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OC43 Strain-Related Coronavirus Antibodies in Different Age Groups

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Serum antibodies against human coronavirus OC43 in different age groups were measured by complement fixation (CF), haemagglutination inhibition (HI), radial diffusion haemolysis-in-gel (HIG), and solid-phase radioimmunoassay (RIA) methods. Antigen grown in suckling mouse brain was used in all tests. Results obtained by the CF and HIG tests, and the RIA, were in good agreement with regard to the presence or absence of antibodies. Similar results were also obtained with the HI test if nonspecific haemagglutination inhibitors were first removed by treatment with phospholipase C and only titers of 1:20 or greater were considered positive.

Children 6–23 months of age (n = 45) were without measurable coronavirus antibodies in all four assays. A rapid increase in the prevalence of antibodies then occurred in subsequent age groups, and practically all persons 6 years of age or older were found to have OC43 antibodies as measured by the HIG test or the RIA. The mean antibody levels determined by these two methods continued to increase, however, up to the age group of 10–14 years. This increase in antibody levels after the initial antibody incidence plateau may be due to boosting effects caused by related coronavirus strains, since OC43 antigens are known to cross-react with antibodies induced by other human coronaviruses. Taken together, these data suggest that OC43 virus, or an antigenically related coronavirus strain, is very common in Finland.

Key words: coronavirus antibodies, cross-reacting antigens, age-group antibody response, serologic techniques

INTRODUCTION

Coronaviruses [Tyrrell, 1968] cause serious infections in many animal species, examples being hepatitis and encephalitis in mice, bronchitis in chickens, encephalomyelitis in piglets, and severe gastroenteritis in both calves and piglets (for references, see McIntosh

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[1974] and Hierholzer [1976]). In contrast, all of the more than 20 coronavirus strains isolated from man were in association with mild upper respiratory tract infections [Bradburne and Tyrrell, 1971]. Our present knowledge of the epidemiology and the real clinical significance of human coronaviruses may be superficial, however, due to technical difficulties in diagnosing coronavirus infections [Bradburne and Tyrrell, 1971]. Indeed, coronaviruses may also cause non-respiratory diseases in man, since they have recently been reported to be associated with nonbacterial gastroenteritis [Caul and Clarke, 1975] and endemic nephritis [Apostolov et al, 1975].

Most seroepidemiologic studies on human coronavirus antibodies have been carried out using the OC43 virus strain [Bradbourne and Tyrell, 1971] as antigen. This strain has been adapted to grow in suckling mouse brain, where it readily produces haemagglutinin [Kaye and Dowdle, 1969]. Haemagglutination inhibition (HI) antibodies are thought to be relatively specific for the OC43 strain and surveys using the OC43 virus HI test suggest that this virus is a common agent in many countries [Bradburne and Tyrrell, 1971; Kaye et al, 1971; Bradburne and Somerset, 1972; Zakstelskaya et al, 1972; Riski and Estola, 1974]. Complement-fixing (CF) antigens produced by the OC43 virus in mouse brain, on the other hand, broadly cross-react with antibodies elicited by most other human coronavirus strains [Bradburne and Tyrrell, 1971], suggesting that OC43 CF antigen may be suitable for use as a "group-antigen" in the general diagnosis of coronavirus infections.

We have started a survey of coronavirus infections in Finland and report here the prevalence of OC43 virus strain-related antibodies in different age groups. For comparative purposes, the antibodies were measured by the conventional CF and HI serologic techniques as well as the more recently developed radial diffusion haemolysis-in-gel (HIG) and solid-phase radioimmunoassay (RIA) methods.

MATERIALS AND METHODS

Specimens

Sera from 295 patients were selected to evenly cover 15 age groups from 5 months or less to more than 60 years of age. The specimens had originally been collected in 1976 and 1977 for the serologic diagnosis of suspected viral infections. They were stored meanwhile at −20°C and kindly made available by the routine diagnostic laboratory of the Department of Virology, University of Helsinki.

Antigen Preparations

The mouse brain-adapted OC43 virus [Kaye and Dowdle, 1969] was propagated as described previously [Riski et al, 1977]. The infected brains were homogenised by vigorous pipetting in ten volumes of cold Melnick A medium and the suspension formed was then centrifuged at 800g for 30 minutes at 4°C. The clarified supernatant was stored at −20°C and used as antigen in the CF, HI, and HIG tests.

