ELISA Serology for Antibodies Against *Chlamydia trachomatis* in Crohn's Disease

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Abstract

Background: Recently we reported IgA anti-*Chlamydia* antibodies in patients with Crohn's disease (CD), in particular in four patients from a single family of six with CD.

Methods: We studied sera from four cohorts from the north of France. These were identified as: EPIMAD (80 pediatric onset CD and 20 pediatric onset ulcerative colitis), MINOTOR (148 adult onset sporadic CD and 50 adult onset ulcerative colitis), Grande Famillies (50) and matched controls for the Grande Famillies cohort (49). Sera were tested using commercial anti-*Chlamydia trachomatis* (LGV2:434) IgG and IgA human enzyme-linked immunosorbent assay (ELISA) kits. Cutoff for positivity was 11.0 standard units.

Results: Patients with sporadic CD, unaffected first degree relatives from multiplex families and ulcerative colitis patients had no greater serologic reactivity than controls. However, multiplex families' patients had twice as many positives as the other groups: for IgG 20% vs. 8%; for IgA 20% vs. 10%.

Conclusions: Though not attaining statistical significance, the data showed that familial CD patients had greater exposure to *C. trachomatis* than sporadic CD patients, supporting our earlier results from one family from the north of France. More specific serologic tests based on outer membrane proteins will need to be employed against the various *Chlamydia* species with zoonotic potential.

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Introduction

In the recent past, we established that obstructed lymphatics represent the fundamental intestinal injury in the pathogenesis of Crohn's disease (CD) [1, 2]. Searching for microbes that might damage lymphatic endothelium and in particular the endothelium of intestinal lymphatics, we discovered the importance of an infectious pig model of CD, in animals inoculated with *Chlamydia suis* [3]. As a consequence, evidence for *Chlamydia* in the resected tissues of patients and antibodies against this microorganism were sought [4].

Employing commercial enzyme-linked immunosorbent assay (ELISA) kits for *Chlamydia trachomatis*, serology for anti-*Chlamydia* IgG revealed two positive values in 24 patients, versus one positive among 15 controls, while serology for anti-*Chlamydia* IgA revealed four positives among the 24 patients, and one positive in 15 controls [4]. One patient and one control had both elevated IgG and IgA titers. The four patients with elevated IgA titers were from a single family of six, all of whom had CD [4]. In an attempt to test the importance of positive serology, we investigated 323 sera from patients registered with an inflammatory bowel disease (IBD) center in northern France, as well as 74 control sera from the same region.

Materials and Methods

Patient's sera were obtained from three different studies: Epimad (100), Minotor (198), and Grande Famillies (50). Control sera (49) were obtained from families without inflammatory bowel diseases matched to the Grande Famillies, for size and number, gender, and ages in the second generation. The Epimad cohort consisted of 80 patients with CD and 20 with ulcerative colitis, all of whom were pediatric onset patients, defined by a diagnosis of IBD before the age of 17. The Minotor comprised 148 patients with sporadic CD, and 50 with ulcerative colitis, all from the tertiary care center at Centre Hospitalier Regional Universitaire de Lille. In the Grande Famillies cohort, there were 25 patients with CD and 25 unaffected first

Table 1. ELISA Serology vs. *Chlamydia trachomatis*

Sample group	Families studies			Sporadic cases			
	Healthy controls for multiplex families (n = 49)	Healthy relatives*, in multiplex families (n = 25)	CD in multiplex families (n = 25)	CD pediatric-onset (n = 80)	UC pediatric-onset (n = 20)	CD adult-onset (n = 148)	UC adult-onset (n = 50)
IgG positives	4 (8%)	2 (8%)	5 (20%)	1 (1%)	2 (10%)	14 (9%)	5 (10%)
IgA positives	5 (10%)	2 (8%)	5 (20%)	8 (10%)	1 (5%)	15 (10%)	3 (6%)
Either IgG or IgA positive or both	7 (14%)	3 (12%)	6 (24%)	9 (11%)	3 (15%)	28 (19%)	7 (14%)
Male %	37%	44%	40%	45%	35%	43%	46%
Age median (min - max)	39 (16 - 78)	41 (17 - 78)	35 (17 - 79)	24 (16 - 37)	26 (20 - 35)	31 (17 - 65)	40 (18 - 70)

CD: Crohn's disease; UC: ulcerative colitis. *Healthy first degree relatives of CD patients in multiplex families.

degree relatives, from 17 families. All the patients and controls were from northern France. Sera were frozen at -80 °C within the registry and later transported by air carrier to the University of Connecticut.

