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Patients with inflammatory bowel disease may have a transforming growth factor-β-, interleukin (IL)-2- or IL-10-deficient state induced by intrinsic neutralizing antibodies

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Summary

Ulcerative colitis (UC) and Crohn's disease (CD) are considered to be immunologically mediated disorders that share certain features with murine models of colitis. Whether any of these models are physiologically relevant to the human condition remains controversial. The hypothesis is that increased amounts of antibodies neutralizing transforming growth factor (TGF)-β, interleukin (IL)-2 or IL-10 create a relative immunodeficient state in inflammatory bowel disease (IBD) that predisposes to disease. To evaluate this, serum samples from patients with UC or CD and from normal healthy individuals were studied by enzyme-linked immunosorbent assays. Antibodies recognizing TGF-β were most prevalent in UC ($P < 0{\cdot}01$); anti-IL-10 antibodies were elevated in CD ($P < 0{\cdot}05$), while anti-IL-2 antibodies were the same for all three groups. Importantly, the percentage of IBD patients with at least one of the antibody levels greater than any control value was 30% for UC and 33% for CD. To verify the presence of these antibodies, immobilized TGF-β was exposed to UC sera and the attached proteins identified by Western blot assay. The proteins proved to be exclusively immunoglobulin (Ig) G. To evaluate the neutralizing activity of these antibodies, cytokine-specific IgG from subjects in each group of patients was incubated with TGF-β, IL-2 or IL-10 before addition to a bioassay with changes in viability determined by a colorimetric analysis. Antibodies from most individuals in all three groups neutralized the action of each cytokine. This study shows that about one-third of IBD patients may have a relative deficiency of TGF-β, IL-2 or IL-10 due to an increase in neutralizing antibodies in their sera.

Keywords: CD, IBD, IL-2, IL-10, TGF, UC

Introduction

Inflammatory bowel disease (IBD) is an immunologically mediated disorder of unknown origin. In order to gain a clearer understanding of the aetiopathogenesis of this disease, numerous animal models have been established that mimic some of the aspects of IBD. Which one of these, if any, are relevant to the human condition is unknown. The premise of this study is that IBD patients have increased amounts of antibodies neutralizing transforming growth factor (TGF)-β, interleukin (IL)-2 or IL-10, as deficiencies of these cytokines in mouse lead to enteritis.

The TGF-β is one of the most widely distributed cytokines. It affects virtually all cell types and carries out pleiotropic functions [1,2]. TGF-β is secreted as a latent inactive complex consisting of a TGF-β dimer linked non-covalently to latency associated peptide (LAP). LAP inhibits binding of TGF-β to its receptors. When LAP is removed by proteases, a biologically active form of TGF-β is formed. TGF-β inhibits the differentiation of T helper type 1 (Th1) and Th2 cells and is important in the generation and maintenance of peripheral tolerance mediated by T regulatory (T_{reg}) cells [1,2].

The TGF-β1-deficient mice develop a severe multiple organ inflammatory disease with increased synthesis of tumour necrosis factor-α and interferon (IFN)-γ [3]. However, when TGF-β signalling was inactivated in the intestinal epithelium, the mice showed increased susceptibility to dextran sodium sulphate-induced colitis, autoantibody production and uncontrolled matrix metalloproteinase enzyme activity [4]. In other experimental models of colitis,

neutralization of TGF-β resulted in development or aggravation of disease [5]. These findings indicate that the decline in TGF-β signalling increases susceptibility to IBD.

There are three isoforms of TGF-β. Although they are localized to distinct chromosomes and have minor variations in sequence, TGF-β1, TGF-β2 and TGF-β3 have nearly identical biological activity and compete for the same receptors [2,6,7]. Intestinal TGF-β1 mRNA is more abundant than mRNA for the other isoforms, although protein expression is similar [8–10]. In IBD, TGF-β2 and TGF-β3, but not TGF-β1, are found in inflammatory cells in active disease.

The IL-2, produced by activated T cells, mediates intercellular communication, promoting T cell proliferation, differentiation of B cells, and activation of macrophages and natural killer cells. IL-2-deficient mice fail to generate the $CD4^+CD25^+$ T_{reg} subset, known to play a key role in maintenance of self-tolerance. They develop a wide spectrum of organ-specific autoimmune diseases as well as an unremitting pancolitis [11,12].

