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Serological Studies with Reovirus-Like Enteritis Agent

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The age distribution of antibody to the human reovirus-like enteritis agent, and to the antigenically related Nebraska calf diarrhea virus (NCDV) was studied in serum specimens obtained from 592 children hospitalized in Boston. Sera were examined for complement-fixation (CF) antibody to the human agent, and for CF and indirect immunofluorescence-staining antibodies to NCDV. The curve of antibody frequency was similar in each of the three assays, showing a steep rise in the 6- to 18-month-old age groups; these results indicate the early acquisition of antibody to the reovirus-like enteritis agent. The majority of children and young adults possessed CF- and immunofluorescence-stainable antibodies. There was a significant association between antibody prevalence data obtained with the human CF antigen and with the two NCDV antigens; this association was closest between the human and NCDV CF antigens.

A reovirus-like agent, referred to as an orbivirus, rotavirus and duovirus by various laboratories, now appears to be a major cause of enteric disease in infants and young children (2, 3, 7, 10, 15). The agent has been visualized by electron microscopy in epithelial cells of duodenal mucosa (1) and in stool extracts from infants and young children with gastroenteritis (2, 3, 7, 10, 15). Furthermore, infection is accompanied by a serological response to the agent, as measured by immune electron microscopy, complement fixation (CF), and indirect immunofluorescence (FA) (3, 8-11, 17). Certain assays for serum antibody to the reovirus-like agent are cumbersome and are unlikely to be suitable for large-scale seroepidemiological studies since they require the use of an electron microscope or sections of human fetal intestinal organ culture infected with the agent. However, serological studies appear to have been greatly facilitated by the observation that this agent is antigenically related to a morphologically similar virus, Nebraska calf diarrhea virus (NCDV) (3, 8-11). NCDV, unlike the human reovirus-like agent, readily grows in cell cultures and therefore has been used successfully as a convenient substitute antigen for the human reovirus-like agent in CF and FA tests. Only one preliminary serological survey of the age prevalence of CF antibody to the human reovirus-like agent and NCDV has been reported (11). This survey indicated that approximately one-half of normal infants admitted to a Washington, D.C. welfare institution possessed antibody by 6 to 12 months of age. The present study was undertaken to determine the age distribution of antibody to the reovirus-like agent in hospitalized Boston area children, and to determine the relationship of antibody responses that were observed by three serological techniques.

MATERIALS AND METHODS

Serum specimens. Aliquots of refrigerated sera, collected 1 to 5 days earlier for diagnostic purposes unrelated to this study, were kindly provided by the clinical chemistry laboratory of the Children's Hospital Medical Center of Boston. All specimens were obtained from children who were hospitalized during the months of February, March, and April of 1975. Children hospitalized with acute or chronic inflammatory gastrointestinal disease or hematological disease requiring transfusion were excluded. All children were residents of the metropolitan Boston area. Five hundred ninety-two sera were studied that tested satisfactorily in CF and FA; one serum specimen was studied per child.

Fluorescent-staining antibody tests. Sera were diluted 1:4 in phosphate-buffered saline (PBS), pH 7.4, and studied in indirect FA tests for reactivity against NCDV. The FA test for human antibody against NCDV has been used in several studies (5, 8, 9, 11). The procedure followed described methods (4-6, 11, 14, 16). Briefly, a passage of the Cody strain (6, 14, 16) of NCDV (kindly provided by C. A. Mebus) was grown on primary bovine embryonic kidney (BEK) cell monolayers that were maintained on cover slips bathed in serum-free medium in petri dishes. Twenty-four hours after infection, cells were acetone fixed (4) and reacted in indirect FA tests (4) with human sera followed by a fluorescein-isothiocyanate-labeled goat anti-human globulin conjugate (4). Conjugate (Antibodies, Inc., Davis, Calif.) was used at a 1:32 dilution in PBS; it failed to produce fluorescence when reacted alone with infected cells.

Uninfected BEK cells did not fluoresce when reacted with human sera and the anti-human conjugate. NCDV infection of BEK cells was verified by their brilliant cytoplasmic fluorescence with a fluoresceinated rabbit NCDV antiserum (13) (kindly provided by C. A. Mebus), whereas uninfected BEK cells failed to react with this reagent. Human sera that were recorded as FA positive produced unequivocal fluorescence; all other sera were recorded as FA negative.

