Articles

Acute *Chlamydia trachomatis* Respiratory Infection in Childhood

Serologic Evidence

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• Serum samples from 184 infants and children whose blood was drawn during a clinic visit were tested for antibody to *Chlamydia trachomatis*, Epstein-Barr virus, and cytomegalovirus. Lifetime illness history was obtained from clinic records. Fifteen percent had anti–*C trachomatis* IgM antibody. Anti–*C trachomatis* IgM without IgG was significantly associated with upper respiratory tract syndromes within the 14 days prior to phlebotomy in 6- to 10-year-old patients. This association was not due to polyclonal activation from Epstein-Barr virus infection. A definitive study of chlamydial illness in children rather than infants appears to be indicated.

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Chlamydia trachomatis is now well established as a sexually transmitted pathogen of major significance. Its role in male urethritis and proctitis, female cervicitis and salpingitis, and infant conjunctivitis and pneumonia has been elucidated. Infant chlamydial infection has been suggested to result in otitis media[1,2] and chlamydial pneumonia in chronic childhood lung disease.[3,4] In addition, three seroepidemiologic studies[5-7] in children have shown an increasing prevalence of antichlamydial antibody with age, indicating the presence of active childhood chlamydial infection beyond infancy. In only the third study[7] was an association between chlamydial serologic findings and a clinical childhood illness identified, namely, pneumonia in children over 6 months of age.

The current seroepidemiologic study was, therefore, performed in an attempt to identify specific illnesses associated with serologic evidence of childhood chlamydial infection. Serologic assays for antibodies to Epstein-Barr virus (EBV) and cytomegalovirus (CMV) were also performed on the serum samples to investigate the role of polyclonal activation of B lymphocytes in the production of anti–*C trachomatis* antibody.

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PATIENTS AND METHODS

Sample

The sample studied included 184 age-selected children whose blood was drawn for any reason during a pediatric clinic visit to Crownpoint Indian Health Service Hospital, a regional Indian Health Service facility located in Crownpoint, NM. An attempt was made to achieve an even distribution of serum samples by age from children from infancy to 15 years old. All children studied were regular patients of this pediatric service, so that complete medical records were available for review. Visits occurred between September 1981 and October 1982.

Medical History

Charts were abstracted for the period from birth to the phlebotomy visit. For each clinic or emergency room visit, the following information was recorded: date, age, physician diagnosis, presence or absence of fever, and disposition. Major illness diagnostic categories were upper respiratory tract infection (URI): acute otitis media, pharyngitis, with or without group A streptococcus identified, conjunctivitis, and viral syndrome; lower respiratory tract infection (LRI): croup, bronchiolitis, pneumonia; gastroenteritis; urinary tract infection; trauma; rash; and miscellaneous other diagnoses, including trachoma. Viral syndrome was included in URI because, in the charts, it was almost exclusively used to describe upper respiratory signs and symptoms without a further specific diagnosis.

Chlamydia trachomatis

Serum samples were tested by the simplified microimmunofluorescence method of Wang and colleagues,[8] using three antigen pools: C'HI, BED, and KGF serovars. A positive IgG was defined as 1:32 or greater on any pool, and a positive IgM as 1:16 or greater. Serologic evidence of acute recent chlamydial infection (within 14 days prior to phlebotomy) was defined as the presence in a serum sample of anti–chlamydial IgM without anti–chlamydial IgG.

EBV–Viral Capsid Antigen IgG

For EBV–viral capsid antigen IgG the test employed was an immunofluorescent test in cells derived from Burkitt's lymphoma.[9] A titer of 1:10 or greater was defined as positive.

EBV–Early Antigen IgG

For EBV–early antigen IgG the method employed was indirect immunofluorescence with NC37 cells on slides. A titer of 1:10 or greater was defined as positive.

EBV–Viral Capsid Antigen IgM

For EBV–viral capsid antigen antibody was detected by immunofluorescence.[10] A titer of 1:10 or greater was defined as positive.

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CMV–Indirect Hemagglutination

Total serum hemagglutinating antibody was detected by the method of Waner et al.[11] A positive test was regarded as a titer of 1:8 or greater.

CMV–Specific IgM and IgG

A modification of the enzyme immunoassay method of Voller et al[12] was used. The AD-169 strain of CMV was employed to sensitize 96-well microtiter plates. Serum samples, alkaline phosphatase–conjugated goat antihuman IgG or IgM, and *p*-nitrophenyl phosphate were added in order. Wells were read at 405 mm. Results were expressed as units read from a standard curve established with reference samples. Serum samples were absorbed with *Staphylococcus aureus* protein A to remove IgG before IgM testing.

