were found to have the essential properties of a rhinovirus. Baylor 5 had a buoyant density in cesium chloride of approximately 1.39 g/cm^3 and its virions were 22–23 mμ in diameter. Virions of Baylor 6, 7, and 8 were <40 mμ in diameter. All of the viruses were ether stable, acid labile at pH 3.0 and uninhibited by treatment with IUDR, suggesting that their nucleic acid is RNA. In addition, the viruses were nonpathogenic for mice and failed to hemagglutinate human group O Rh positive, pigeon, guinea pig, or chicken red blood cells.

Neutralization tests. Isolates were originally screened with antisera prepared against rhinoviruses 1A, 1B, and 2 through 55. At least 20 antibody units of each serum was used in all tests against approximately 100 $TCID_{50}$ of virus. When no neutralization was observed, purified antigens were prepared and antisera made (Table I). Reciprocal neutralization tests were then performed using rhinoviruses 1A, 1B, and 2 through 55 and their antisera. In addition, the candidate viruses were tested against 62 enterovirus antisera (polioviruses 1, 2, and 3; group A coxsackieviruses 1–22 and 24; group B coxsackieviruses 1–6; echoviruses (1–7, 9, 11–27, 29–33) and against reovirus 1 antiserum. No neutralization was noted. In a cross-neutralization test, the candidates were also shown to be distinct from each other (Table II).

Candidate rhinoviruses Baylor 5, 6, and 7 and antisera were then submitted to phase II of the Rhinovirus Collaborative Program and tested in reciprocal neutralization tests against other candidate viruses and antisera. Rhinovirus Baylor 8 was not submitted at this time because of insufficient antiserum. Baylor 5 was found to be similar to candidate viruses CH82 and 6660-CV38, Baylor 6 was found to be similar to candidate viruses 6258-CV44 and 1647-63 and Baylor 7 was found to be similar to candidate virus 191-1 (6). In completed reciprocal neutralization tests, Baylor 8 was not neutralized and appears to be distinct from the 89 numbered rhinovirus serotypes.

Discussion and Summary. The isolation and characterization of 4 new rhinovirus serotypes, including the prototype strain of RV83 (Baylor 7), strains of RV56 (Baylor 5) and RV64 (Baylor 6), and a new rhinovirus serotype to be submitted to phase III of the Rhinovirus Collaborative Program have

TABLE II. Cross-Neutralization Testing of Baylor Rhinoviruses.

Virus strains[a]	Antisera[b]			
	Baylor 5	Baylor 6	Baylor 7	Baylor 8
Baylor 5	10,000	<20	<10	<20
Baylor 6	<50	4800	<10	<20
Baylor 7	<50	<20	200	<20
Baylor 8	<50	<20	<10	400

[a] 50–100 $TCID_{50}$ used in test.

[b] ≧20 antibody units used in test.

been described. All of the viruses exhibited the essential properties of a rhinovirus: small size, RNA core, acid lability, and ether stability. All were nonpathogenic for mice and failed to hemagglutinate red blood cells. All were isolated from young adults with the common cold in tissue culture cells of human origin (WI-38, HEK, and human aorta) and all grew best rolled at 33°. Baylor 5 had a buoyant density in cesium chloride of approximately 1.39 g/cm^3. Of particular note is the fact that although 34 new rhinovirus serotypes were just recently added to the numbering system to bring the total number of rhinovirus serotypes classified to 89, Baylor 8 assures the continued expansion of the numbering system.

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