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Sero-epidemiological study of reovirus infection amongst the normal population of the Chandigarh area—northern India

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Reoviruses are widely distributed throughout the world, in man and a large number of animal species. Serological evidence of reovirus infection has been reported in non-isolated human communities of many different countries of the world (Taylor-Robinson, 1965) and it has recently been reported even in remote isolated communities such as Hottentots, Eskimos and Micronesians (Brown & Taylor-Robinson, 1966).

Not much is known about the epidemiology of reovirus infection in India. Small numbers of sera were tested by Ramos-Alvarez & Sabin (1956) and Taylor-Robinson (1965), but no definite sero-epidemiological data in different age groups are available to determine its prevalence. The present investigation was undertaken to examine normal persons belonging to different age groups for presence of haemagglutination-inhibiting (HAI) antibodies in their sera against three types of reoviruses with the idea: (i) of studying the incidence of latent or inapparent infection, (ii) of assessing the rate of infection in different age groups, and (iii) of establishing the normal basal titre of HAI antibodies to reoviruses in different age groups.

MATERIALS AND METHODS

Sera

A total of 264 serum samples was collected from apparently normal persons of different age groups.

One hundred and three sera (from the age group 6 months–5 years) and 34 sera (from the age group, > 5–10 years) were collected mainly from healthy children attending a polio-vaccination clinic for the first time at the Institute of Post-Graduate Medical Education and Research, Chandigarh, and also from healthy children of hospital workers.

Twenty-seven sera were collected from children attending a surgical clinic for operation of congenital abnormalities, and from new trainee technicians of the Institute, and also from household servants and relatives of Chandigarh population. These formed the samples from the age group > 10–20 years.

One hundred serum samples of the age group above 20 years came from doctors, nurses, students, technicians and grade IV employees of the Institute. These sera represented a mixed population of different socio-economic status; some came

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from persons of different states of India, who were residing in Chandigarh temporarily.

All sera were stored at −20° C. if they were not tested immediately.

Virus strains

Three prototype strains of reoviruses, type 1 'Lang' strain, type 2 'D 5 (Jones)' strain and type 3 'Abney' strain were kindly supplied by Dr Leon Rosen (Pacific Research Section, National Institute of Allergy and Infectious Diseases, Honolulu, Hawaii).

Culture system and preparation of haemagglutinating antigen

The primary Rhesus monkey kidney cells were grown in monolayer and were maintained in serum-free Eagle's MEM medium as previously described (Pal, Banerjea & Aikat, 1966). The three prototype strains, after four passages in static MK tissue culture tubes and bottles according to the method of Rosen (1964), were passaged for the fifth time in roller drum culture (12 rev./hr.). After complete degeneration of the cell sheet, tissue culture fluid with degenerated cells served as seed virus and haemagglutinating antigen, and was stored at −20° C.

Haemagglutination-inhibition test

Antigen titrations for the prototype strains were made at each passage level with 0·7% human group O red blood cells according to the method of Rosen (1964). The tissue culture fluids at the fourth and fifth passage level were used as antigens in the HAI test, when the HA titres were 1/128, 1/64, and 1/64 for reoviruses type 1, type 2 and type 3 respectively at the fourth passage level, and 1/256, 1/512, and 1/128 for reoviruses types 1, 2 and 3 respectively at the fifth-passage level. To ensure the amount of antigen used in the HAI test during each run, antigen titrations for the three types of viruses were always made on the same day prior to the HAI test. Haemagglutination-inhibition tests were also performed according to the method of Rosen (1964) with the slight modification of using perspex trays instead of tubes. The sera were inactivated at 56° C. for 30 min., and later adsorbed with 25% acid-washed kaolin. Twofold dilutions of sera in 0·2 ml. volumes starting from 1/10 were tested against 4 units of HA antigen contained in 0·2 ml. using unit volume (0·2 ml.) of 0·7% human group 'O' red blood cells. The dilution of serum showing partial haemagglutination was taken as the end point.

RESULTS

Incidence of reovirus infection

A positive haemagglutination-inhibition at 1/10 or higher was considered as evidence of infection according to the method followed by Rosen (1964). The results of the HAI test in Table 1 showed that infection with reoviruses was not infrequent in India. It was found that antibodies to reoviruses irrespective of their types and multiplicity were present in 191 sera out of 264 sera tested (72%).