The IL-10 is produced mainly by antigen-presenting cells (APCs), but also by epithelial cells and activated T and B cells. It inhibits the antigen-presenting function of APCs, thereby reducing T cell activation. IL-10-deficient mice lack T_{reg1} cells as well as TGF-β/Smad signalling due to pathogenic $CD4^+$ T cells [13]. The Th1-mediated inflammation is most severe in the colon, but also involves the small intestine.

One of the possible mechanisms by which cytokine levels can be decreased is through the neutralizing effects of intrinsically produced antibodies against specific cytokines. In fact, anti-cytokine antibodies have been found in autoimmune diseases (such as rheumatoid arthritis and systemic lupus erythematosus) and infectious diseases (such as hepatitis and varicella). Such antibodies have not been studied in IBD [14,15]. The most commonly described autoantibodies are directed against IL-1α and IFN-α [16]. Autoantibodies against TGF-β have not been described. Neutralizing anti-IL-2 antibodies have been found in some studies, but not others [17,18]. Antibodies against IL-10 are either rare or of low avidity [19,20]. This study shows that a subset of IBD patients have increased amounts of neutralizing antibodies against TGF-β, IL-2 and IL-10, which may predispose them to disease.

Methods

Patient population

Serum samples were obtained from patients with ulcerative colitis (UC) ($n = 136$), Crohn's disease (CD) ($n = 81$) and normal individuals ($n = 58$) and stored at –70°C in aliquots until use. Informed consent and approval by the Institutional Review Board for UMDNJ were obtained. Results were correlated with age (more or less than 40 years old) and duration of disease (greater or less than 10 years). These separations were chosen because they resulted in similar numbers of patients in each group. Extent of disease was defined as proctitis (disease limited to the first 15 cm from the anus), left-sided colitis (from the rectum to the splenic flexure) or pan-colitis (extending proximal to the splenic flexure) according to endoscopic and histological changes. Active UC was defined as a disease activity index score of greater than 2, and for CD a CD activity index score of greater than 150. Immunomodulators were defined as prednisone (at least 15 mg daily), 6-mercaptopurine (6-MP), infliximab or their equivalents. The ages of the patients averaged 43 ± 10 years for UC, 45 ± 8 for CD and 44 ± 8 for normal individuals. The male-to-female ratio averaged 1·1 for UC, 1·2 for CD and 0·9 for normal individuals.

Enzyme-linked immunosorbent assay

Flat-bottomed microtitre wells were coated overnight at 4°C with TGF-β (isoforms 1, 2 or 3), IL-2, IL-4, IL-6, IL-8, IL-10, IL-12, IL-18, IFN-α or IFN-γ (0·5 ug/ml each) (R&D Systems, Minneapolis, MN, USA) suspended in 50 mM sodium carbonate buffer (pH 9·6). The excess cytokines were removed by washing with phosphate-buffered saline (PBS) lacking detergent in order not to dissociate the cytokines from the plastic surface. RPMI-1640 with 10% fetal calf serum (0·04 ml) (Gibco, Grand Island, NY, USA) was then added to each well for blocking. Test sera (1:200 dilution) were then added, and the plate was incubated for 1 h at room temperature before washing with PBS. This dilution provided the greatest difference between IBD and Nl serum values. The next step was the addition of goat anti-human immunoglobulin (Ig)G (1:5000 dilution) or murine anti-human IgG1 or IgG2a (both at 1:250 dilution) conjugated to alkaline phosphatase followed by a 1-h incubation. After washing with PBS, phosphatase substrate solution (Sigma-Aldrich, St Louis, MO, USA) was introduced and, after 30 min, colour development (optical density: OD) was read spectrophotometrically by absorbance at 405 nm. The control value (without serum) was subtracted from each test value to obtain the final reading. Each serum sample was tested in at least three different experiments and the results averaged. In general, the intra-experimental variation in OD levels averaged 0·015 ± 0·005, while the interexperimental variation was 0·025 ± 0·01.