Sera were tested using anti-*C. trachomatis* (LGV 2:434) IgG human ELISA kits, and anti-*C. trachomatis* (LGV 2:434) IgA human ELISA kits (Abcam, Cambridge, MA), according to the manufactures instructions. Briefly, all materials were equilibrated at room temperature prior to use. For initial detection of specific antibodies, serum samples were diluted 1 to 100 with sample diluent as indicated in the manufacturer's instructions. Limited cross reactivity data are available for the kits used. Cutoff for positivity was 11.0 standard units.

Statistical analysis used Chi-square or Fisher's exact tests for categorical data, and Kruskal-Wallis tests for quantitative data. Effect of age, sex and disease status on positive *C. trachomatis* serology was tested on IgG positivity, IgA positivity and positivity of either IgA or IgG. Additional comparisons were done within the families' studies, among controls, healthy relatives and CD patients; within sporadic cases, between CD and ulcerative colitis (UC) patients.

The study was conducted in accord with University of Connecticut IRB approval, protocol HO8-128.

Results

Results are summarized in Table 1. Employing these ELISA tests, patients with sporadic CD, unaffected first degree relatives from multiplex families, and ulcerative colitis patients had no greater serologic reactivity than controls.

On the other hand, multiplex families had twice as many positives, i.e., above the cutoff, as the other groups: for IgG, CD in Grande Famillies vs. controls (20% versus 8%, P = 0.22); for IgA, CD in Grande Famillies vs. controls (20% versus 10%, P = 0.29) (Fig. 1).

Positive IgG and IgA/IgG serologies increased with age (P < 0.01 and P = 0.01, respectively), but not IgA (P = 0.10). Positive IgG serology was more often observed in females than in males (P = 0.06), but not for IgA or IgA/IgG.

No significant difference was observed among the three groups of subjects within the families' studies, and between
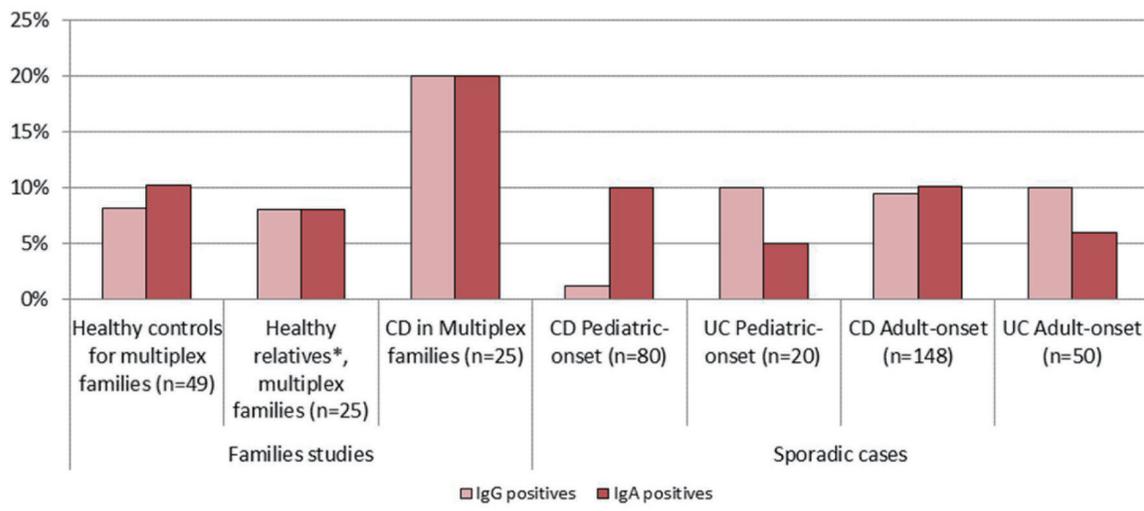
Figure 1. Positive serology vs. *Chlamydia trachomatis* in patients and control groups. *Healthy first degree relatives of CD patients in multiplex families

