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Diagnosis of Human Coronavirus Infection by Immunofluorescence: Method and Application to Respiratory Disease in Hospitalized Children

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Rabbit antisera were prepared against coronavirus strains 229E and OC43 and used successfully to detect viral antigen in epithelial cells shed from the nasopharynx of symptomatic volunteers who had received coronavirus inocula three to four days before. The same serologic reagents were applied to nasopharyngeal secretion cells obtained from 106 infants and children hospitalized with respiratory tract disease and apparently not infected with conventional respiratory viruses. No coronavirus infections were detected by this method. It appears that coronavirus OC43 or 229E infections were not common in children in Tyneside hospitals during the period of study. However, fluorescence is a useful method for detection of coronavirus infections in symptomatic human subjects.

Key words: coronavirus infection, immunofluorescence, respiratory disease

INTRODUCTION

Human coronaviruses were first recovered from adults with colds in the early 1960s [5, 16]. Their culture required unusual measures: either blind passage in tissue culture or inoculation of human embryonic tracheal organ cultures. Since that time, seroepidemiological studies have implicated coronavirus infection in approximately 15% of all adult colds [12]. Volunteers receiving suspensions of coronavirus developed colds differing in certain minor respects from those following rhinovirus inoculation [2]. Infants and children are also subject to natural infection, but the disease spectrum in this age group has been difficult to define.

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Because of the formidable difficulties of culturing human coronaviruses from clinical specimens [10, 16], all the previous studies in infants and children depended primarily on seroepidemiologic methods. We considered that rapid diagnostic immunofluorescence methods [4] might offer a unique opportunity to survey a large pediatric population for coronavirus infection while avoiding the insensitivity and inaccuracy of seroepidemiology and the expense and tedium of organ culture virus recovery [10, 16] or multiple blind passage in tissue culture [5].

MATERIALS AND METHODS

Coronavirus Antisera

Strain 229E [5], originally obtained from Dr D. Hamre and purified by terminal dilution, was grown in 32-oz bottles containing human embryonic lung fibroblast (HELF) cells with Eagle's minimal essential medium (MEM) supplemented with 2% fetal calf serum (FCS). Rabbits were immunized using the method of Gardner and McQuillin, and sera were absorbed with cell packs of both uninfected HELF cells and HEp-2 cells [4].

Strain OC43 [10] was grown in suckling mouse brain using mouse hepatitis virus-free Swiss CD-1 mice from Charles River Farms [9]. Ten percent brain suspensions were clarified by low-speed centrifugation and the virus adsorbed to and eluted from human type O erythrocytes [6]. This material was then used for immunization of rabbits. Absorption of sera was carried out both with mouse brain-liver homogenates and HEp-2 cells.

Specificity of both sera was assured by strong fluorescence in homologous systems (MRC-5 cells grown on coverslips and infected with 229E, and MRC-5 cells or frozen sections of mouse brain from suckling mice infected with OC43) and absence of fluorescence in tissue cultures or frozen human nasopharyngeal secretion (NPS) specimens infected with influenza A or B, parainfluenza 1, 2, 3, 4a, or 4b, adenovirus, respiratory syncytial virus, rubella virus, measles virus, or mumps virus. A cross-reaction was shown between anti-229E antiserum and either OC43-infected mouse brain or OC43-infected MRC-5 cells. Antiserum to OC43 did not, however, cross-react with 229E.

Patients and Specimens

Adult volunteers at the Common Cold Research Unit, Salisbury, were used; the general methods, including inoculation of virus-containing nasal drops, have been described [1, 15].

Viruses for inoculation of volunteers were prepared from nasal washings from other experimentally infected, isolated volunteers. 229E had undergone two passages in human embryonic nasal or tracheal organ culture and four passages in isolated volunteers since receipt from Dr D. Hamre. OC38 (antigenically identical to OC43 [11]) and OC44 (antigenically closely related to OC43 [11]) had each been passaged once in human embryonic nasal or tracheal organ culture and once in volunteers. Specimens for staining by the fluorescence method were obtained from nasal washings collected on the third or fourth day after virus inoculation. Phosphate-buffered saline (10 ml) was instilled in small amounts alternately into each nostril, and the expelled fluid and nasal secretions were collected; these were kept on melting ice for not more than 30 minutes before centrifugation at 1,500 rpm for ten minutes at 4°C. The deposited cells were thereafter prepared as described by Gardner and McQuillin [4].

