Clinical and Serologic Responses in Volunteers Given Vacuolating Virus (SV_{40}) by Respiratory Route. (26843)

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During studies on experimental infection in adult volunteers with respiratory syncitial (RS) virus(1,2) grown in cultures of rhesus monkey kidney cells it was found that the RS virus pool prepared for use in this work was contaminated with vacuolating virus (SV_{40})(3). This paper describes inoculation by the respiratory route of volunteers with the dually infected inoculum and also inoculation with the dually infected material in which RS component had been neutralized by addition of RS antiserum, reports recovery of SV_{40} from throat swab specimens obtained from inoculated volunteers, and presents serologic data indicating that SV_{40}, when administered by the respiratory route, evokes subclinical infection in man.

Materials and methods. Volunteers. Thirty-five test and 11 control subjects employed were male volunteers between the ages of 21 and 35 years. All were given physical examinations including chest roentgenograms, blood and urine tests before and periodically after exposure to test preparations. For several days before and for 2 weeks after inoculation, all volunteers were isolated, usually 3 to a room in the Clinical Center at the Nat. Inst. of Health, under careful medical surveillance; thereafter, they were available for follow-up studies on an out patient basis.

Virus and virus administration. SV_{40} virus employed in the laboratory aspects of the current study was isolated as a contaminant from a pool of RS virus which had been grown in bottle cultures of rhesus monkey kidney cells. Preparation of the RS virus pool is described in detail elsewhere(4). Briefly, 6 to 11 days after inoculation of RS virus, at a time when specific RS cytopathogenic changes were noted in about 50% of the cells in an inoculated culture, the infected cultures were harvested, and the supernatant fluid obtained following low speed centrifugation was filtered through a 830 mμ gradacol membrane and the filtrate stored in convenient amounts at –60°C until used. This material contained in addition to 320 $TCID_{50}$ of RS virus, 5000 $TCID_{50}$ of SV_{40} virus per ml as measured in grivet kidney cell cultures. It may be noted that RS virus unlike SV_{40} does not readily produce cytopathogenic changes in grivet kidney cell cultures. In one volunteer experiment, the RS component of a sample of the dually infected inoculum was neutralized by addition of undiluted guinea pig serum containing RS antibody and in another sample of the infected material the SV_{40} was neutralized by use of guinea pig serum containing SV_{40} antibody. Infectivity titrations performed before and after addition of the antisera showed that neutralization of the homologous agent was accomplished with no appreciable loss of heterologous virus.

Infected and control (Hanks' BSS) inocula were administered to volunteers by the respiratory route. Approximately 1 ml of the original RS-SV_{40} inoculum was nebulized into the nose and mouth, and with the volunteer in a prone position, an additional 0.5 ml was dropped into each nostril. The nose was massaged between thumb and forefinger by the volunteer following inoculation. After this manipulation excess liquid was discharged. In the experiment employing the virus pool to which was added either monotypic SV_{40} or monotypic RS guinea pig antiserum, the volume of the test material administered was increased to 3.0 ml (2 ml of virus mixture + 1.0 ml of serum) to permit the volunteer to receive the full dosage of 10,000 $TCID_{50}$ of SV_{40} virus, or the full dosage of 640 $TCID_{50}$ of RS virus. In presence of the specific SV_{40} antiserum complete suppression of SV_{40} cytopathogenic changes was demonstrated in tube cultures of grivet kidney cells; similarly, in presence of the RS antiserum, RS virus was completely neutralized in cultures of rhesus kidney and Hep-2 cells.

Safety tests. Aerobic and anaerobic cultures of a sample of the dually infected pool on blood agar and thioglycolate medium failed to show the presence of bacterial contaminants. Tests to detect extraneous viruses in the pool material gave negative results in rabbit kidney cultures; no significant cytopathogenic changes occurred during 3 weeks following inoculation. Rabbits, mice and guinea pigs inoculated by multiple routes with the test material remained free of signs of illness during a 3-week observation period. Inoculation of the test pool into cultures of grivet kidney cells demonstrated the presence of SV_{40} virus. Detailed account of safety tests is given elsewhere(4).

Virus recovery. SV_{40} virus isolation attempts were performed in duplicate tube cultures of grivet kidney cells. Throat and rectal swab specimens were collected just prior to and daily (throat swabs) or every other day (rectal swabs) for 12 days after administration of test and control preparations. The swabs were immediately immersed in 3 ml of Hanks' salt solution containing 0.5% gelatin. After storage for a few days to several weeks at −60°C in plastic capped glass vials, 0.2 ml amounts of each specimen were inoculated into grivet kidney cell cultures. If no specific cytopathogenic effect was observed during 21 days of incubation at 36°C the cells and fluids were passed to new tubes: when such subcultures failed to show changes characteristic of SV_{40} infection the isolation attempt was considered negative. Recovered virus was identified in neutralization tests with 2 antisera, one prepared by us in guinea pigs inoculated with the strain of SV_{40} virus recovered from the original RS virus pool and the other in Dr. Hilleman's laboratory against the prototype strain of SV_{40} virus.