Both OC43 virus-infected and uninfected brain antigens were prepared for use in the RIA. Pooled brain material was placed in 0.02 M sodium phosphate (pH 7.4) containing 0.14 M NaCl (PBS), and the cells were disrupted by 20 strokes in a tight-fitting glass homogenizer at 4°C. The suspensions were then centrifuged at 100,000g for 60 minutes at 4°C. The pellets obtained were resuspended in PBS and stored at −20°C. Upon thawing, homogenous suspensions were obtained by sonication at 4°C. Protein concentrations were deter-

mined by the method of Lowry et al [1951] using bovine serum albumin (BSA) as a standard.

Complement Fixation Assay

The standard method [Palmer and Casey, 1969; Bradburne and Tyrrell, 1971] was used with minor modifications. Serial two-fold dilutions of heat-inactivated sera were incubated overnight at 4°C with 4 units of antigen and 2 units of complement. Binding of complement was assayed by means of sensitized sheep red blood cells. A titer of 1:4 or greater was considered positive.

Haemagglutination Inhibition Test

Nonspecific inhibitors of the OC43 virus haemagglutination were first removed from the sera by treatment with phospholipase C (type I, Sigma, St. Louis) as described by Haukenes and Blom [1975] for rubella virus nonspecific HI inhibitors. Serial two-fold dilutions were then made in Dulbecco's phosphate buffered saline containing 0.4% BSA. Rat erythrocytes (0.75%) in the same buffer were added and haemagglutination was scored after 90 minutes at room temperature. A titer of 1:20 or greater was considered positive.

Radial Diffusion Haemolysis-in-Gel Test

OC43 antigen was kindly mounted in Orivir HIG plates by Mr. P. Heinonen and Mrs. T. Väänänen of Orion Diagnostica Ltd., Helsinki, according to published methods [Väänänen et al, 1976; Riski et al, 1977]. Heat-inactivated sera (5 μl) were pipetted into the wells, and after 20 hours at 37°C the diameter of the haemolysis ring, if any, was measured with a ruler. The smallest haemolysis ring scored positive had a diameter of 2.5 mm.

Solid-Phase Radioimmunoassay

The procedure used was an adaptation of previous assays [Kalimo et al, 1976, 1977; Arstila et al, 1977] with the iodinated antibodies against human Ig and the assay dilution buffers prepared as described more recently [Meurman and Ziola, 1978]. Briefly, OC43 virus antigen and control antigen were diluted in PBS and adsorbed on 6.4-mm-diameter polystyrene balls (Precision Plastic Ball Co., Chicago) at a concentration of 6 μg per ball. After an overnight incubation at room temperature, unadsorbed antigen was removed and the balls were air-dried. Serum dilutions (200 μl) in disposable plastic tubes were tested in duplicate with virus and control antigen balls. After 1 hour at 37°C, unbound immunoglobulins were aspirated away and the balls were washed twice with 5 ml of tapwater. ^{125}I-labeled antibodies against human IgG (heavy-chain-specific) were then added. After 1 hour at 37°C, unbound iodinated antibodies were removed and the balls were again washed. The balls were finally rolled into clean tubes, and bound radiolabel was counted in a Wallac LKB 1280 gamma counter.

Preliminary tests revealed that specific IgG binding to the virus antigen-coated balls did not show a prozone at low dilutions. Consequently, all sera were tested at the single dilution of 1:32 and the cpm difference between virus and control antigen balls was used as a semiquantitative measurement of the actual OC43 virus IgG levels. The assay was standardized in two ways. First, the absolute amount of iodinated antibodies against human IgG per tube was that needed to give 2,500 cpm bound when incubated for 1 hour at 37°C with a ball adsorbed with 5 μg of DEAE-Sepharose chromatography-purified human IgG.

Second, interassay variation was compensated for by relating the cpm difference of the serum specimens to that of a reference serum pool which was included in each assay. A binding difference of 100 cpm or greater was then taken to indicate seropositivity.

RESULTS

Prevalence of Coronavirus Antibodies in Different Age Groups

Sera from individuals of different ages were tested for the presence of coronavirus antibodies by the CF, HI, HIG, and RIA methods. The percentage of individuals found positive in each of the age groups studied is shown in Figure 1. Only one of 20 infants 5 months of age or less had CF antibodies (titer of 1:4) against the OC43 virus. Similarly, only one infant had an HI antibody titer of 1:20. In contrast, the HIG test and the RIA detected OC43 virus antibodies in sera from 8 (40%) and 18 (90%) of these infants, respectively. All 25 children in the two subsequent age groups (6–11 months and 12–23 months) were negative for OC43 virus antibodies in each of the four tests.