Biochemical characterization of detected anti-cytokine antibodies

To determine which serum proteins bind TGF-β, beads were coated with TGF-β, IFN-γ or no protein. To accomplish this, magnetic beads (Dynal, Oslo, Norway) were washed with 0·1 M sodium phosphate buffer (pH 7·4). They were then resuspended in the same buffer containing TGF-β, IFN-γ (100 ng/50×10^6 beads) or no cytokine and incubated while rotating at 4°C for 72 h and blocked by 100 ng/0·1 ml of

bovine serum albumin. The washed beads were incubated for 1 h with pooled sera from three UC patients or three normal individuals, all with the greatest values for anti-TGF-β or anti-IFN-γ antibodies by enzyme-linked immunosorbent assay (ELISA). After washing, the beads were suspended in Laemli buffer and boiled for 10 min. The dissociated proteins were subjected to non-reduced 10% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) followed by transfer to nitrocellulose paper. Total proteins were visualized by ponceau staining (Sigma-Aldrich). The presence of IgG that bound to TGF-β or IFN-γ was detected by staining the blot with an alkaline phosphatase-conjugated goat anti-human IgG (Sigma-Aldrich) (1:40 000 dilution) and visualized by enhanced chemiluminescence light detection kit (Perkin Elmer, Boston, MA, USA).

In order to obtain IgG specific for TGF-β, IL-2, IL-4 or IL-10, magnetic beads were coated with each cytokine as described above. IgG fractions from seven patients with UC, seven with CD or seven normal individuals were obtained using a Protein G column (Sigma Aldrich) and then incubated with the cytokine-coated beads. The beads were washed, and the bound IgG eluted using 0·1 M glycine, pH 3. The recovered protein was neutralized, concentrated and the quantity determined by spectrophotometry.

Determination of bioactivity of anti-cytokine antibodies using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay

HT-29 colonic adenocarcinoma cells (1×10^4 cells/0·1 ml) (ATCC, Manassas, VA, USA) were incubated with TGF-β (10 ng/ml) in the presence or absence of TGF-β-specific IgG (10 μg/ml). Alternatively, cytotoxic T cell line (CTLL) cells (ATCC) were incubated with IL-2 or IL-10 (10 ng/ml) in the presence or absence of cytokine-specific IgG (10 ug/ml). After 18 h for TGF-β or IL-10 or 48 h for IL-2, MTT (Sigma-Aldrich) (final concentration of 0·5 mg/ml) was added, and the cells incubated for 6 h at 37°C. Colour development was initiated by the addition of dimethylsulphoxide (0·1 ml) to 0·1 ml in each well. The amount of yellow MTT that was reduced to purple formazan was measured spectrophotometrically at an absorbance of 550 nm. This reduction takes place only when mitochondrial reductase enzymes are active, and relates directly to the number of viable cells. The results were categorized as complete inhibition of the cytokine action (60–100%), partial inhibition (31–59%) and minimal inhibition (0–30%).

Statistical analysis

Two groups of data were compared with the Student's *t*-test if the distribution was Gaussian; otherwise the Wilcoxon rank sum test was used. For more than two groups of data, analysis of variance was used with the Tukey test to compare pairs of data sets. The median values are shown in the graphs involving patient numbers.

Results

The presence of anti-cytokine antibodies in the serum of IBD patients was determined by ELISA (Fig. 1). For anti-TGF-β antibodies, the OD reading, corresponding to the concentration of anti-TGF-β antibodies in the serum, was significantly higher ($P < 0{\cdot}01$) with UC, but not CD, compared with normal sera. Importantly, the percentage of IBD patients with OD values greater than the highest normal value (those above the dotted line in Fig. 1a) was 17% for UC and 8% for CD (Table 1).

Similar to TGF-β, anti-IL-2 antibodies in IBD patients and normal individuals were determined by ELISA. When the groups were compared, the corresponding OD levels were not statistically different among the three patient groups (Fig. 1b). However, the percentage of IBD patients with OD values greater than the highest value in the normal control group was 6% for UC and 21% for CD (Table 1).

Anti-IL-10 antibody levels were also measured by ELISA and found to be greater ($P < 0{\cdot}01$) in CD than in normal individuals (Fig. 1c). Again, the percentage of IBD patients with OD values greater than the highest normal value was 11% for UC and 17% for CD (Table 1).

There were no differences in the median antibody levels among the three groups when studying IL-2, IL-6, IL-8, IL-12, IL-18, IFN-α and IFN-γ (not shown). With IL-4, however, there was a significant difference among the groups with values for CD patients exceeding those for normal individuals (Fig. 1). In addition, with IL-4, there were IBD patients with OD values greater than the highest normal value (9·9% with UC and 10·5% with CD).