Amongst these 19 (7%) had antibodies against reovirus type 1, 9 (4%) against type 2, and 56 (21%) against type 3. Antibodies to any two types and to all three types were also recorded (table 1).

Table 1. *Distribution of sera containing reovirus antibodies in different age groups*

		Age group				
		6 months to 5 years	>5–10 years	>10–20 years	Over 20 years	Total
Total no. of sera tested		103	34	27	100	264
Sera with no antibody		47 (46)	10 (29)	3 (11)	13 (13)	73 (28)
No. of sera with antibody against reovirus type:	1	5 (5)	3 (9)	3 (11)	8 (8)	19 (7)
	2	3 (3)	2 (6)	1 (4)	3 (3)	9 (4)
	3	28 (27)	7 (21)	5 (19)	16 (16)	56 (21)
	1+2	3 (3)	2 (6)	4 (15)	6 (6)	15 (6)
	1+3	5 (5)	3 (9)	3 (11)	24 (24)	35 (13)
	2+3	3 (3)	1 (3)	0	3 (3)	7 (3)
	1+2+3	9 (9)	6 (18)	8 (30)	27 (27)	50 (19)
Total positive sera		56 (54)	24 (70)	24 (89)	87 (87)	191 (72)

Figures in parentheses indicate percentages.

Table 2. *Antibody pattern in different age groups according to multiplicity of infection with reoviruses*

	Age group				
	6 months to 5 years	>5–10 years	>10–20 years	Over 20 years	Total
Total no. of sera tested	103	34	27	100	264
Sera with no antibody	47 (46)	10 (29)	3 (11)	13 (13)	73 (28)
Sera with antibody to any one type of reoviruses.	36 (35)	12 (35)	9 (33)	27 (27)	84 (32)
Sera with antibody to any two types of reoviruses.	11 (11)	6 (18)	7 (26)	33 (33)	57 (22)
Sera with antibody to three types of reoviruses.	9 (9)	6 (18)	8 (30)	27 (27)	50 (19)

Figures in parentheses indicate percentages.

Multiple infections in different age groups

After rearranging the results obtained in Table 1, according to the age group and multiplicity of infection, irrespective of type or types of viruses involved, the rate of infection with any one type of reovirus did not differ markedly in different age groups, and did not decrease significantly with increasing age, suggesting thereby that infection with a single virus type might still be occuring in adults. The rate of double or triple infection increased very slowly with age.

Incidence of various types of reovirus antibodies in different age groups

Table 3 shows that, for all ages combined, the commonest reovirus infection was with type 3 and the least common was with type 2.

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Reovirus type 1. The incidence of the type 1 sero-positive persons rose steadily with age up to 20 years, beyond which there was no further increase (Tables 3 and 4). The geometric mean (GM) antibody titre did not vary significantly in the different age groups ($P > 0{\cdot}05$). These findings suggest that infection with type 1 virus had been occurring at all ages up to 20 years and was not confined to childhood. The GM titres between the ages of 25 and 65 showed a slight fall ($P > 0{\cdot}05$).

Reovirus type 2. The incidence of type 2 sero-positive persons was low in all age groups (Tables 3 and 5). It showed a steady rise up to the age of 20, followed by a slow fall between 20 and 65 years. The GM antibody titres were high in the earliest age group and fell steadily throughout life. These differences were highly significant ($P < 0{\cdot}01$). The GM antibody titre against type 2 reovirus was at its lowest in the >30–65 years age group, supporting the findings of Lerner (1963) that type 2 antibody did not persist throughout life at a high titre. It is evident that most infections with type 2 occur before the age of 10 years.

Reovirus type 3. With reovirus type 3 nearly half the children showed antibody before the age of 5 years (Tables 3 and 6). The incidence increased slowly with age up to a maximum in the >20–25 years age group. Above 25 years there was no significant change. The GM titres in different age groups showed no significant difference ($P > 0{\cdot}05$) suggesting that infection with type 3 reovirus occurred at all ages, probably beyond 20 years.

Time of onset of infection

The more or less exact time for the onset of infection with different serotypes was obtained after further splitting the number of sera in the age group 6 months–5 years into three subgroups (Tables 4–6). The GM titre values in these subgroups were not calculated as the frequency distribution of the number of positive sera was too small in different dilutions. The figures in Tables 4–6 show that infection with all three types started between 6 months and 1 year. The numbers of positives had increased considerably for all types in the >2–5 years age group, and it appears that a heavy shower of infection probably occurred after the age of 2 years. This might be attributed to the more frequent exposure of children over 2 to the risk of infection.

