Comparing the type and severity of inflammatory bowel disease in relation to IgG4 immunohistochemical staining

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Abstract

Background and study aim : **The role of immunoglobulin (Ig) G4 in the etiopathogenesis of inflammatory bowel disease (IBD) and its association with endoscopic and pathological activity are not yet completely understood. The purpose of this study was to determine the possible relationship between IgG4 status and IBD.**

Patients and methods : **Endoscopic colon biopsies of 55 patients with ulcerative colitis (UC) and of 17 patients with Crohn's disease (CD) were examined. Numbers of IgG4-positive plasma cells stained immunohistochemically were counted in a minimum of 5 high power fields (HPFs) for each specimen. The presence of > 10 cells/HPF IgG4-positive PCs was considered positive.**

Results : **The prevalence of IgG4-positive plasma cells in the lamina propria of the colonic mucosa was significantly higher in patients with UC than in those with CD (p :0.01). Additionally, the prevalence of IgG4-positive plasma cells increased in line with endoscopic and pathological activity in UC patients. Conversely, we determined no significant correlation between IgG4 positivity and pathological activity in the CD group. IgG4-positive UC patients also exhibited findings of more severe disease compared to IgG4-negative UC patients.**

Conclusions : **Immunohistochemical IgG4 staining may predict disease severity in UC and may be a useful marker for distinguishing between UC and CD.** (Acta gastroenterol. belg., **2016**, 79, **216-221**).

Key words : IgG4, immunohistochemistry, inflammatory bowel disease.

Introduction

Inflammatory bowel disease (IBD) is a chronic intestinal inflammatory disorder with two major forms, ulcerative colitis (UC) and Crohn's disease (CD). Although the etiology and pathogenesis of UC and CD are still unknown, several immunological mechanisms play significant roles in both (1). While differences between histological findings, sites of involvement and endoscopic features help to distinguish these two entities, differences in immunological pathways are the main reason for their different clinical outcomes (2). No immunological parameters have yet been developed to differentiate between these two conditions.

Plasma cells (PCs) are one of the major cellular components of the gut lamina propria. In healthy individuals, immunoglobulin (Ig) A-producing PCs are dominant in the gut lamina propria. However, in IBD there is a massive accumulation of IgG-containing PCs (3). IgG has four subgroups. Severely inflamed mucosa in UC and CD patients has been shown to have a different IgG subclass distribution at histological examination (4). However, the precise roles of different IgG subtypes have yet to be identified.

Today, due to awareness of IgG4-related disease (IgG4-RD), a systemic sclerosing condition that affects various organs, including the pancreas, bile duct and gastrointestinal tract (5), investigations have focused on the association between IgG4 and other immunological diseases. Autoimmune pancreatitis (AIP) and IgG4-related sclerosing cholangitis, a similar entity to primary sclerosing cholangitis (PSC) with few differences, are the best known clinical forms of IgG4-RD and have been shown to be related to IBD.

Due to this relationship, IBD has been suspected to be a part of IgG4-RD (6, 7). Limited studies have shown a high prevalence of IgG4-positive PCs in the lamina propria of the colonic mucosa in IBD (8-10). However, the validity of this has not yet been confirmed, and its usefulness in the setting of IBD is unclear.

This study evaluated the presence and the significance of IgG4-positive plasma cells in colonic biopsies of patients with established diagnosis of IBD.

Patients and Methods

The study was approved by the institutional review board at the Kecioren Research and Training Hospital in Turkey, in concordance with the principles of the Helsinki documents.

We used a pathology database to identify IBD patients who had undergone endoscopic colon biopsy between October 2012 and July 2014 at a single center in Turkey. Two groups were included in the study, patients with biopsy-proven CD and patients with biopsy-proven UC. Patients with AIP or PSC or who had received biological treatments were excluded. Patients who were receiving mesalazine and/or azathioprine therapy, or else no treatment were included into study. In all cases, diagnosis

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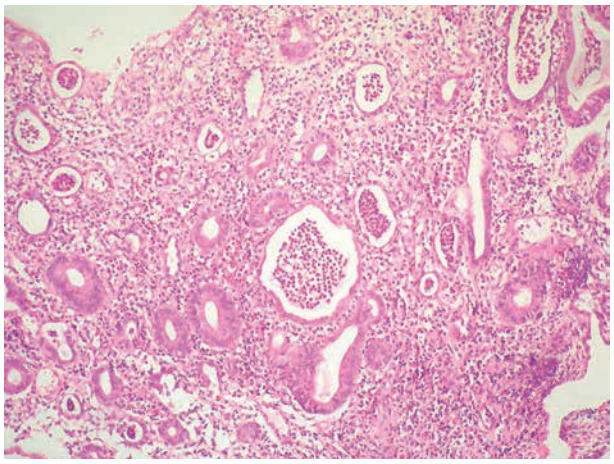

Fig. 1. — Histopathological examples of inflammatory bowel disease : Severely active ulcerative colitis (200× H&E).