CF tests. Sera were tested at a 1:4 dilution in CF against the human reovirus-like agent, according to described technique (10). A 2% stool filtrate (10), derived from a 16-month-old child hospitalized with gastroenteritis, was used as antigen. This filtrate contained the reovirus-like particle, as determined by immune electron microscopy reaction (10) with convalescent serum from the child. Acute illness and 3-week convalescent (paired) sera from this child demonstrated an eightfold rise in CF-antibody titer against the homologous stool particle and a fourfold rise in FA-staining antibody titer against NCDV. Paired sera from two other children hospitalized with gastroenteritis, whose stool filtrates contained the reovirus-like particle, also showed a fourfold or greater rise in CF-antibody titer to the CF antigen and in FA-staining antibody titer against NCDV. Paired sera from six children, whose stool filtrates were negative for the reovirus-like particle, failed to demonstrate a rise in antibody titer to the CF and FA antigens. CF tests were performed by the microtiter method, using 16 to 32 U of antigen.

Sera were also tested at a 1:4 dilution in CF against NCDV according to described technique (11). A strong, positive association between human CF-antibody responses to the human reovirus-like agent and NCDV has already been reported (11). NCDV stock pools were grown and concentrated 25-fold, and the concentrates were used undiluted in CF as described (11). All three children described above, who seroconverted to the human reovirus-like CF antigen, also developed a fourfold or greater CF-antibody rise to the concentrated NCDV antigen.

RESULTS

Age distribution of human antibody. Prevalence of human antibody to the reovirus-like agent and NCDV in different age groups was examined as shown in Fig. 1. Five hundred ninety-two sera were tested for FA-staining antibody to NCDV and for CF antibody to the human reovirus-like agent (Fig. 1, A and B); 428 of these sera were also examined for CF antibody to NCDV (Fig. 1, C). For Fig. 1, A and B, the point for each age group represents results obtained with 50 sera, with the exception of the five age groups from 3 months through 2 years; for each of these age groups, between 28 and 48 sera were tested. For Fig. 1 C, the point for each age group represents results obtained with 28 to 36 sera.

The similar patterns observed with the three serological tests are apparent (Fig. 1). Antibody present in the 0- to 3-month-old age group presumably was of maternal origin. The percentage of sera with antibody diminished for the next several months, and was then followed by a sharp rise in the frequency of antibody. CF antibody to the human reovirus-like agent of presumed maternal origin diminished at a precipitous rate, so that only 3 of 28 sera in the 3- to 6-month-old age group were positive. A fall in FA staining and CF antibodies to NCDV was also apparent; however, the decline was not as precipitous as with antibody to the human agent, and thus the lowest levels of antibody to NCDV were noted in the 6- to 9-month-old age group. It is clear from Fig. 1 that antibodies to the human reovirus-like agent and NCDV were commonly acquired in the 6- to 18-month-old age groups. CF antibodies to the human and calf agents were present in a little more than 50% of the children and young adults tested. FA-staining antibody to NCDV was present in a high percentage of sera tested in the age groups older than 18 months of age (62 to 84%).

Relationship of three antibody tests. The examination of several hundred human sera for antibody responses to the human reovirus-like agent and NCDV, provided an opportunity to compare the prevalence of positive reactions to the human agent with those obtained with NCDV. As shown in Table 1, there was a significant association in prevalence data obtained with the human antigen on the one hand and the two NCDV antigens on the other. This association was closer between the two CF antigens than between the human CF antigen and NCDV FA-staining antigen. Concordance was noted in 333 (56%) of 592 sera examined with the human antigen and NCDV FA-staining antigen, whereas concordance was noted in 286 (67%) of 428 sera tested with the human and NCDV CF antigens. The highest percentage of seropositivity (61%) was noted with NCDV FA-staining antigen, and the lowest percentage of seropositivity (45%) was observed with NCDV CF antigen. The human reovirus-like agent CF antigen detected more positive reactions (56%) than the NCDV CF antigen (45%) among the 428 sera examined by both techniques.

DISCUSSION

These studies indicate that antibody to the reovirus-like agent is acquired at an early age and support the concept that a high infection rate occurs early in life. A majority of the older children and young adults studied possessed antibody to the agent as measured by three separate assays; however, it is not known