The nasal washings from volunteers inoculated with 229E and OC44 were cultured in a 229E-sensitive continuous cell line (MRC-C) obtained from Dr A.F. Bradburne.

NPS specimens from infants and children admitted with respiratory tract disease to the Tyneside hospitals during 1969–1974 were inoculated into three tissue culture types (HELF, HEp-2 or Bristol HeLa, and rhesus monkey kidney). In addition, slides of shed nasopharyngeal cells were prepared for and examined by immunoflourescence methods for respiratory viruses [4]. Extra acetone-fixed slides were stored at –40°C. Coronavirus studies were performed only in specimens where routine virus tests had failed to uncover a virus. The tests for coronaviruses were performed in 1974 after storage at –40°C for four months to five years. An effort was made to test children of all ages, with a variety of respiratory syndromes, and entering the hospital at all seasons of the year.

The clinical diagnoses used in this analysis were those written on laboratory forms by physicians at the time of submission of the specimen.

Examination of Slides

All slides were stained by the indirect method using anti-coronavirus rabbit sera and fluorescein-conjugated anti-rabbit globulin prepared by Burroughs-Wellcome. Specimens described as negative had been carefully examined by at least two of us (K.M. and either J.M. or P.S.G.).

Serologic Survey

Paired sera from 66 infants and children obtained during 1970–1974 and single sera from a further 81 were successfully tested by complement fixation for antibody to coronavirus strains 229E and OC43. Sera were screened at a 1:8 dilution and, if positive, titrated from 1:4.

RESULTS

As shown in Table I, specimens were examined from three adult volunteers who received strain OC38, three who received 229E, and four who received OC44. Positive specimens from volunteers receiving all three coronaviruses stained identically when a homologous or closely related antiserum was used. Epithelial cells contained bright-green particulate cytoplasmic fluorescence (Fig 1). Shed cells from all three volunteers receiving OC38 and one receiving OC44 stained with anti-229E rabbit serum. In these instances heterologous fluorescence was judged 2+ in brightness, whereas that observed with the homologous serum was rated 4+.

Nasal washings from volunteers given 229E or OC38 were cultured in MRC-C cells; samples from two of the three 229E-inoculated subjects gave typical 229E cytopathic effect, and in the third subject the result was equivocal.

Serologic screening by complement fixation (CF) antibody to 229E and OC43 showed a low level of antibody prevalence among children in the Newcastle area. Seven of 147 sera (5%) contained CF antibody to strain 229E and 17 of 147 (12%) to OC43. No rises in coronavirus antibody were detected among the 66 paired sera tested.

NPS specimens from 106 infants and children were examined by fluorescence methods for coronavirus antigens. However, none of the pediatric specimens contained detectable coronavirus antigens (Table II). The stability of coronavirus antigens under the storage conditions used was unknown at the time of the study, but we have subsequently

TABLE I. Inoculation of Human Coronaviruses Into Volunteers: Shedding of Fluorescence-Stainable Cells During Colds

Virus inoculated	Severity of experimental colds					Day of specimen	Volunteers with NPS cells staining with indicated antiserum (No. positive/No. tested)			Number of washings producing cytopathic effect in 229E-sensitive tissue cultures
	Severe	Mod.	Mild	Very mild	Nil		Anti-229E	Anti-OC43	Human convalescent	
OC38[a]	0	0	1	1	1	4 or 5	3/3	3/3	NT[c]	NT
229E	0	0	0	3	0	4	2/3	0/3	NT	2/3
OC44[b]	1	1	1	1	0	3	1/1	1/1	3/4	0/2

[a] Antigenically identical to OC43 [11].

[b] Antigenically closely related to OC43 [11].

[c] NT = not tested.

