

Elevated basal intestinal mucosal cytokine levels in asymptomatic first-degree relatives of patients with Crohn's disease

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Subject headings crohns disease; cytokine/analysis; intestinal mucosa

Abstract

AIM To determine levels of cytokines in colonic mucosa of asymptomatic first degree relatives of Crohn's disease patients.

METHODS Cytokines (Interleukin (IL) 1-Beta, IL-2, IL-6 and IL-8) were measured using ELISA in biopsy samples of normal looking colonic mucosa of first degree relatives of Crohn's disease patients ($n = 9$) and from normal controls ($n = 10$) with no family history of Crohn's disease.

RESULTS Asymptomatic first degree relatives of patients with Crohn's disease had significantly higher levels of basal intestinal mucosal cytokines (IL-2, IL-6 and IL-8) than normal controls. Whether these increased cytokine levels serve as phenotypic markers for a genetic predisposition to developing Crohn's disease later on, or whether they indicate early (pre-clinical) damage has yet to be further defined.

CONCLUSION Asymptomatic first degree relatives of Crohn's disease patients have higher levels of cytokines in their normal-looking intestinal mucosa compared to normal controls. This supports the hypothesis that increased cytokines may be a cause or an early event in the inflammatory cascade of Crohn's disease and are not merely a result of the inflammatory process.

INTRODUCTION

Numerous studies have proven that cytokines play an integral role in the pathogenesis of inflammatory bowel disease (IBD). These protein mediators have been shown to regulate the immune response, induce tissue injury, and mediate complications such as fibrosis and obstruction in patients with IBD^[1]. In our institution, we have measured cytokines IL-1B (Interleukin-1 Beta), IL-2, IL-6, and IL-8 from mucosal biopsies obtained during colonoscopy in patients with IBD and other colitides to see if there is any predictive pattern of cytokine elevation^[2-5]. Since there is evidence for a genetic predisposition in IBD^[6-10], we were also interested in determining if asymptomatic first-degree relatives of Crohn's disease (CD) patients exhibit elevated intestinal mucosal cytokine levels as well. If they do, is there any pattern to the cytokine elevations?

To our knowledge, there are no studies examining specific cytokine levels in first-degree relatives. Some studies suggest there is increased intestinal permeability in healthy relatives of CD patients^[11-14], pointing to a possible genetic defect in these patients. This study may add that even in asymptomatic relatives, there is evidence of subclinical expression of the disease at a cellular level. Elevated cytokine levels would also support the hypothesis that cytokines are a cause or an early event in the inflammatory cascade of CD and not a result of the inflammatory process, since these patients have no pathological signs or symptoms of disease.

MATERIALS AND METHODS

After obtaining prior approval of the Institutional Review Board in our hospital, we studied ten people without a family history of IBD who acted as controls, and nine first-degree relatives of patients with Crohn's disease, who did not exhibit signs or symptoms referable to CD.

The control patients included four males and six females ranging in age from 40 - 82 years (mean-64.3). Each patient underwent a routine colonoscopy. (Olympus, Lake Success, NY) after appropriate consent. Table 1 shows further data regarding the group. These patients underwent colonoscopy for causes other than IBD such as screening, Guaiac positive stools, abdominal pain (Table 1).

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Eleven first-degree relatives were initially studied but only nine were used in the final analysis. Two relatives were excluded as one had a prior history of radiation proctitis and the other had a prior sigmoid biopsy consistent with nonspecific inflammation. These relatives included two males and seven females ranging in age from 21-79 years (mean-50.1). Two relatives were from the same family (i.e. brother and sister of a CD patient). Otherwise, the relatives were unrelated to each other. After obtaining informed consent each underwent a flexible sigmoidoscopy (Olympus) upto splenic flexure without a prior bowel prep. (Refer to Table 1). There was one patient in this group with abdominal pain and weight loss who was diagnosed with depression and anxiety but no pathologic condition was found. Also, as shown in the table, endoscopic findings were of minimal significance in both groups.

Demographic data of the eight CD patients (the index cases of the first-degree relatives) were analyzed and compared (Table 2).