Table 2. Previously Reported Anti-*Chlamydia* Antibodies in Crohn's Disease

Author	Year	Country	Ab	Antigen	Test	N +	N Total	N %	Ctr +	Ctr Total	Ctr %
Rodaniche et al [5]	1943	US		*C. trachomatis* LGV	SN	0	4	0			
Swarbrick et al [6]	1979	England	IgG	*C. trachomatis* A-K, 207, LGV 1-3	Micro FA	4	54	7.4*	10	75	13
Taylor-Robbinson et al [7]	1979	England		*C. trachomatis* B, D-J, LGV 1-3	Micro FA	8	55	14.5	5	23	22
Schuller et al [10]	1979	Holland	IgG	*C. trachomatis* A-K, LGV 1-3	Micro FA	38	55	69	1	50	2
Mardh et al [11]	1980	Sweden	IgG	*C. trachomatis* D-K, LGV 1-3	IIF	83	107	78	38	50	76
Gump et al [12]	1981	US		*C. trachomatis* LGV 2	IIF	35	58	60	33	58	57
Elliot et al [8]	1981	England		*C. trachomatis* A-K, LGV 1-3	Micro FA	0	62	0	3	160	2
Orda et al [13]	1990	Israel	IgG	*C. trachomatis*	IIP	14	15	93	4	15	26
Orda et al [13]	1990	Israel	IgA	*C. trachomatis*	IIP	5	15	33	1	15	6
McGarity et al [9]	1991	England	IgG	*C. trachomatis* L1	ELISA	7	48	15	14	48	29
Van Kruiningen et al [4]	2016	France	IgG	*C. trachomatis* LGV 2: 434	ELISA	2	24	8.3	1	15	6.6
			IgA	*C. trachomatis* LGV 2: 434	ELISA	4	24	16.6	1	15	6.6
Gump et al [12]	1981	US	IgA	*Chlamydia* group antigen	CF		58	9.5	1	58	1.7
Swarbrick et al [6]	1979	England	IgG	*C.psittaci*	Micro FA	0	54	0	0	75	0
Munro et al [14]	1979	Wales	IgG	*C. psittaci*	CF	30	62	48	18	57	32
Elliot et al [8]	1981	England	IgG	*C. psittaci*	Micro FA	0	62	0	0	160	0
Van Kruiningen et al [15]	2000	France	IgG	*C. psittaci*	CF	5	13	38	1	13	8

Ab: antibody; Ctr: control; ELISA: enzyme-linked immunosorbent assay. *7.4% were positive to serovars A-K or 207.

CD and UC patients within sporadic cases. Nonetheless, positive IgA serology was 10% (23/228) for sporadic CD patients and 6% (4/70) for sporadic UC patients (P = 0.26).

Discussion

At first glance, our data (Table 2 [4-15]) appear to show no serologic relationship between *C. trachomatis* and CD. This finding, of a lack of or little antibody response in CD patients, agrees with Rodaniche et al [5], Swarbrick et al [6], Taylor-Robinson et al [7], Elliot et al [8] and McGarity et al [9] of previous studies done by other methods, and it disagrees with Schuller et al [10], Mardh et al [11], Gump et al [12] and Orda et al [13]. This test would not serve to identify patients with CD.

On the other hand, though not attaining statistical significance, the data appear to show that familial CD patients have had greater exposure to *Chlamydia* antigen than sporadic CD patients, which supports our earlier results with one family from the north of France (in the latter instance, in IgA levels) [4]. If the difference is real, what possible reasons might explain the increased number of positives in familial disease? Was there important genetic susceptibility, or, was there a greater level of exposure to *Chlamydia* within family settings? Were the Grande Famillies exposed to a different, and cross-reacting, *Chlamydia* species? Or is there a geographic reason for the greater proportion of positives? One wonders how our data would compare to sporadic and familial disease data from the south of France.

The test employed was designed to discriminate patients exposed to *C. trachomatis* lymphogranuloma venereum (LGV) serotypes. Based on reactivity to lipopolysaccharide, sera with antibodies to any species of *Chlamydia* should react [16]. During the development of the Abcam serologic test, it was established that cross-reactivity with *C. pneumoniae* does not occur. Sera from 14 patients who had recovered from infection with *C. pneumoniae* all failed to react in the Abcam test. Cross-reactivities to other *Chlamydia*, such as those harbored in farm species (*C. psittaci, C. abortus, C. suis,* and *C. pecorum*), and to Gram-negative bacteria, have never been tested.

From experimental work in monkeys, it is known that different antibody responses occur to *C. trachomatis* LGV serotypes and E-serotypes [17]. Had the Abcam ELISA test, with its defined cutoff, been applied to the experimental monkeys, those with LGV proctitis would have been regarded as positive, whereas those infected with serovar E would have fallen below detection. Extent of disease in an affected organ is of additional concern; women with acute salpingitis have been shown to have IgG responses (to serotypes D-K) proportional to severity of disease [18]. Antibodies have a finite life, thus duration of disease from onset to the time of testing (very variable and often prolonged in CD) must influence titers against any putative organism. And, the use of antibiotics early in the illness has the potential to abort antibody responses. Patients with CD report greater use of antibiotics during childhood and adolescent years than control subjects [19]. These factors ameliorate conclusions from this study.

There is concordance between the enteric lymphatic pathology of CD and that of the *Chlamydia suis*-infected pig [3]. The various species and serotypes of *Chlamydia* that occur

in disease states [16, 20-22], plus the variable production of antibody, in target tissues, e.g., the lung, conjunctiva, uterus, placenta, urethra, joints or gastrointestinal tract, suggest that more specific serologic tests, based on outer membrane proteins might yield better results. All of the *Chlamydia* species of animal origin have zoonotic potential [23-39]. We continue to hold the possibility that one of them is responsible for CD.

Acknowledgments

Conflict of Interest

The authors declare no conflict of interest.

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