When TGF-β, IL-2 and IL-10 were compared, 30% of UC patients and 33% of CD patients had at least one antibody level that was greater than the highest normal value (Table 1). This indicates that 4% of UC patients and 13% of CD patients had more than one antibody. Patients with high antibody levels did not differ clinically from those with antibody levels within the normal range when examining disease

Table 1. Percentage of ulcerative colitis (UC) and Crohn's disease (CD) patients with anti-cytokine antibodies (measured by OD) greater than the highest normal value.

	UC	CD
	% of sera	
TGF-β	17	8
IL-2	6	21
IL-10	11	17
Any of the three cytokines	30	33

This table shows the percentage of UC and CD sera that have values greater than the highest normal values (those lying above the dotted lines in Fig. 1). IL, interleukin; TGF, transforming growth factor.

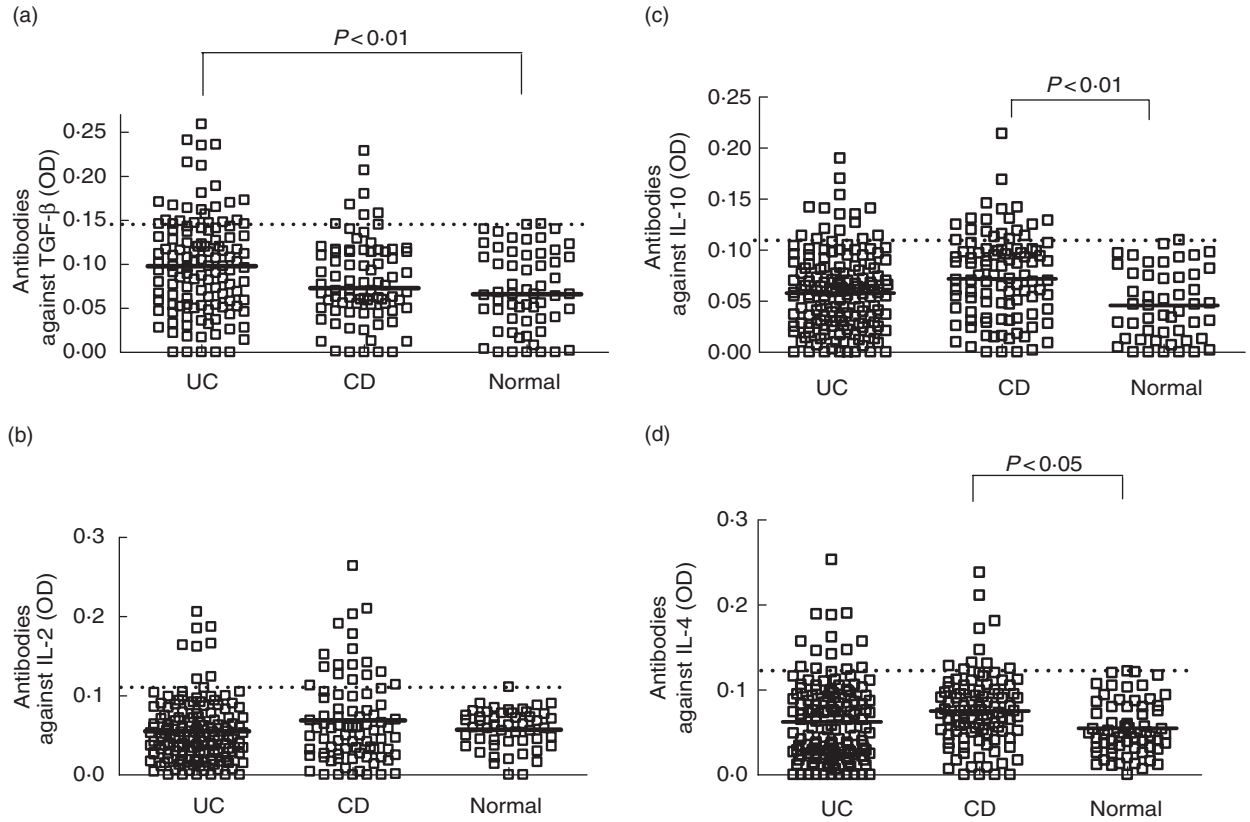

Fig. 1. Serum samples (1:200 dilution) from patients with ulcerative colitis or Crohn's disease or from normal individuals were tested by enzyme-linked immunosorbent assay for antibodies recognizing (a) transforming growth factor-β, (b) interleukin (IL)-2, (c) IL-10 or (d) IL-4. The solid horizontal lines are median values. The dotted lines are the highest values reached by normal sera. Statistical analysis was performed using analysis of variance followed by the Tukey test to evaluate pairs of data within the group.

duration, extent of disease, age and use of immunosuppressive medications.