Virus identity tests. Neutralization tests using monotypic guinea pig sera were carried out in Hep-2 and rhesus kidney or grivet kidney cell cultures as described(4); these served to identify the presence of RS and SV_{40} viruses in the dually infected inoculum and showed the absence of other detectable viruses.

Neutralization tests. Serial 2-fold dilutions of serum (inactivated 56°C for 30 minutes) in 0.5 ml amounts were mixed with approximately 250 $TCID_{50}$ of SV_{40} virus contained in 0.5 ml of infected tissue culture material. The mixtures were incubated in a water bath at 37°C for 30 minutes after which 0.4 ml of each mixture which contained about 100 $TCID_{50}$ of virus was added to each of 2 tubes containing grivet kidney cells. The cultures were examined on the 10th day for presence or absence of specific vacuolating effect characteristic of SV_{40} infection. Serum titers are expressed as the highest initial dilution of serum, before addition to equal volume of virus and inoculation into cell culture tubes containing 1.0 ml nutrient-fluid, which completely inhibited specific cellular degeneration.

Results. Clinical evidence of SV_{40} infection. All 8 volunteers inoculated by the respiratory route with a preparation containing 10,000 $TCID_{50}$ of SV_{40} virus in which the RS virus component had been neutralized by RS antiserum remained free of signs and symptoms of illness in the month following inoculation. Two of 8 subjects inoculated by the same route with the RS preparation in which the SV_{40} component had been neutralized by SV_{40} antiserum developed respiratory disease (4). Sixteen of 27 individuals inoculated with a mixture of 640 $TCID_{50}$ of RS virus and 10,000 units of SV_{40} experienced respiratory disease described in detail elsewhere(4), none of the 27 test subjects, however, developed signs or symptoms which differed from those observed in volunteers who received the RS virus mixture in which the SV_{40} component had been neutralized.

Serologic. Neutralizing antibody responses elicited in 35 test subjects by inhalation of 10,000 $TCID_{50}$ of SV_{40} virus are given in Tables I and II. All preinoculation sera obtained from volunteers listed in Tables I and II were devoid of SV_{40} neutralizing antibody at a 1:5 dilution. All 8 recipients of the dually infected material in which the RS constituent was neutralized by addition of RS antibody, developed SV_{40} neutralizing antibody in titers ranging from 1:5 to >1:20 (Table I). Fourteen of the 27 volunteers given inoculum containing both viruses in the active, unneutralized state developed neu-

tralizing antibody against the SV_{40} component in titers comparable to those seen in the volunteers receiving material in which the RS constituent had been neutralized with specific RS antiserum (Table II). None of the 8 subjects given the dual material mixed with SV_{40} antiserum developed SV_{40} antibody during the month following inoculation. Six of the persons listed in Tables I and II who developed SV_{40} antibody following inoculation supplied additional sera 6-9 months later. Four of these still possessed antibody of the same or lower titers; 2, whose original titers had been low, no longer possessed detectable antibody.

Virus isolation. From throat swabs of 2 of the test subjects, SV_{40} virus was recovered on the 7th day, and from another, on the 11th day (Table I). None of the throat or rectal swab specimens taken at other times yielded SV_{40} virus. Each of the 3 isolates was shown to be identical with SV_{40} in neutralization tests with specific antiserum provided by Dr. Hilleman and with antiserum prepared in our laboratory in guinea pigs

TABLE I. Virus Isolation and Serologic Findings in Volunteers Inoculated with Dually Infected Inoculum to Which Was Added Guinea Pig Serum Containing Antibody against One or the Other of the Infected Viruses.

Inoculum*	Volunteer	SV_{40} virus isolation: Throat swab	SV_{40} neutralizing antibody titer: 3 wk	5 wk
SV_{40} and RS viruses + RS antiserum	1	Pos. day 7	>20	ND
	2	" " 7	>20	"
	3	" " 11	20	"
	4	Neg.	10	"
	5	"	10	"
	6	"	10	"
	7	"	10	10
	8	"	5	5
SV_{40} and RS viruses + SV_{40} antiserum	9	Neg.	0	ND
	10	"	0	"
	11	"	0	"
	12	"	0	"
	13	"	0	"
	14	"	0	"
	15	"	0	"
	16	"	0	"

0 = No neutralization at 1:5 serum dilution, the lowest dilution tested.