A rapid increase in the antibody prevalence was observed in the three age groups comprised of children 2, 3 and 4, and 5 and 6 years old (Fig. 1). By the age of 6 years practically all children had antibodies against the OC43 virus as measured by the HIG test and the RIA. Slightly lower positive incidences were obtained with the CF and HI tests. The high frequency of antibody positivity seen by age 6 years was sustained through older age groups up to the age of 60 years; thereafter, a tendency for the frequency to decline was observed.

Coronavirus Antibody Levels in Different Age Groups

The mean OC43 virus antibody levels were calculated for each age group and are shown in Figure 2. The mean antibody titers measured by both the CF and HI test were low, since titers higher than 1:8 in the CF test or 1:40 in the HI test were uncommon. In contrast, a much greater variation in antibody levels was seen if the HIG test or the RIA were used.

The mean OC43 antibody levels of the different age groups were found to form a distinctive pattern for each of the four antibody assays (Fig. 2). The highest CF titers were found between ages 3 and 9 years, after which there was a slight tendency for the mean titers to decrease. Mean titers obtained by the HI test steadily increased up to the group 10–14 years old and then remained constant until a drop was seen in the over 60 age group. A similar pattern of mean antibody levels was obtained with the HIG test. The change of the mean antibody curve from the increase-phase to the plateau-phase at the age group 10 to 14 years was much more distinct for the HIG test than for the HI test, owing to the greater antibody range measured with the HIG test. An apparently biphasic mean antibody curve was obtained with the RIA. The mean antibody level increased sharply with children 24–35 months old and then plateaued for the 3- to 10-year-olds. A second increase in the mean antibody levels was then observed after 10 years of age.

DISCUSSION

Several points concerning the present results deserve comment. It is obvious that coronavirus infections are widespread in Finland, as essentially everyone older than 5 years had measurable antibodies against the OC43 virus antigen. In light of this, the complete lack

of coronavirus antibodies in the 6- to 23-month-olds was an unexpected finding. This observation may be explained by two different epidemiologic situations: Either children younger than 2 years do not contact coronavirus infections or, more likely, there has been very little coronavirus circulation in Finland during the last two years. If the latter is true, sera collected some years ago from children younger than 2 years (ie, from the age-mates of the 5-to 6-year-old children of this study) should possess coronavirus antibodies much more frequently than was found for the 6- to 23-month-olds in this study. This question will be answered as soon as appropriate specimens have been assembled from various serum archives.

Another epidemiologic question arises when the age group plots of antibody frequency and the mean antibody levels are compared. While the antibody frequency curve reaches a maximum at about 6 years, the mean antibody levels continue to increase up to the 10–14 years group. This difference can readily be seen with the HIG test results and is particularly evident with the RIA results owing to the biphasic nature of the mean antibody level curve. It has been suggested that the OC43 virus typically infects preschool children while the 229E strain is more frequently associated with infections of adolescents and young adults [Bradburne and Tyrrell, 1971]. If this is true, the two rapid increases in the RIA mean antibody

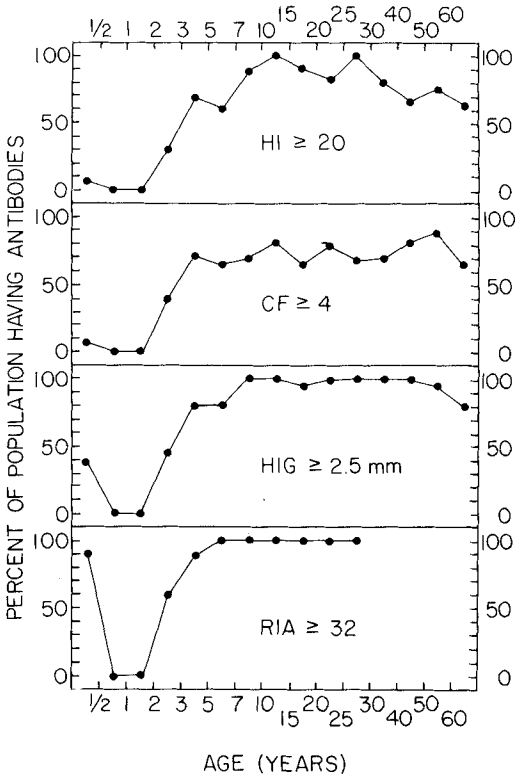

Fig. 1. Occurrence in different age groups of serum antibodies reacting with coronavirus OC43 antigen. The number of sera tested was 18 or more in each age group, except for the groups 6–11 months and 12–23 months of age, where the number of specimens was 15 and 10, respectively. HI) haemagglutination inhibition; CF) complement fixation; HIG) radial diffusion haemolysis-in-gel; RIA) solid-phase radioimmunoassay.