To confirm the presence of anti-cytokine antibodies in the serum, TGF-β or IFN-γ were linked to beads, then incubated with pooled sera from either three UC patients or three normal individuals, with the highest OD readings for each cytokine. IFN-γ was chosen as a negative control because its antibody levels in all three groups of patients were low. The bound proteins were collected by boiling, then run on SDS-PAGE followed by Western blot analysis with anti-human IgG (Fig. 2). The ponceau stain showed that the major proteins that bound TGF-β or IFN-γ were at the molecular weight corresponding to IgG, specifically about 140 kD. Western blot analysis confirmed that these proteins were human IgG. UC patients had more TGF-β antibodies than did normal individuals in this analysis.

It has been reported that the three isoforms of TGF-β differ in their concentration in IBD [8–10]. In order to evaluate such preferential isotype specific antibodies in IBD sera, ELISAs were performed with TGF-β1, -β2 or -β3 attached to the bottom of microwells (Fig. 3). Using sera from patients with UC, there proved to be no difference in the median values of antibodies for each TGF-β isotype. In only a few cases were there wide individual variations in antibody levels for each isotype. For the most part, however, antibody levels for the three isotypes were similar for each individual. When serum samples from patients with CD or normal individuals were tested, the same results for the three isoforms of TGF-β were obtained (not shown).

The UC is characterized by a predominance of IgG1-secreting inflammatory cells in the mucosa, so an

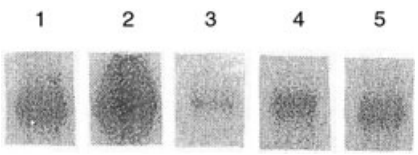

Fig. 2. Transforming growth factor (TGF)-β or interferon (IFN)-γ were attached to beads, then incubated with serum samples from normal individuals or patients with ulcerative colitis (UC) (pooled samples from three patients each). The bound proteins were subjected to sodium dodecyl sulphate-polyacrylamide gel electrophoresis. A Western blot assay shown below was accomplished with goat anti-human immunoglobulin G. Lane 1: immobilized TGF-β1 with normal sera. Lane 2: immobilized TGF-β1 with UC sera. Lane 3: beads with no cytokine or sera. Lane 4: immobilized IFN-γ with normal sera. Lane 5: immobilized IFN-γ with UC sera.

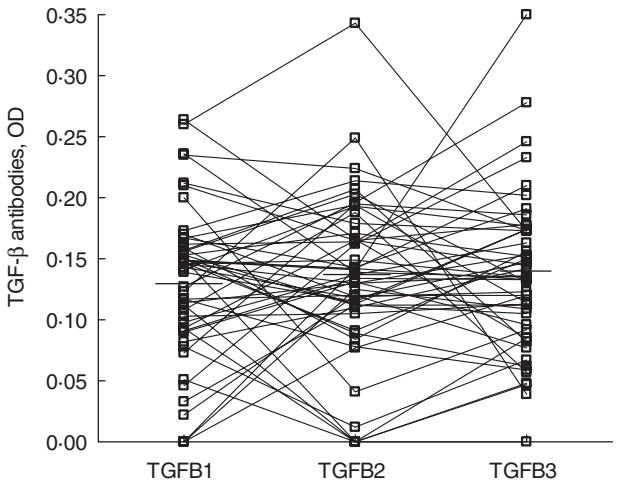

Fig. 3. Serum samples from patients with ulcerative colitis were tested by enzyme-linked immunosorbent assay for antibodies against the three transforming growth factor-β isotypes. The lines connect values from each patient. The horizontal lines represent the median values.