* In each instance the virus mixture contained 10,000 $TCID_{50}$ of SV_{40} virus and 640 $TCID_{50}$ of RS virus.

TABLE II. Virus Isolation and Serologic Findings in Volunteers Inoculated with SV_{40} and RS Viruses and Control Preparation.

Inoculum*	Volunteer	SV_{40} neutralizing antibody titer: 3 wk	34-38 wk
RS + SV_{40} viruses	17	80	20
	18	80	5
	19	>20	ND
	20	10	"
	21	10	"
	22	5	0
	23	5	0
	24	5	ND
	25	0	"
	26	0	"
	27	0	"
	28	0	"
Hanks' BSS	29	0	"
	30	0	"
	31	0	"
RS + SV_{40} viruses	32	>20	ND
	33	20	"
	34	20	"
	35	10	"
	36	5	"
	37	5	"
	38	0	"
	39	0	"
	40	0	"
	41	0	"
	42	0	"
	43	0	"
	44	0	"
	45	0	"
	46	0	"

0 = No neutralization at 1:5 serum dilution, the lowest dilution tested.

* In each instance the virus mixture contained 10,000 $TCID_{50}$ of SV_{40} virus and 640 $TCID_{50}$ of RS virus.

against the SV_{40} strain recovered from rhesus monkey kidney cell preparation infected with RS virus. All 3 isolates were recovered in cell cultures inoculated with the original clinical specimens. Blind passage attempts failed to yield additional isolates.

Discussion. Our interest in SV_{40} is centered around its potentialities as an infectious agent for man. Sabin has reported evidence which leads him to conclude that SV_{40} when administered orally fails to infect man(5).*

* Our studies on materials made available to us by Dr. Sabin support his idea. These materials consisted of a sample of type 1, live, attenuated, oral, polio vaccine, strain LSC, which had been administered to 12 children whose paired sera were also submitted to us for examination in SV_{40} neutralizing antibody tests. The vaccine contained at least 500

The present experience provides data suggesting that SV_{40} when given by the respiratory route induces subclinical infection in man, manifested by presence of virus in the throats of a few of the subjects 7 to 11 days after exposure, and by development of specific neutralizing antibody in about two-thirds of the volunteers in the weeks following inoculation. The generally low levels of serum antibody detected in the volunteers after exposure to SV_{40} and failure to recover virus from most of the volunteers in the present work suggest that SV_{40} induces only a low grade infection in adults when introduced by the respiratory route.

Summary. SV_{40} virus present as a contaminant in a pool of respiratory syncitial virus prepared in cultures of rhesus monkey kidney cells was inoculated by the respiratory route into 35 adult volunteers: 8 of these received SV_{40} virus preparation in which the RS virus component was neutralized by addition of RS antiserum. None of the test subjects developed signs or symptoms attributable to inhalation of SV_{40}. SV_{40} virus was recovered from throat swab specimens of 3 of the volunteers 7 or 11 days after exposure. SV_{40} neutralizing antibody in titers ranging from 1:5 to 1:80 appeared in the blood of 22 of the 35 test subjects.

$TCID_{50}$ of SV_{40} per ml. None of the 12 pairs of serum, however, contained SV_{40} neutralizing antibody when examined at a 1:5 dilution against 100 $TCID_{50}$ of our strain of SV_{40} virus.

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Effect of Acid Mucopolysaccharides on Hair Growth in the Rabbit.* (26844)

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Histologic studies on the cyclic hair growth in animals have shown a close correlation between activity of the hair follicles and metachromatically staining material in and around the hair bulb(1). The same areas are heavily labeled on injection of $S^{35}O_4$ in the rat(2), though not in the mouse(3). The nature of the presumed sulfated mucopolysaccharide (MPS) has never been determined. The starting point of the present studies was the observation that most, if not all, children with Hurler's syndrome had extremely thick hair and appeared to have a faster rate of hair growth than children of comparable age. Since in Hurler's syndrome there is a characteristic storage and urinary excretion of 2 sulfated polysaccharides, chondroitin sulfate B and heparitin sulfate(4), the possible effect of intradermal injection of these and other polysaccharides on hair was studied in pigmented adult rabbits.

Experimental. The rabbits were kept in individual cages and fed a standard laboratory diet. The hair of the back was clipped. The rabbits were used only if the underlying skin was uniformly unpigmented, to insure as much as possible that all areas of the skin were approximately at the same stage of the hair cycle(5). Several areas of about 2 × 2 cm were marked off in each rabbit. 0.5 ml of different solutions of polysaccharide in

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