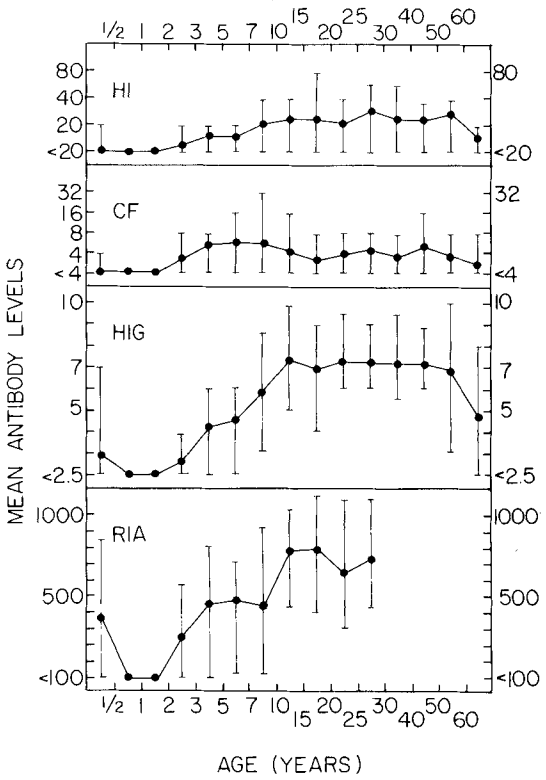

Fig. 2. Mean levels of OC43-related coronavirus antibodies in different age groups. Two top panels show the geometric means of HI and CF titres, respectively. In the HIG, an arithmetic mean of the diameters of hemolysis rings (mm) induced by individual sera is shown for each age group. In the RIA, all sera were tested at a single dilution (1:32) and bound Ig was quantitated by ^{121}I-labeled antihuman Ig. The figures shown are arithmetic means of bound ^{121}I cpm. Vertical bars represent the 95% confidence limits of variation. For further details see Methods.

levels could be due to two age-related coronavirus epidemics. It is more likely, however, that the second epidemic wave was not caused by the 229E virus, but by a third human coronavirus strain, since cross-reactions between the OC43 and 229E strains were not very strong in the RIA. For instance, we found several sera with relatively high antibody levels against the 229E virus which did not react at all with OC43 virus antigen-coated balls and vice versa (unpublished results). It is also possible, of course, that the second sharp mean antibody increase seen with the RIA results is simply due to reinfection by the OC43 virus strain.

The present results provide further evidence of the presence in sera of nonspecific inhibitors of coronavirus haemagglutination [Hovi, 1978]. For example, most of the sera of children 6–23 months of age negative in the RIA all had a moderate titer in the HI test if the sera were not pretreated before testing (data not shown). The phospholipase C treatment does not, however, completely remove the nonspecific inhibitors, since all children 6–23 months of age had an apparent HI titer of 1:10 even after treatment.

An important aspect of the present study concerns the comparison of the different serologic methods employed. The HI test has been used relatively widely for serologic screening of OC43 virus antibodies. Rooster red blood cells were originally used in our labora-

tory to titer the virus haemagglutinin [Riski and Estola, 1974]. Large variation in the sensitivity of erythrocytes from different birds was subsequently found, however, and a change to rat erythrocytes was made [Hovi, 1978; also see Kaye and Dowdle, 1969]. Use of rat erythrocytes and phospholipase C pretreatment of the sera has resulted in a more accurate and reproducible test [Hovi, 1978].

Although most of the individuals in the present study had coronavirus OC43 HI antibodies, the titers were low, suggesting that most of the OC43 infections had occurred some time before. The low HI titers in the present sera thus were in contrast to the moderately high OC43 virus HI titers of 1:160 to 1:320 that have been found more recently in some convalescent sera. Titer variation found with the OC43 virus CF test was similar to that found with the HI test. The majority of the sera had CF antibodies, but titers higher than 1:16 were rare. As in the case of HI antibodies, titers as high as 1:128 or 1:256 have been measured in certain convalescent sera.

Much greater variation in OC43 virus antibody levels was found when the HIG or RIA methods were used. This probably is a reflection of the greater sensitivity of these two methods compared to the HI and CF tests. It is also possible that the HIG test and the RIA detect antibodies against more of the OC43 virus antigens than do the two older serologic methods. Consequently, apart from usefulness in seroepidemiologic surveys such as the present one, the HIG and RIA methods may also be valuable tools in the diagnosis of acute coronavirus infections. Preliminary application of these two assays in this regard has yielded very promising results.

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