STATISTICAL ANALYSIS

Children positive and negative for *C trachomatis* antibody were compared for illness history using Fisher's exact test. Serologic studies were compared using Fisher's test and *t* tests for the differences in geometric mean titers. Illnesses were compared by individual category and where appropriate (URI, LRI) by group.

RESULTS

Ninety-five (51.6%) of the 184 children in the sample were girls. Acute illness was the reason for phlebotomy in 118 children (64.1%) and routine physical examination in another 49 children (26.6%). Thus, these two categories accounted for 90.7% of phlebotomies. One hundred sixteen (63%) of the blood samples were drawn in February and March 1982; 91 (78%) of these were for illness visits. The remainder of the bleedings were relatively evenly distributed through the remainder of the study period. The most common illness diagnoses for which blood samples were drawn were pharyngitis (45 of 118), URI (35 of 118), otitis media (eight of 118), viral syndrome (three of 118), and gastroenteritis (three of 118).

The sample was evenly distributed by age group (9% to 20% per two-year interval), except for some underrepresentation of 0- to 2-year-old children (7%) and 15-year-old children (4%). The majority of phlebotomy visits in all age groups except 3- to 4-year-old children were for illnesses, with an excess of routine visits for preschool examinations in the 3- to 4-year-old and 5- to 6-year-old children.

CHLAMYDIAL SEROLOGIC RESULTS

Fifty-eight children (31.5%) had antibody to *C trachomatis*. Within this group, 51.7% (30/58) had specific IgG but not IgM, 31.0% (18/58) had IgM but no IgG, and the remaining 17.3% (10/58) had both classes of antibody measurable. There were no statistically significant differences in the antibody prevalence by sex or reason for visit.

AGE- AND SEX-SPECIFIC SEROPREVALENCE RATES

Sample sizes by two-year age groups ranged from a low of three children aged 0 to 1 year to 30 children aged 3 to 4 years, with all other groups containing between eight and 30 patients. No significant trends in either IgM or IgG antibody could be identified for either age or sex.

RELATIONSHIP OF SEROLOGIC STATUS TO ILLNESS

We attempted to define serologically a subgroup of children undergoing acute, primary chlamydial infection. We assumed, based on infant and animal data, that children with an acute chlamydial infection could produce specific IgM within seven days and measurable specific IgG by 14 days. Consequently, a chart review for illnesses in the 14 days prior to phlebotomy was performed. Illnesses in IgM-positive, IgG-negative children (those who had an acute, recent infection) were compared with those in IgM-negative children. Excluded from this analysis were the ten children with both IgM and IgG antibody present, since we could not be sure of the timing of their infection.

Of all illnesses examined, only respiratory ones yielded associations with serologic status (Table 1). Evidence of acute chlamydial infection was associated with clinical URI in 6- to 10-year-old children, and with URI and pharyngitis in girls regardless of age. An association of IgM antibody and URI within two weeks in the whole sample was seen, but did not quite reach significance at the .05 level.

There did not appear to be an excess of acute EBV or CMV infection in the chlamydial IgM–positive group compared with the IgM-negative group (Table 2). Although there were significantly more elevated EBV–early antigen titers in the IgM-positive group, the elevations were borderline, and the difference in geometric mean titer between the two groups was not significant. Thus, the elevated antichlamydial antibody in children with respiratory disease did not appear to be due to polyclonal activation, either by EBV or by CMV infection. Polyclonal activation by another, but unidentified agent, would have been expected to result in well-correlated elevations in IgM titer to all three agents investigated.

COMMENT

An appreciable prevalence (15%) of specific antichlamydial IgM antibody was observed in a sample of children beyond infancy. The presence of this antibody was associated with the recent clinical diagnoses of URI, suggesting acute chlamydial respiratory disease in childhood. Although trachoma was endemic among schoolchildren in this area as late as the 1960s, the chlamydial antibody response was not due to this clinical illness (only four children had a clinical history of trachoma; only one of these had chlamydial antibody [IgG only]). The association between chlamydial antibody and illness appeared to be strongest in those children 6 to 10 years old, and in girls.

This association did not appear to be due to polyclonal activation by EBV infection in children with prior chlamydial immunologic memory due to infant chlamydial infection, and the EBV and CMV serologic results indicate that there was not a polyclonal activation due to some other agent. This would have been expected to result in well-correlated elevations of IgM antibody to all three agents, an effect that was not observed.