DISCUSSION

The etiologic role of reoviruses in human diseases has not been well established. They have been associated with undifferentiated febrile illness with enteritis (Rosen *et al.* 1960 *a*), common cold (Jackson, Muldoon & Cooper, 1961), hepatoencephalomyelitis (Joske *et al.* 1964), diarrhoea in children (Sabin, 1956; Ramos-Alvarez & Sabin, 1958), febrile illness with or without morbiliform rash (Lerner, Cherry, Klein & Finland, 1962), upper respiratory tract infection (Rosen *et al.* 1960 *b*). They have also been isolated from the intestinal tract of healthy children (Sabin, 1959). Probably the majority of reovirus infections run an inapparent course.

A few sero-epidemiological surveys which have been carried out suggest that the infection with reoviruses is world-wide. The incidence of neutralizing antibodies against Cincinnati 'HE' type 4 virus (now known as 'Lang' strain of

Table 3. *Incidence and geometric mean antibody titre of reoviruses in different age groups*

	Age group					
	6 months to 5 years	> 5–10 years	> 10–20 years	Over 20 years	Total	
Total no. of sera tested	103	34	27	100	264	
Sera with antibody against reovirus type 1	22 (21)	14 (41)	18 (67)	63 (63)	117 (44)	
G.M. titre	41	59	49	43	—	$P > 0{\cdot}05$
Sera with antibody against reovirus type 2	18 (17)	11 (32)	13 (48)	39 (39)	81 (30)	
G.M. titre	52	24	21	16	—	$P < 0{\cdot}01$
Sera with antibody against reovirus type 3	45 (44)	17 (50)	16 (59)	72 (72)	150 (57)	
G.M. titre	30	30	20	31	—	$P > 0{\cdot}05$

Figures in parentheses indicate percentages.

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Table 4. *Results of haemagglutination-inhibition (HAI) test with reovirus type* 1

		Age group									
		Age group									
		6 months to 1 year	> 1–2 years	> 2–5 years	6 months to 5 years	> 5–10 years	> 10–20 years	> 20–25 years	> 25–30 years	> 30–65 years	Over 20 years
Total no. of sera tested		34	23	46	103	34	27	44	24	32	100
No. of sera with titres of:	< 10	30	20	31	81	20	9	16	11	10	37
	10	2	1	4	7	1	3	1	4	5	10
	20	1	—	3	4	4	3	8	—	2	10
	40	1	1	3	5	2	3	6	1	11	18
	80	—	—	—	—	3	6	7	6	3	16
	160	—	—	1	1	2	1	3	2	1	6
	320	—	—	3	3	1	2	3	—	—	3
	640	—	1	1	2	1	—	—	—	—	—
Total positive sera		4	3	15	22	14	18	28	13	22	63
		(12)	(13)	(33)	(21)	(41)	(67)	(64)	(54)	(69)	(63)
G.M. titre		—	—	—	41	59	49	54	44	36	43
								$P < 0{\cdot}05$			

Figures in parentheses indicate percentages.

Since the class frequencies in the age group 6 months to 1 year, 1–2 years, and 2–5 years are very small, statistical analysis and geometric mean titre may not be significant and reliable.

Table 5. *Results of haemagglutination-inhibition (HAI) test with reovirus type* 2

		Age group									
		6 months to 1 year	>1–2 years	>2–5 years	6 months to 5 years	>5–10 years	>10–20 years	>20–25 years	>25–30 years	>30–65 years	Over 20 years
Total no. of sera tested		34	23	46	103	34	27	44	24	32	100
No. of sera with titres of:	<10	30	21	34	85	23	14	24	15	22	61
	10	—	2	—	2	5	5	7	3	7	17
	20	1	—	—	1	2	3	8	3	3	14
	40	1	—	6	7	1	3	5	1	—	6
	80	2	—	3	5	2	2	—	2	—	2
	160	—	—	2	2	1	—	—	—	—	—
	320	—	—	1	1	1	—	—	—	—	—
	640	—	—	—	—	—	—	—	—	—	—
Total positive sera		4	2	12	18	11	13	20	9	10	39
		(12)	(9)	(26)	(17)	(23)	(48)	(45)	(37)	(31)	(39)
G.M. titre		—	—	—	52	24	21	19	23	12	16
								$P > 0{\cdot}05$			

Figures in parentheses indicate percentages.

