

Serologic Assessment for Inflammatory Bowel Disease in Patients with Chronic Granulomatous Disease

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Defects in innate immune responses appear to predispose to inflammatory bowel disease (IBD) similar to Crohn's disease (CD), and 30% of patients with chronic granulomatous disease (CGD), a genetic disease of the innate immune system, develop IBD. Antibody panels are used to assist in the diagnosis of IBD in symptomatic patients. The aim of this study was to determine the usefulness of serologic testing for IBD in children with CGD. The medical records of 9 patients with CGD were reviewed for symptoms of IBD, and a serologic panel for IBD was performed. The panel measures ASCA IgA and IgG ELISA, Anti-Omp C IgA ELISA, Anti-CBir 1 ELISA, and IBD-specific pANCA. A proprietary formula is used to report IBD likely. All 9 patients had IBD serologic findings consistent with CD; nevertheless, only 2 of the 9 patients had IBD symptoms: diarrhea and mucoid stools in one, and mild diarrhea and growth failure in the other. Endoscopy performed in these 2 patients revealed granulomatous colitis in one individual only. These findings indicate that IBD serologic testing does not identify CGD patients with IBD. The consistently positive IBD panels in our CGD patients suggest that this innate immune deficiency predisposes to antigen sensitization that is seen in CD. However, other factors appear to determine which CGD patients progress to clinical granulomatous IBD.

Introduction

MUTATIONS IN COMPONENTS of the phagocyte oxidase cause chronic granulomatous disease (CGD), a disorder characterized by recurrent, life-threatening infections with catalase-positive microorganisms.¹ The primary defect in CGD is an inability of phagocytic cells to kill selected microbes by oxidative means while antigen and cellular recognition remains intact.¹ Up to one-third of CGD patients develop a form of granulomatous inflammatory bowel disease (IBD) that is clinically and histologically similar to Crohn's disease (CD).² The etiology of CD is unknown, although an aberrant immune process, including innate immune responses, may contribute to the development of disease.³

The diagnosis of CD is based on clinical and histologic findings.⁴ However, selected IBD serology panels, antibodies directed against self and microbial antigens, have been assessed to assist in the diagnosis of suspected patients.⁵ Nevertheless, the presence of positive serologies alone does not establish a diagnosis of IBD. The purpose of this study was to evaluate the clinical usefulness of IBD serology testing in CGD patients.

Materials and Methods

A convenience sample of 8 males and 1 female, 2–23 years of age with CGD were studied regardless of the presence or

absence of IBD symptoms. The diagnosis of CGD was confirmed in each subject with a colorimetric nitroblue tetrazolium test and genetic mutation analysis. All subjects were receiving prophylactic trimethoprim-sulfamethoxazole and itraconazole except for patient no. 5 (see Table 1), who had disseminated aspergillosis at the time of study and who was receiving intravenous amphotericin B. Retrospective chart review and prospective data collection were performed according to a protocol approved by the Institutional Review Board to test the hypothesis that IBD serology panel findings are correlated with clinical IBD. Written and informed consent was obtained from parents for those subjects studied prospectively. Blood was obtained from subjects on 1 occasion at the time of standard laboratory testing.

Medical records were reviewed to identify: age, sex, CGD genotype, symptoms of IBD, and surgical pathology reports of bowel biopsies if available. A serologic panel for IBD (IBD panel; Prometheus Laboratories, San Diego, CA) was obtained for each patient. The IBD panel measures ASCA IgA ELISA, ASCA IgG ELISA, Anti-Omp C IgA ELISA, Anti-CBir 1 ELISA, and IBD-specific pANCA (including AutoAb ELISA, IFA perinuclear pattern, DNase sensitivity), and a proprietary algorithm is used to report "IBD likely" or "IBD unlikely." Normal values are defined by the reference laboratory. ASCA ELISA is an indirect solid phase enzyme immunoassay for quantitative measurement of IgG and IgA

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TABLE 1. INFLAMMATORY BOWEL DISEASE SEROLOGY PANELS IN 9 PATIENTS WITH CHRONIC GRANULOMATOUS DISEASE

Patient no.	Age (years)	Sex M/F	CGD genotype	IBD symptoms	IBD serology panel results ^a							IBD serology panel interpretation ^a
					ASCA IgA (<20) ^b	ASCA IgG (<40) ^b	Anti-OmpC IgA (<16.5) ^b	Anti-CBir1 (>21) ^b	pANCA AutoAb (<12.1) ^b	IFA perinuclear pattern	DNase sensitivity pANCA	
1	2	M	XL	Absent	<12	45.7	8.7	178	20.3	Detected	Sensitive	Likely
2	8	M	XL	Absent	24	112.3	4.1	40.4	14.6	Detected	Sensitive	Likely
3	9	M	AR	Absent	13.7	33.8	25.1	123.9	13.8	Not detected	Not applicable	Likely
4	10	M	AR	Present	99.9	84.9	28	87.7	15.8	Detected	Sensitive	Likely
5	12	F	AR	Absent	>114.1	155.4	110.4	123.5	<12.1	Not detected	Not applicable	Likely
6	9	M	AR	Absent	63.5	24.8	28.3	32.7	<12.1	Not detected	Not applicable	Likely
7	23	M	XL	Present	+ ^c	+ ^c	+ ^c	<21	<12.1	Not detected	Not applicable	Likely
8	8	M	AR	Absent	>114.1	96.7	48.5	<21	<12.1	Not detected	Not applicable	Likely
9	12	M	XL	Absent	54.1	90.9	74.3	35	<12.1	Not detected	Not applicable	Likely

^aSee Materials and Methods for details of specific antibodies and definition of "IBD likely."^bNormal values in EU/mL.^cQuantitative values not available.

