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A Survey of Reovirus Antibodies in Sera of Urban Children

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ABSTRACT

The incidence of antibodies to reovirus types 1, 2 and 3 was estimated on sera from 512 children between the ages of 1 month and 10 years. Delineation of age groups at intervals of one year allowed the inclusion of 25 to 30 sera per group. The decline of maternal antibody to types 2 and 3 was demonstrated but the pattern of type 1 suggested an epidemic of infantile infection by that virus during the time the sera were collected. The initial gradual rise in incidence of antibodies changed to a more rapid rise during early school years which, however, settled to a more moderate increase as the children approached 10 years of age. At that time, the incidence of antibodies for all three types of reovirus was between 50 and 60%.

SOMMAIRE

La fréquence des anticorps au réovirus des types 1, 2 et 3 a été évaluée sur des sérums de 512 enfants âgés de un mois à 10 ans. La fixation des groupes d'âge à un an d'intervalle a permis d'inclure 25 à 30 sera par groupe. La chute de l'anticorps maternel aux types 2 et 3 été constatée, tandis que la courbe du type 1 a permis de croire à une épidémie d'infection infantile par ce virus pendant la période où les sérums étaient prélevés. L'augmentation graduelle initiale dans la fréquence des anticorps a été suivie d'une augmentation plus rapide pendant les premières années scolaires, à laquelle succéda une augmentation plus modérée à mesure que l'enfant allait vers ses 10 ans. A ce moment, la fréquence des anticorps pour les trois types de réovirus s'établissait entre 50 et 60%.

REOVIRUSES contain three distinctive hemagglutinins corresponding to the three types of virus in the group. An antigen common to members of the group may be determined by complement fixation tests.[1, 2] The original member of the group, reovirus 1, was formerly designated as ECHO 10 virus.

Reoviruses have been isolated from a great variety of animals, including man.[3-12] Further evidence of the wide host range is found in the demonstration of serologic evidence of reovirus infection in bats, trout, and Australian wallabies and quokka.[13, 14]

Investigations of human populations have revealed a high incidence of reovirus infection. Fifty per cent of adult sera and 25% of childhood sera from patients hospitalized for respiratory disease have been reported to contain reovirus antibodies.[15] Pooled adult human sera from Australian populations[14] showed a steady increase in incidence of reovirus antibodies to all types with increasing age. Lerner *et al.*[16] tested antibodies to reovirus 2 in 235 randomly selected patients, from premature infants to adults over 60 years of age, admitted to the Boston City Hospital during 1959-1961. Comparative tests of mothers and their newborn infants in this group showed conclusive evidence that antibodies to all three reovirus types pass freely through the placenta. Passively acquired antibodies to reovirus 2 were largely lost by the age of 6 months, after which these antibodies were found with increasing frequency during early childhood, about 50% of the sera having antibodies to this type by the age of 10 years. The peak incidence was reached in the age group 41-60 years, in which more than 80% of the patients had antibodies to reovirus 2 in their serum.

Because of the high incidence of reovirus infections reported during childhood in countries other than Canada, this study was undertaken to determine whether a similar situation was current in Metropolitan Toronto.

MATERIALS AND METHODS

Serum

Sera from children of the age of 1 month to 10 years were obtained through the courtesy of Dr. L. E. Elkerton and Mr. H. Smith of the Central Laboratory, Department of Health for Ontario. The sera were not associated with virus infections in the donor and were drawn in the nine months from September 1963 to May 1964.

They were heated at 56° C. for 30 minutes and stored at –20° C. until tested at dilutions of 1:16 and 1:64. Each serum was tested individually for antihemagglutinins against reovirus types 1, 2 and 3.

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Virus

Reovirus type 1 "Lang", type 2 "D5 Jones" and type 3 "Dearing" were obtained through the courtesy of Drs. N. Labzoffsky and E. Zalan of the Central Laboratories, Department of Health for Ontario. These viruses were propagated in primary rhesus monkey kidney cultures (Connaught Medical Research Laboratories) containing a maintenance medium of Earle's saline with lactalbumin hydrolysate (0.5%) and yeast extract (0.1%) (ELY) together with calf serum (0.5%). Cultures infected with reoviruses were incubated in roller drums for enhancement of the hemagglutination (HA) titre.[17] After incubation they were frozen and thawed three times and the fluids were then pooled. The HA titre of types 1 and 2 usually was 1:128 or greater and the HA titre of type 3 varied between 1:8 and 1:16. Occasionally a titre of 1:32 could be obtained for type 3. The virus pools were stored at –70° C. For the hemagglutination-inhibition test four hemagglutinating units were used.