Table 6. *Results of haemagglutination-inhibition (HAI) test with reovirus type* 3

		Age group									
		6 months to 1 year	>1–2 years	>2–5 years	6 months to 5 years	>5–10 years	>10–20 years	>20–25 years	>25–30 years	>30–65 years	Over 20 years
Total no of sera tested		34	23	46	103	34	27	44	24	32	100
No. of sera with titres of:	<10	21	16	21	58	17	11	12	7	9	28
	10	4	2	6	12	5	2	3	3	5	11
	20	5	2	5	12	3	7	7	5	4	16
	40	2	1	6	9	4	3	13	6	12	31
	80	1	1	4	6	4	2	8	2	1	11
	160	1	1	4	6	1	1	—	1	—	1
	320	—	—	—	—	—	1	—	—	1	1
	640	—	—	—	—	—	—	1	—	—	1
Total positive sera		13 (38)	7 (31)	25 (54)	45 (44)	17 (50)	16 (59)	32 (73)	17 (71)	23 (72)	72 (72)
G.M. titre		—	—	—	30	30	20	39	30	30	31
								$P > 0{\cdot}05$			

Figures in parentheses indicate percentages.

reovirus type 1) among the normal population of Cincinnati was 10 and 63 % in normal children (1–5 years) and adults (20–30 years) respectively. It was 26 % among Indian children, 1–4 years of age (Ramos-Alvarez & Sabin, 1956). In Boston and in San Juan the incidence of infection with reovirus types 1, 2 or 3 was as high as 60 % (Lerner, 1963). In England, a significant titre of HAI antibody against reovirus type 1 was observed in about 44 % of donors in the age group 11–30 years. A little higher incidence (61 %) with type 2 was noticed in the corresponding age group (Taylor-Robinson, 1963). A similar higher incidence of reovirus type 2 (87·9 %) was noted by Schmidt, Tauchnitz & Kuhn (1965). It is of interest that 80–100 % of sera collected from tropical countries including India (Taylor-Robinson, 1965) had antibodies against reovirus types 1 and 2, whereas a lower proportion of sera from temperate zones had antibodies against type 1 (47–67 %) and type 2 (67–68 %).

In the present series, children below 6 months of age were excluded. It is evident that infection with all three serotypes occurred as early as 6 months to 1 year. The presence of serum antibodies in this age group probably does not represent the maternal antibody because passively acquired antibody to reoviruses was lost by the age of six months (Lerner, 1963). A heavy shower of infection probably occurred after the age of 2 years, which might be attributed to the more frequent exposure to infection. With reovirus types 1 and 2, a maximum incidence of 67 % and 48 % respectively was attained by the age of 20 years, whereas with type 3 infection the maximum incidence of 73 % was reached by the age of 25 years. The GM antibody titres to types 1 and 3 also supported the rate of infection at different ages, and persisted almost throughout life at the same level which was attained earlier at the time of the maximum rate of infection. The reovirus type 2 antibody did not persist throughout life like the antibodies to the other two serotypes. This is borne out by a fall in GM titres to reovirus type 2 in higher age groups. The present findings show that the incidence of reovirus type 1 infection is similar to and the incidence of type 2 infection is even much lower than that in Western countries (Taylor-Robinson, 1965).

SUMMARY

Two hundred and sixty-four samples of sera obtained from normal healthy persons belonging to different age groups were examined for haemagglutination-inhibiting antibodies against reovirus types 1–3 to assess the prevalence of reovirus infection in the northern part of India.

Reovirus infection appeared as early as 6 months to 1 year of age. With reovirus types 1 and 2, the maximum incidence of 67 and 48 % respectively occurred by the age of 10–20 years, whereas with type 3 infection the maximum incidence (73 %) was reached by the age of 25 years. The incidence of reovirus type 2 infection in all the age groups was remarkably low in this series. A drop in the incidence of reovirus type 2 infection was also noticed after the age of 20 years.

The difference in geometric mean titre of antibody in different ages against reovirus type 2 was highly significant, suggesting probably that most of the infec-

tion occurred by the age of twenty years. The difference in the geometric mean value of the titres of antibody against types 1 and 3 was not significant in different age groups. The levels of antibody against types 1 and 3 in all the age groups were almost the same, suggesting that infection, specially with reovirus type 3, was occurring in all the age groups even beyond 20 years of age.

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