Table 1 Study population: demographic data

Clinical data	Control group	First degree relative
Number (n)	10	9
Mean age (Range)	64.3 (40-82)	50.1 (21-79)
Males	4	2
Females	6	7
Indications		
Anemia	1	None
Abdominal pain	1	Pain/Weight loss (1)
Guaiac (+)	3	None
Surveillance	5	None
Study	0	3
IBS	0	3
Endoscopic findings		
Normal	5	6
Hemorrhoids	3	3
Polyps	2	None
Family His of colitis	None	All
History of IBD	None	None

Table 2 Demographics of Crohn's disease patients (n = 8)*

Clinical data	Number
Mean age	44 (26.93)
Males	2
Females	6
Extent of the disease	
Ileum and colon	5
Ileum alone	2
Colon alone	1
Duration of the disease	
<10yrs	1
10-30yrs	6
>30yrs	1
Present medications	
6MP, steroids, ASA	4
ASA, steroids	2
ASA	1
None	1
History of Surgery	
Yes	6
No	2
Number of first degree relatives	
One relative	7
Two relatives	1

ASA = Aminosalicic acid; 6MP-6 Mercaptopurine

*One of the patients had two first-degree relatives.

Tissue collection and analysis

Three mucosal biopsies were obtained from the sigmoid or descending colon in each patient in both groups. The biopsy samples, weighing between 15 mg and 30 mg each, were immediately wrapped in aluminum foil, placed in a container of liquid nitrogen and stored at -70°C entigrade until they were processed. Tissue was crushed and homogenized in diluent from IL-kits (Quantikine, R & D systems) for 30 seconds, then centrifuged for 15 minutes at 3000 r/min. The supernatant fluid was used for assaying interleukins. Histologic evaluation was only performed if endoscopic abnormalities were noted as it would have, otherwise, delayed the procedure.

A solid phase ELISA using Quantikine kits (Research and Diagnostic Systems, Minneapolis, MN, USA) was used to measure IL-1B, IL-2, IL-6, and IL-8. Results were expressed as mean \pm SEM. The Quantikine method had a sensitivity of 0.03 pg-0.08 pg and a standard curve linearity of 0.03 pg to 3.0 pg. A Student's independent test was used to compare data from the two groups and P value <0.05 was considered as statistically significant.

RESULTS

The concentrations of IL-2, IL-6, and IL-8 were significantly ($P < 0.05$) higher in the colonic mucosa of first-degree relatives (3.40 ng/g \pm 0.56 ng/g, 1.19 ng/g \pm 0.21 ng/g, 11.98 ng/g \pm 2.62 ng/g, respectively, Figure 1) when compared to controls (1.86 ng/g \pm 0.30 ng/g, 0.69 ng/g \pm 0.01 ng/g, 5.41 ng/g \pm 1.87 ng/g, respectively). No significant difference was found in the concentration of IL 1B between relatives (0.85 ng/g \pm 0.17 ng/g) and controls (1.03 ng/g \pm 0.15 ng/g).

Individually, seven of the nine relatives had significantly higher levels of IL -2 and IL-6 and six relatives had significantly higher IL-8 levels than the mean value of controls. Interestingly, mean IL-1B and IL-6 levels of the two relatives from the same family (0.23 ng/g \pm 0.09 ng/g and 0.21 ng/g \pm 0.09 ng/g, respectively) were significantly lower ($P < 0.05$) than that of the other relatives (1.03 ng/g \pm 0.16 ng/g and 1.47 ng/g \pm 0.14 ng/g, respectively).

We also compared various subsets of the CD index cases to see if there were any differences in the corresponding first-degree relative cytokine levels (Table 2). In analyzing age, severity, duration or extent of disease, medications, and history of surgery, there were no significant differences between the subsets of the relative group in any category.

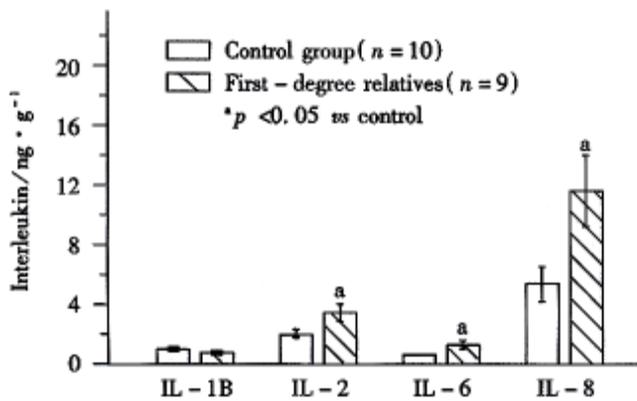


Figure 1 Interleukin levels of first-degree relatives and controls.