Erythrocytes

Human erythrocytes were obtained by mixing fresh blood with an equal amount of Alsever's solution. This suspension was stored at 4° C. and used as a 1% washed saline suspension within one week. Human red cells of donors belonging to blood groups O, A, B and AB were tested to ascertain any differences in their reaction with the three reovirus types. Using any of these blood groups, no difference was observed with any virus type. However, the hemagglutinin tests to be reported were all carried out with type O.

Hemagglutination Inhibition Test

The test was conducted in plastic hemagglutination plates. Each well contained four units of hemagglutinin per 0.2 ml., and 0.2 ml. of the dilution of serum to be tested. Controls for non-specific agglutination by the serum alone contained 0.2 ml. of saline in place of the virus inoculum. Erythrocyte controls containing only 0.4 ml. of saline plus red cells were also included.

The serum-virus mixtures and controls were incubated at room temperature for one hour before the addition of 0.4 ml. of a 1.0% saline suspension of erythrocytes. The reactants were kept at room temperature, and read by sedimentation pattern after 75 minutes. Sera which showed non-specific agglutination were eliminated from the survey.

Interpretation of Reovirus Hemagglutinin Inhibition Tests

Preliminary experiments with reovirus antisera prepared in rabbits indicated that a considerable degree of cross-reaction between the three reovirus types would occur in antihemagglutinin tests. Consequently, in order to make a reasonable estimate of whether the presence in a serum of any particular titre of antibody to a specific reovirus type signified an infection of the donor with that type, it was necessary to appreciate the extent of these cross-reactions. With rabbits hyperimmunized to a single reovirus type, heterologous reactions (if present) occurred at titres at least eight-fold less than the maximum homotypic titre. From the above findings we assumed that antibody titres of 1:16 to a particular reovirus serotype occurred following infection with that virus only in the absence of heterologous antibody.

TABLE I.—THE INCIDENCE OF ANTIBODIES TO REOVIRUS TYPES 1, 2 AND 3 IN SERA FROM CHILDREN IN METROPOLITAN TORONTO

		Per Cent Incidence of Antibodies											
		Single infections			*Total of*	*Double infections*			*Total of*	*Triple infections*	*Any sera with antibodies to:*		
Age	*Number of sera*	*Reo 1*	*Reo 2*	*Reo 3*	*any one*	*Reo 1+2*	*Reo 2+3*	*Reo 3+1*	*any two*	*total*	*Reo 1*	*Reo 2*	*Reo 3*
(Months)													
1 - 2	27	11	22	7	41	19	0	7	26	11	48	52	76
3 - 4	151	23	9	5	37	4	4	3	11	3	32	19	15
5 - 6	55	35	6	15	55	4	2	0	6	0	38	11	16
7 - 8	19	37	5	0	42	0	11	0	11	5	42	21	16
9 - 11	4*	—	—	—	—	—	—	—	—	—	—	—	—
(Years)													
1	25	8	12	0	20	0	20	0	20	8	16	40	28
2	22	9	9	5	23	14	5	5	24	18	45	45	32
3	33	12	12	18	42	3	18	3	24	9	27	42	48
4	21	10	10	24	43	19	10	0	29	10	38	48	43
5	25	8	12	24	44	0	4	4	8	12	24	28	44
6	28	11	21	18	50	0	14	4	18	7	21	43	43
7	25	8	12	20	40	16	8	16	40	12	52	48	56
8	26	15	15	19	42	0	8	8	15	23	46	38	58
9	26	8	16	16	38	8	12	8	27	19	42	54	54
10	25	8	20	8	40	4	20	0	24	16	28	60	48
Sub-total (years)	256	10	13	16	39	6	12	5	23	13	34	44	46
Total:	512	18	11	11	40	5	8	4	17	8	35	33	31

*Too few sera for calculations of incidence.