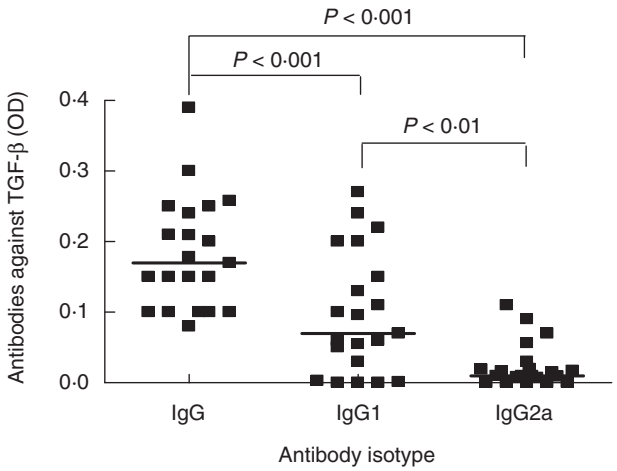

Fig. 4. Serum samples from patients with ulcerative colitis were tested by enzyme-linked immunosorbent assay for immunoglobulin (Ig)G, IgG1 or IgG2a using the appropriate alkaline phosphatase-conjugated anti-human antibody. The horizontal lines represent median values. This shows that the majority of the IgG was IgG1.

abnormally high level of anti-TGF-β antibodies could be derived from such cells. To evaluate the IgG isotype of anti-TGF-β antibodies, an ELISA was developed using murine antibody to human IgG1 and IgG2a conjugated with alkaline phosphatase (Fig. 4). These experiments showed that IgG1 predominated, although IgG2a was present in a few individuals. This parameter was the same for CD and normal individuals. Similarly, anti-IL-2 and anti-IL-10 antibodies were predominantly, but not exclusively, IgG1 (not shown).

To determine whether there is a relationship between the amounts of anti-cytokine antibodies and clinical features, comparisons were made between the OD readings representing the amounts of anti-cytokine antibodies and age, disease duration, extent of disease, the presence or absence of immunosuppressive drugs and disease activity. In addition, comparisons were made between the OD readings and the date of collection of the samples as IgG is known for aggregating with time, giving variable results. These analyses all proved to be negative (not shown), indicating that the rise in anti-cytokine antibodies was not found in particular patient subsets.

Finally, the neutralizing ability of the anti-cytokine antibodies from seven UC patients, seven CD patients and seven normal individuals was investigated. TGF-β, IL-2 and IL-10 were studied because depletion of these cytokines led to intestinal inflammation. IL-4 was not studied, as depletion of this cytokine has been found to ameliorate colitis [20]. First, TGF-β (10 ng/ml) was preincubated for 10 min with TGF-β-specific IgG antibodies from each subject before addition to HT-29 colon adenocarcinoma cells. The TGF-β-induced reduction in the number of viable cells, as measured by the MTT assay after 18 h, was reversed by TGF-β-specific IgG from most individuals in all three patient groups (Table 2). Next, IL-2 was preincubated for 10 min with anti-IL-2 antibodies from each subject before addition to the CTLL cells. The associated increase in numbers of viable cells with IL-2 was reversed by the cytokine-specific IgG from most individuals in all three patient groups. Finally, IL-10 was preincubated for 10 min with anti-IL-10 antibodies from each subject before addition to the CTLL cells. The associated increase in numbers of viable cells with IL-10 was reversed by the cytokine-specific IgG from most individuals in all three patient groups.

Table 2. Numbers of ulcerative colitis (UC) or Crohn's disease (CD) patients or normal individuals (NI) with neutralizing antibodies against transforming growth factor (TGF)-β, interleukin (IL)-2 or IL-10.

	TGF-β			IL-2			IL-10		
	UC	CD	NI	UC	CD	NI	UC	CD	NI
Degree of neutralization	Numbers of individuals								
Complete	5	4	3	6	5	5	5	4	3
Partial	1	2	3	0	2	1	2	2	1
None	1	1	1	1	0	1	0	1	3

Antibodies against IL-2, IL-4, IL-10 and TGF-β were isolated from seven individuals in each patient group and tested for their ability to neutralize the actions of these cytokines in bioassays.

Discussion

The role of autoantibodies against cytokines is unknown [14,15]. They may arise due to an imperfect discrimination between self and non-self, a process that dictates the balance between immunity and autoimmunity. Alternatively, there may be a break in tolerance by molecular mimicry, such as the IL-10-like molecules encoded by some poxviruses [14,15]. Cytokines may serve as haptens on proteins, eliciting an immune response. An alternative thought is that these autoantibodies serve a function. They may act as carrier proteins for cytokines, extending their circulation time from minutes to hours. This would be due to protection against proteolytic degradation as well as delayed glomerular filtration by the kidney. In addition, these antibodies may buffer rapid changes in the concentrations of cytokines. Finally, autoantibodies may inhibit, activate or have no effects on cytokine actions.