TABLE II. Results of Tests for Coronavirus Infection of Children

Age (years)	No. of infants and children in each category whose NPS specimens were examined for 229E and OC43 antigens.							
	Upper respiratory tract infection		Pneumonia		Other lower respiratory tract disease[a]		All	
	Examined	Positive	Examined	Positive	Examined	Positive	Examined	Positive
< 1	26	0	10	0	15	0	51	0
1–2	21	0	4	0	5	0	30	0
3–12	8	0	14	0	3	0	25	0
All	55	0	28	0	23	0	106	0

[a] Includes bronchitis, bronchiolitis, exacerbations of cystic fibrosis, croup.

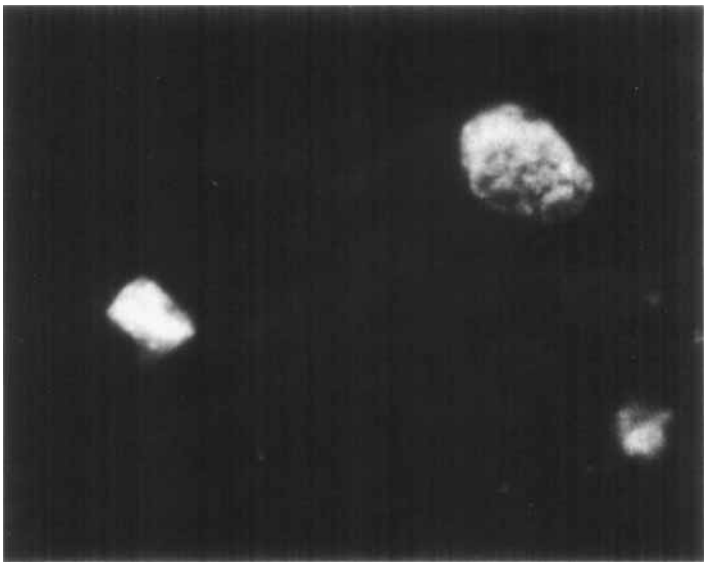

Fig 1. Bright cytoplasmic fluorescence in a nasal epithelial cell shed on the fourth postinfection day in a volunteer inoculated with OC38; stained with anti-OC43 rabbit serum and anti-rabbit conjugate.

examined NPS specimens from the volunteer study outlined above after 3.5 years in storage, and fluorescence has not noticeably faded.

DISCUSSION

This study has shown that infection with coronaviruses 229E and OC43 was an uncommon event among infants and children hospitalized with respiratory tract infections in Tyneside between 1969 and 1974. Moreover, coronavirus activity during this time was not completely absent in children, since CF antibody was found to both 229E and OC43 in a small proportion of the sera tested. The proportion of those with anti-229E CF antibody (5%) was greater, and those with anti-OC43 antibody (12%) somewhat less, than those found among children in Washington, DC by McIntosh et al [12] (0.6% and 29%, respectively).

Previous studies of coronaviruses in children have disclosed a variable incidence of infection and have not clearly established the disease spectrum. One seroepidemiologic study failed to show a significant association of infection with lower respiratory tract disease (LRTD) in children [12]. In another study which examined only hospitalized infants under 18 months there was an 8.3% incidence of coronavirus infection (detected by serology) in those with LRTD, and viruses resembling strain 229E were recovered from respiratory tract cultures obtained on admission to hospital from two infants with pneumonia [13]. Still another serologic survey disclosed that asymptomatic coronavirus infection was common in children [7, 8]. It appears from these and our own studies that coronaviruses are probably not an important cause of serious respiratory tract diseases in children, although lesser infections are, at least in some parts of the world, common in this age group [14].

In view of the excellent results from volunteers inoculated with the two viruses and the closely related strain OC44, it is clear that immunofluorescence of respiratory tract

cells in coronavirus infection represents a potent new tool for the study of suitable adult and pediatric patients with respiratory infection. The method, if properly controlled, and preferably with serologic confirmation, obviates the need for expensive and tedious organ culture systems for virus isolation.

The demonstration of a one-way cross-reaction between strains 229E and OC43 in tissue culture, infected mouse brain, and nasopharyngeal cells from volunteers confirms the findings of Bradburne [3]. It gives further evidence of the serologic relatedness of many coronaviruses of both man and animals [3, 11].

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