RESULTS AND DISCUSSION

The data collected from all age groups are shown in Table I. Considering the sera collected from ages 1 to 10 years, 75% had antibodies to one or more reovirus types. Of these, 39% were single, 23% double and 13% triple positives. When calculations were made on the basis of whether or not

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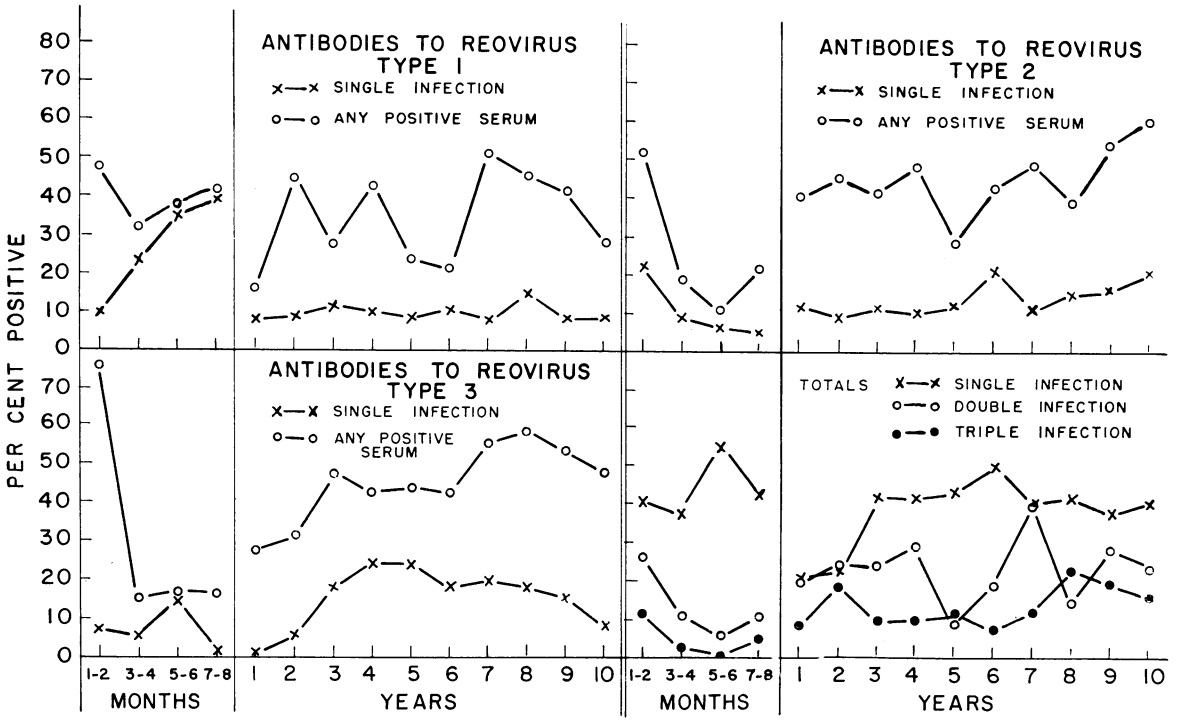

Fig. 1.—Incidence of reovirus types 1, 2 and 3 antihemagglutinins in children aged 1 month to 10 years.

a particular antibody was present without regard to the presence of any other antibody, 34% of sera contained antibodies to reovirus 1, 44% to reovirus 2 and 46% to reovirus 3. Calculations which will include the effect of maternal antibodies, the 1- to 11-month age group, have little effect on these percentages with the exception of decreasing the incidence of reovirus 2 and reovirus 3 antibodies to 33% and 31%, respectively.

Fig. 1 presents a graphic illustration of these results. The fall in incidence of antibodies to reovirus 2 and 3 during the first 12 months after birth indicates the decay of maternal antibody. This is not apparent with reovirus type 1 and probably is a reflection of active infantile infection at this time, by this virus, in this age group. The graph plotting single infection with reovirus 1 bears out this suggestion. After one year the incidence of antibodies may be considered to rise in all three cases to levels approximating 50 to 60% by 10 years of age. The epidemiological significance of dips and peaks in the graph is difficult to assess. They would appear, however, to suggest a prompt increase in incidence shortly after children begin school, which thereafter levels out to a more moderate rate of increase.

In estimating the extent of cross-reactions that occurred, it is possible that the criteria for a specific homotype reaction were too stringent. If this is the case, this survey presents an underestimate of the actual situation. However, our experience with many antisera produced to a single reovirus type has persuaded us that the estimate of cross-reaction, as we have reported, is essentially accurate.

SUMMARY

A survey of the incidence of reovirus antibodies in sera of children in Metropolitan Toronto is presented. The decline of maternal antibody to types 2 and 3 reovirus is demonstrated, whereas the pattern of type 1 antibody suggests a process of active infantile infection during the time sera were collected. The gradual rise in antibody incidence after one year is disturbed by a more rapid rise during the early school years, which, however, settles to a more moderate rate of increase as the children approach 10 years of age.

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