The presence of antibodies against TGF-β, IL-2 or IL-10 was determined initially by ELISA. This is an efficient and sensitive assay to screen large numbers of patients. The results may be altered, however, by other components of the serum and by the non-specific binding of IgG. Therefore, biochemical means were utilized to prove the presence of anti-TGF-β antibodies. The proteins in UC sera that bound to immobilized TGF-β were identified by Western blot assay; only IgG bound TGF-β. Although LAP also attaches to TGF-β, there was no band at 100 kDa, the molecular weight of LAP. This could be due to a greater affinity of TGF-β for anti-TGF-β autoantibodies than for LAP or to an inability of the autoantibodies to select TGF-β already bound to LAP.

The TGF-β inhibits the proliferation of most epithelial cells and some cancer cells [7]. In the present study, TGF-β reduced the numbers of viable HT-29 cells. Antibodies from most individuals neutralized this effect. It is possible that this contributes to the development of cancer, particularly in those IBD patients with high levels of TGF-β antibodies. IL-2 or IL-10 increased the numbers of viable CTLL cells. This, too, was neutralized by antibodies from most individuals. There is no difference in neutralizing capacity among the three patient groups.

The TGF-β transcripts and protein have been found to be elevated in active IBD, as demonstrated by Northern blot analysis, immunohistochemistry and *in situ* hybridization [8–10], although one study showed no increase [21]. The TGF-β-producing cells were found mainly in the lamina propria. This may not, in fact, be accompanied by increased TGF-β activity if high concentrations of neutralizing antibodies are present locally. TGF-β has divergent effects in the bowel. While it promotes epithelial cell healing and suppresses the immune response, it could be pathogenic by causing intramural fibrosis.

The amount of IL-2 in the mucosa of IBD patients is controversial. In some studies, messenger RNA has been shown to be increased in active CD while both mRNA and protein are reduced in both CD and UC in other studies [21–24]. Although the present analysis shows no difference in the amounts of anti-IL-2 antibodies between the different patient groups, there was a substantial number of CD patients with levels greater than the highest normal value. This may reduce the availability of active IL-2 in CD.

The IL-10 mRNA and protein are elevated in active IBD mucosal tissues due to excess production by $CD4^+$ T cells and macrophages in the intestine [21,22,25]. In the present study, CD, as a group, had a higher average amount of anti-IL-10 antibodies in the serum than did normal individuals. One explanation for this dichotomy is that the elevated IL-10 production by mucosal leucocytes may be a feedback response to excess anti-IL-10 antibodies in the sera. Alternatively, IL-10 may be generated with chronic inflammation in response to stimuli such as IL-12. It may be less effective in those with neutralizing anti-IL-10 antibodies.

This study shows that a subset of IBD patients have high amounts of neutralizing antibodies against TGF-β, IL-2 or IL-10. This suggests that IBD is not a uniform process, but rather one associated with a variety of immune abnormalities. The lack of variation with disease activity is consistent with the idea that the excess anti-cytokine antibodies represent an innate characteristic of certain patients, perhaps predisposing these individuals to destructive mucosal inflammation. Although this study does not prove conclusively that the anti-cytokine antibodies are pathogenic, they are easily measurable in serum and may affect cytokine actions. The degree of cytokine action, rather than its production, would be a true measure of its deficiency or excess in IBD.

References

1 Gorelik L, Flavell RA. Transforming growth factor-β in T cell biology. Nat Rev Immunol 2002; **2**:46–53.

2 Ohtsuka Y, Sanderson IR. Transforming growth factor-beta: an important cytokine in the mucosal immune response. Curr Opin Gastroenterol 2000; **16**:541–5.

3 Shull MM, Ormsby I, Kier AB *et al*. Targeted disruption of the mouse TGFβ1 gene results in multifocal inflammatory disease. Nature 1992; **359**:693–99.

4 Hahm K-B, Im Y-H, Parks TW *et al*. Loss of transforming growth factor β signaling in the intestine contributes to tissue injury in inflammatory bowel disease. Gut 2001; **49**:190–8.

5 Ludviksson BR, Ehrhardt RO, Strober W. TGFβ production regulates the development of the 2, 4, 5-trinitrophenol-conjugated keyhole lipet hemocyanin-induced colonic inflammation in IL-2-deficient mice. J Immunol 1997; **159**:3622–28.