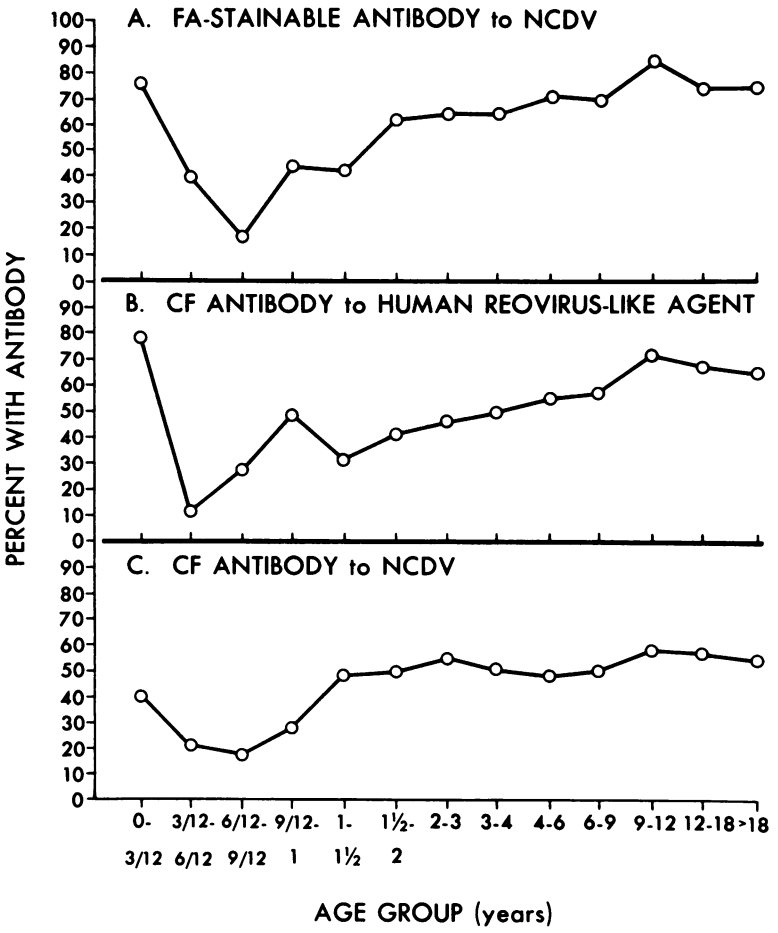

FIG. 1. *Age distribution of human antibody to the reovirus-like agent and NCDV, as measured by three assays.*

whether the antibody present in adults is due to a prolonged antibody response or to reinfection. These results support and expand the findings of a preliminary serological survey of CF antibody in specimens collected from infants and young children in Washington, D.C. during 1954 through 1969 (11); the Washington, D.C. survey has been the only reported study of the age prevalence of antibody to the reovirus-like agent.

Although there was a highly significant association between the results of CF tests with the human and calf antigens, this concordance was not observed with one-third of the sera tested. Analysis of the sera of 110 Washington, D.C. children produced similar findings (11). It is clear that use of NCDV as a CF antigen will tend to miss more serological responses than will use of the human CF antigen. Moreover, there clearly are major differences in the antibody responses detected by the three assays used in this study. It is currently not known whether these differences may reflect varying serological responses to more than one serotype of a human agent.

The widely prevalent human antibody activity to NCDV, particularly exemplified by the high percentage of sera positive for FA-staining antibody, could conceivably have implications for immunoprophylaxis against gastroenteritis produced by the human reovirus-like agent. Should the calf agent be shown to infect man asymptomatically, it could be evaluated as a candidate vaccine strain and the protective nature of circulating antibody to NCDV could later be assessed.

This serological study was performed with specimens obtained from hospitalized children and the results cannot necessarily be extended to other population groups. The purpose of this study was not to perform a detailed survey of the demographic characteristics of the study

TABLE 1. *Comparison of prevalence of serum CF antibody against human reovirus-like agent with FA and CF antibodies against NCDV*

Titer against NCDV	CF-antibody titer against human reovirus-like agent	
	≥1:4 (Positive)	<1:4 (Negative)
FA-antibody		
≥1:4 (Positive)	205[a]	158
<1:4 (Negative)	101	128
CF-antibody		
≥1:4 (Positive)	145[b]	49
<1:4 (Negative)	93	141

[a] Number of serum specimens from children hospitalized at Children's Hospital. $\chi^2 = 8.6$, $P < 0.01$ for concordance between CF antibody against human reovirus-like agent and FA antibody against NCDV; V = +0.121 Kendall coefficient of association (16).

[b] $\chi^2 = 52.6$, $P < 0.001$ for concordance between CF antibodies against human reovirus-like agent and NCDV; V = +0.351 Kendall coefficient of association.

population. However, even though a selected population was studied, the data do help to provide a valuable overview of the age prevalence of antibody in the Boston metropolitan area, and of the inter-relationships between three serological assays. The findings are in substantial agreement with the preliminary findings noted in the Washington, D.C. population (11). Most of the sera examined in this Boston Children's Hospital series were obtained within 3 days of hospital admission, and all but a few patients were hospitalized for nonfatal disease. There were no in-hospital outbreaks of diarrhea during the 3 months that sera were collected, and children with inflammatory gastrointestinal disease or hematological disease requiring transfusion were excluded from the study. Further studies will be necessary to compare serological results obtained in patients from high and low socioeconomic backgrounds.

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