DISCUSSION

There is evidence that inflammatory bowel disease is determined by genetic predisposition. Supporting data include familial aggregation^[6,15] and an increased concordance rate in monozygotic twins compared with dizygotic twins^[16]. CD patients have a more frequent positive family history than ulcerative colitis (UC) patients^[17]. Relatives of CD patients have a higher risk of IBD than those of UC patients^[18,19]. There is also a higher concordance rate for CD than for UC among either monozygotic or dizygotic twins^[16]. This suggests that hereditary predisposition may play a more important role in CD than UC.

Genetic studies have used subclinical markers in unaffected family members of IBD to either indicate the genetic abnormality predisposing to a disease or identify those in whom a subclinical phase of the disease process is occurring. These include serum antibodies (i.e. ANCA^[20,21], antibodies to viruses, bacteria, mycoplasma, food antigens^[22], and colonic epithelial cells^[23]), colonic mucin abnormalities^[24], obligate anaerobic fecal flora^[25], mucosal production of IgG subclasses^[26], and C₃ dysfunction^[27]. Abnormal elevations of these markers have been seen in healthy relatives of either CD or UC patients or both.

Most studies on relatives of CD patients have analyzed intestinal permeability in patients with CD and their first-degree relatives by measuring urinary excretion of poorly absorbed, water-soluble compounds such as polyethylene glycol (PEG-400) and large sugars, including lactulose, mannitol, and rhamnose^[28], 51 Cr-labelled ethylene diaminetetraacetic acid (EDTA)^[29] and luminal prostaglandin^[30] and hyaluronin^[31] release. Although initial studies have shown increased permeability in patients with CD^[29,32-34] and their first-degree relatives^[35,36], several additional studies^[37-39] have been inconsistent, showing no increased permeability in either group.

Hollander^[40] and May *et al.*^[41] in 1993,

reviewed earlier studies and found that most studies showed a significant increase in permeability in 10% of first-degree asymptomatic relatives as it is possible that only a subgroup of relatives of CD patients are genetically susceptible. A recent study^[42] has confirmed this by showing that the subset of asymptomatic relatives with increased intestinal permeability had increased CD45 (common leukocyte antigen) isoform in peripheral blood cells similar to CD patients. These findings suggest that the permeability defect in CD patients may serve as a subclinical and genetic marker for CD and is not secondary to intestinal inflammation^[34] or may indicate early intestinal damage (preclinical expression).

Our study is the first to our knowledge to determine cytokine levels, known to be elevated in the colonic mucosa of CD patients and in asymptomatic relatives of CD patients. The fact that IL-2, IL-6, and IL-8 were significantly elevated suggests a possibility of early intestinal damage in the relatives group similar to the studies of increased permeability in these patients. Although the number of cases were small ($n = 9$), six of the nine patients had significant elevations of all three cytokines (IL-2, IL-6, and IL-8) versus controls (the others had significant elevations of at least one or two of these cytokines). Whether a subset of these relatives are actually more predisposed to the disease than controls, as suggested in the permeability studies, has yet to be known. It is also of interest that we could not find any significant differences in cytokine levels between subsets of the relatives with regard to the various parameters of the index CD patients (listed in Table 2). The fact that the two relatives from the same family had significantly lower levels than the rest of the group is of questionable value. Future studies including more patients and the evaluation of other cytokines would be of interest and would help in determining the significance of these alterations in this group.

This study shows that asymptomatic relatives of CD patients have increased levels of cytokines in normal-looking colonic mucosa. Studies with intestinal permeability have been inconsistent. It may be possible that increased cytokines are an early event in the pathogenesis of the disease. Whether these increased cytokines lead to increased permeability and then to CD is something that needs further investigation.

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