AR, autosomal recessive; CGD, chronic granulomatous disease; IBD, inflammatory bowel disease; XL, X-linked recessive. Shaded areas reflect positive (abnormal) antibody results.

autoantibodies against mannan from *Saccharomyces cerevisiae* in human serum or plasma. These are targeted to epitopes on the cell wall mannan of this particular yeast and are 95% specific for CD versus ulcerative colitis. ANCA is the anti-neutrophil cytoplasmic autoantibody directed against components of neutrophil granules. pANCA is most commonly used in testing for IBD, as it has 56% sensitivity and 89% specificity in ulcerative colitis patients. OmpC is an outer membrane protein originally isolated from *E. coli*. There is excessive production of OmpC autoantibodies in CD. Anti-Omp C IgA antibodies are found in ~37%–55% of CD patients but only in 2%–11% of ulcerative colitis patients. C-Bir 1 is a colitogenic antigen of microbial flora in C3h/HeJ/Bir mice. It is closely related to flagellin from multiple bacteria, including clostridial species. Immune responses to this antigen can be seen in up to 50% of CD patients, but only ~6% of ulcerative colitis patients. Endoscopy with biopsies were performed on the 2 patients clinically suspected of having CGD-associated IBD.

Results

Four of the 9 patients had X-linked CGD. Two patients had symptoms consistent with IBD: patient no. 7 had diarrhea and mucoid stools and patient no. 4 had mild diarrhea and growth failure, and these patients underwent clinically indicated endoscopy. However, all 9 subjects had strongly positive serologic evidence of CD (Table 1). Seven patients had elevated ASCA IgA ELISA, 8 patients had elevated ASCA IgG ELISA, 7 patients had elevated Anti-Omp C IgA ELISA, 6 patients had elevated Anti-CBir1 ELISA, and 3 patients had elevated IBD-specific pANCA. Endoscopy and biopsies of the 2 symptomatic patients revealed granulomatous colitis in patient no. 7, but only *H. pylori* in patient no. 4.

Discussion

CGD, a clinical syndrome caused by a genetic defect in 1 of the subunits of the NADPH oxidase resulting in the inability of CGD phagocytes to efficiently eliminate catalase-positive microbes, is characterized by recurrent life-threatening bacterial and fungal infections, but also by pathologic inflammatory processes, including obstructive granuloma formation¹ and CD-like IBD.² Recently, innate immune dysregulation has been described in CGD.³ Other studies strongly suggested that the absence of the oxidative pathway in CGD dramatically enhances nonoxidative inflammatory pathways.^{4,5}

The immunopathophysiology of IBD remains to be completely elucidated. Genetic predisposition to IBD via a number of pathways (reviewed in Cho⁶), alterations in the gut microbiome,⁷ disrupted epithelial barrier functions (reviewed in Xavier and Podolsky⁸), and variations in innate and adaptive immunity (reviewed in MacDonald et al.⁹), are all likely to be involved. Of particular interest is a recent genome-wide association study that identified *NCF4* as a distinct susceptibility locus for CD. This gene encodes p40, 1 of the subunits of the phagocyte oxidase (p40^{PHOX}).¹⁰ Most recently Smith et al. identified a primary disorder of cytokine secretion by CD macrophages¹¹; these findings led Casanova and Abel to suggest that CD represents a primary immunodeficiency of macrophages.¹²

In the present study, the clinical usefulness of serum immunologic assessment for IBD in CGD patients was reviewed. Serologic markers for IBD reflect increased adaptive immune responses to microbial and self-antigens. Although not recommended for routine diagnosis,⁵ serologic responses in IBD may provide insight into the immunopathophysiology of CGD. Our current findings are in agreement with the recent description of a variety of autoimmune disorders in patients with CGD,¹³ and support the concept that the dysfunction of the phagocyte oxidative pathway predisposes to CD, particularly in regard to the generation of potentially pathologic antibodies associated with CGD. Our data clearly show that the IBD serologic panel employed in this study does not differentiate CGD patients who have IBD. It is possible, however, that at some point in time all subjects with CGD will develop bowel inflammation that may not be clinically apparent to warrant diagnostic endoscopy. Noninvasive techniques for the evaluation of IBD are warranted. Additional factors, yet to be determined, must play a role when selected CGD patients go on to develop active IBD. Of relevance to this issue, Cadwell et al.¹⁴ recently demonstrated in a mouse model of CD that a confluence of factors, including a genetic susceptibility allele, norovirus infection, and the presence of normal gut commensal bacteria, are required for the emergence of active disease.

Limitations of the present study include the small number of subjects with none <2 years of age, and their study at only one point in time. In conclusion, IBD serology testing does not discriminate between CGD patients with and without clinical IBD. However, the innate immune deficiency in CGD appears to predispose CGD patients to antigen sensitization that is seen in CD. The prospective study of young infants with CGD, the demonstration of when specific IBD-associated antibodies become detectable, and the potential of other noninvasive measures to identify CGD patients with emerging IBD are areas for study.

Author Disclosure Statement

No competing financial interests exist.

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