6 Massague J, Chen YG. Controlling TGFβ signaling. Genes Dev 2000; **14**:627–44.

7 Grainger DJ, Mosedale DE, Metcalfe JC. TGFβ in blood: a complex problem. Cytokine Growth Factor Rev 2000; **11**:133–45.

8 Babyatsky MW, Rossiter G, Podolsky DK. Expression of transforming growth factors α and β in colonic mucosa in inflammatory bowel disease. Gastroenterology 1996; **110**:975–84.

9 DiMola FF, Friess H, Schreuren A *et al*. Transforming growth factor-β and their signaling receptors are coexpressed in Crohn's disease. Ann Surg 1999; **229**:67–75.
10 Xian CJ, Xu X, Mardell E *et al*. Site-specific changes in transforming growth factor-α and -β1 expression in colonic mucosa of adolescents with inflammatory bowel disease. Scand J Gastroenterol 1999; **34**:591–600.
11 Sadlack B, Merz H, Schorle H, Schimpi A, Feller AC, Horak I. Ulcerative colitis-like disease in mice with a disrupted interleukin-2 gene. Cell 1993; **75**:253–61.
12 Elson CO, Cong Y, McCracken VJ, Dimmitt RA, Lorenz RG, Weaver CT. Experimental models of inflammatory bowel disease reveal innate, adaptive, and regulatory mechanisms of host dialogue with the microbiota. Immunol Rev 2005; **206**:260–76.
13 Ruiz PA, Shkoda A, Kim SC, Sartor RB, Haller D. IL-10 gene-deficient mice lack TGF-beta/Smad signaling and fail to inhibit proinflammatory gene expression in intestinal epithelial cells after the colonization with colitogenic *Enterococcus faecalis*. J Immunol 2005; **174**:2990–99.
14 Bendtzen K, Hansen MB, Ross C, Svenson M. High-avidity autoantibodies to cytokines. Immunol Today 1998; **19**:209–11.
15 Coutinho A, Kazatchkine MD, Avrameas S. Natural autoantibodies. Curr Opin Immunol 1995; **7**:812–18.
16 Meager A, Wadhwa M, Dilger P *et al*. Anti-cytokine autoantibodies in autoimmunity: preponderance of neutralizing autoantibodies against interferon-alpha, interferon-omega and interleukin-12 in patients with thymoma and/or myasthenia gravis. Clin Exp Immunol 2003; **132**:128–36.
17 Tiberio L, Caruso A, Pozzi A *et al*. The detection and biological activity of human antibodies to IL-2 in normal donors. Scand J Immunol 1993; **38**:472–6.
18 Svenson M, Hansen MB, Ross C *et al*. Antibody to granulocyte-macrophage colony-stimulating factor is a dominant anti-cytokine activity in human IgG preparations. Blood 1998; **91**:2054–61.
19 Menetrier-Caux C, Briere F, Jouvenne P, Peyron E, Peyron F, Banchereau J. Identification of human IgG autoantibodies specific for IL-10. Clin Exp Immunol 1996; **104**:173–9.
20 Stevceva L, Pavil P, Husband A, Ramsay A, Doe WF. Dextran sulfate sodium-induced colitis is ameliorated in interleukin-4 deficient mice. Genes Immun 2001; **2**:309–16.
21 Melgar S, Yeung M-W, Bas A *et al*. Over-expression of interleukin 10 in mucosal T cells of patients with active ulcerative colitis. Clin Exp Immunol 2003; **134**:127–37.
22 Niessner M, Volk BA. Altered Th1/Th2 cytokine profiles in the intestinal mucosa of patients with inflammatory bowel disease as assessed by quantitative reversed transcribed polymerase chain reaction (RT-PCR). Clin Exp Immunol 1995; **101**:428–35.
23 Mullin GE, Lazenby AJ, Harris ML, Bayless TM, James SP. Increased interleukin-2 messenger RNA in the intestinal mucosal lesions of Crohn's disease but not ulcerative colitis. Gastroenterology 1992; **102**:1620–7.
24 Kusugami K, Matsuura T, West GA, Youngman KR, Rachmilewitz D, Fiocchi C. Loss of interleukin-2-producing intestinal CD4+ T cells in inflammatory bowel disease. Gastroenterology 1991; **101**:1594–605.
25 Autschbach F, Braunstein J, Helmke B *et al*. *In situ* expression of interleukin-10 in noninflamed human gut and in inflammatory bowel disease. Am J Pathol 1998; **153**:121–30.