These data support the seroepidemiologic studies of Black et al[5] and San Joaquin et al[6] in showing the presence of antichlamydial antibody in child-

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Table 1.—Antibody Status (IgM+/IgM− or IgM−) vs Syndromes in Last 14 Days

Group	Illness	Proportion (%) With Illness in Last 14 Days		P Value*
		IgM+/IgG−	IgM−	
All	All URI†	14/18 (78)	86/156 (55)	.053
Girls	All URI	12/13 (92)	44/76 (58)	.014
	Pharyngitis	7/13 (54)	19/76 (25)	.041
Boys	All URI	2/5 (40)	42/80 (53)	>.1
	Pharyngitis	1/5 (20)	19/80 (24)	>.1
Aged 1-5 y	All URI	1/4 (25)	18/56 (32)	>.1
Aged 6-10 y	All URI	8/8 (100)	34/54 (63)	.035
Aged 11+ y	All URI	5/6 (83)	32/43 (74)	>.1

*One-sided Fisher's exact test.
†URI indicates upper respiratory tract infection, including otitis media, viral syndrome, pharyngitis, and conjunctivitis.

Table 2.—Proportion Seropositive for EBV and CMV in Relation to Serologic Status for *Chlamydia trachomatis**

	C trachomatis Serologic Status, Proportion (%)		Geometric Mean Titer of Positives	
	IgM+/IgG−	IgM−	IgM+/IgG−	IgM−
EBV-VCA IgG	17/17 (100)	134/140 (98)	86.2	72.2
EBV-EA IgG	3/16 (19)	3/121 (2)†	15.9	10.0
EBV-VCA IgM	0/10 (0)	0/11 (0)	...	...
Acute EBV infection‡	0/16	0/121	...	...
CMV-IHA	11/17 (65)	111/144 (77)	397.9	304.6
CMV-EIA IgG	12/17 (71)	109/143 (76)	380.0	512.9
CMV-EIA IgM				
Negative	4/10	3/10	...	...
Intermediate	4/10	3/10	...	...
Positive	2/10	4/10	...	...

*EBV indicates Epstein-Barr virus; CMV, cytomegalovirus; VCA, viral capsid antigen; EA, early antigen; IHA, indirect hemagglutination; and EIA, enzyme immunoassay.
†$P = .021$; Fisher's two-sided exact test.
‡Either VCA IgM positive or VCA IgG ≥1:320 and EA-IgG ≥1:40.

hood outside of infancy. Our association of antibody with respiratory disease supports that of Grayston et al,[7] who found seroconversions to *C trachomatis* in a small proportion of children and in up to 9% of adults with "pneumonias." They are also supported by a recent seroepidemiologic study in adults[13] that suggested a role for *C trachomatis* in adult pharyngitis and by a preliminary report by Shehab and colleagues,[14] who isolated *C trachomatis* from the nasopharynx of older children with LRI.

Inferences about chlamydial disease in childhood based on serologic findings must, however, be drawn with caution. Antigenic cross-reactivity with *Chlamydia psittaci* in the microimmunofluorescence test has been observed, as has simultaneous seroconversion to *Mycoplasma pneumoniae* and *Legionella pneumophila* in sporadic patients.[15] Further concern is generated by the work of Gerber and colleagues,[16] who failed to take cultures of *C trachomatis* from the throats of 95 randomly selected university students with the chief complaint of a sore throat, and by the study of Huss et al,[17] who obtained the same result in 126 patients with sore throats. Limitations in our study include lack of paired serum samples, lack of a priori defined and standardized diagnoses, and the confinement to a sample of subjects seen at a clinic rather than drawn from the general population.

Some component of the antichlamydial antibody rise, especially in girls, is likely due to genital infection as the result of sexual abuse.[18,19] This cannot be ruled out in our data, even though no complaints of sexual abuse were recorded in the charts, since such events might not have been reported. Abuse, however, is unlikely to account for all the antibody in girls or for the antibody found in boys.

An attractive new hypothesis to explain all these disparate findings is that the illnesses are due in large part to atypical psittacosis agents dubbed "TWAR" agents by Grayston et al.[20] The two prototype strains of this agent are TW 183 and IOL 207.[21] These agents have been associated with pneumonias in adolescents and adults, and their epidemiology is unknown except that they may be confined to humans.

These strains are as yet difficult to isolate in cell culture and are not stained by either iodine or the Syva culture-confirmation reagent, which is a monoclonal antibody to the *C trachomatis* species-specific major outer membrane protein. Thus, the agents would not have been detected by the methods employed by Gerber et al[16] and Huss et al[17] in their two studies of patients with sore throats. On the other hand, serum antibody to these agents does cross react to some degree with *C trachomatis* strains in the microimmunofluorescence test (Julius Schachter, PhD, oral communication March 19, 1986). Thus, serologic identification of infection, in the absence of culture detection, would have been possible in both the childhood and adult studies performed to date.

In summary, our data plus those of others suggest a role for chlamydial infection in childhood respiratory disease, with potentially both *C trachomatis* and atypical *C psittaci* strains being contributing pathogens. The work emphasizes the need for a definitive tissue culture isolation study of chlamydiae and respiratory disease in childhood.

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