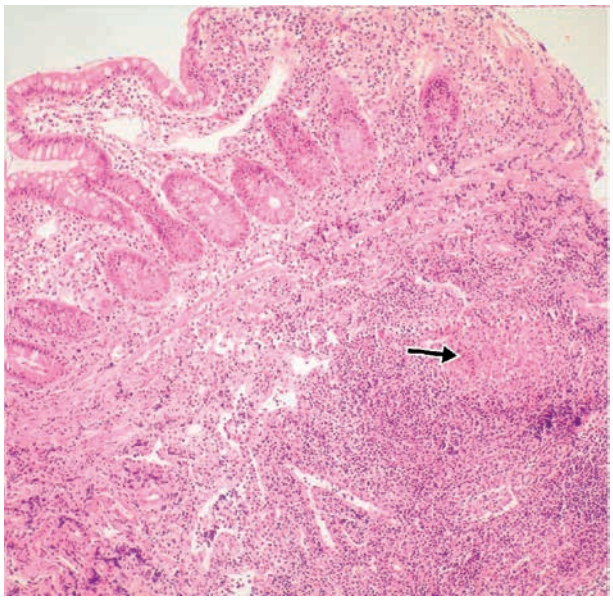

Fig. 2. — Histopathological examples of inflammatory bowel disease : "naked granuloma (arrow)" in Crohn's disease (100× H&E).

was confirmed using established clinical, radiological and endoscopic criteria with confirmatory histology.

Patients' medical records were reviewed for demographic and laboratory data including hemoglobin (Hb), white blood cell count (WBC), platelet count (PLT), C-reactive protein (CRP) and sedimentation (Sed). Endoscopic findings of disease were recorded on the basis of endoscopy reports in our gastroenterology clinic. Extent and severity of disease in UC patients were evaluated based on endoscopic findings. The Rachmilewitz Endoscopic Activity Index (EAI) was used to evaluate disease severity in UC patients (11). Patients were grouped according to EAI. A score of 0 was defined as inactive colitis, scores of 2-6 as mild colitis and scores of 6-12 as severe colitis. Extent of UC was recorded as left-sided colitis and pancolitis. UC patients were also divided into two subgroups based on IgG4 positivity.

Paraffin blocks of endoscopic colon biopsies were available for all 72 cases (55 cases of UC and 17 cases of CD). Hematoxylin and eosin (H & E) stained slides of all biopsies were reviewed by two blinded pathologists (Figs. 1 and 2). A histopathological evaluation was performed based on epithelial neutrophil infiltration, cryptitis, crypt abscesses, ulceration and erosion. A Harpaz HSS score of 0 was categorized as inactive, a score of 1 as mild, a score of 2 as moderate, and a score of 3 as severe. The Harpaz HSS was scored according to the parameters shown in Table 1 (12). Table 1 shows a histopathological inflammation scoring system for IBD that can be used for both CD and UC (Table 1).

A biopsy fragment with the highest amount of chronic inflammation was selected for inclusion on the paraffin-embedded tissue sections. Immunohistochemistry for IgG4-positive plasma cells was performed on formalin fixed paraffin-embedded tissue sections. Monoclonal anti-human IgG4 antibody (Abcam&Cambridge [EP4420] [ab109493], UK, 1/200 dilution) was applied to 4-mm-thick sections, and antigen retrieval was performed using protease digestion. Antigen detection was conducted

Table 1. — **The histopathological inflammation scoring system of IBD**

Score	Definition	Histopathologic finding
0	Inactive colitis	No cryptitis
1	Mildly active colitis	Cryptitis in < 50/ of crypts
2	Moderatelt active colitis	Cryptitis in > 50/ of crypts
3	Severely active colitis	Ulcerations or erosions

using diaminobenzidine chromogen. We did not used secondary antibody. Fields with the highest subjective density of IgG4-positive cells were photographed using a ×40 objective lens, as were the corresponding regions on IgG- stained sections. We counted cells with plasmacytoid morphology and cytoplasmic immunostaining. Numbers of immunohistochemically identified IgG4-positive PCs in the lamina propria were counted in a minimum of 5 high power fields (HPFs) in each specimen, and the mean value was calculated as the average point. Degrees of IgG4-positive PCs infiltration based on average points were categorized into two groups, IgG4-positive and IgG4-negative. IgG4 positivity was defined as the presence of > 10 IgG4-positive PCs/HPF and IgG4-negativity as < 10 IgG4-positive PCs/HPF (13).

Statistical Analysis

Statistical analyses were performed on SPSS version 16.0 (SPSS, Chicago, IL, USA) software. The chi-square test was used for categorical data and one-way analysis of variance (ANOVA) for continuous variables. Numbers and percentages were used to express categoric data, mean plus SD for normally distributed data and median

Table 2. — **Demographic and clinical data of study group**

Parameters		UC	CD	p
Age (years)		49.0 ± 15.6	50.8 ± 12.5	0.666
Sex	Male (n, %) Female (n, %)	33 (60.0%) 22 (40.0%)	10 (58.8%) 7 (41.2%)	0.931
Hb (gr/dl)		12.8 ± 1.8	12.2 ± 1.6	0.231
WBC (/mm3)		9310 ± 3833	10788 ± 3364	0.158
Plt (/mm3)		288381 ± 85007	348352 ± 81863	0.013
CRP (mg/dl)**		2.1 (0.12-75)	1.7 (0.23-8.5)	0.542
Sedim (mm/h)		34.3 ± 20.5	39.6 ± 13.6	0.327

Hb, Hemoglbine ; WBC, white blood cell count ; PLT, Platelet count ; CRP, C- Reactive Protein ; Sedim, Sedimentation ; UC, Ulcerative colitis ; CD, Crohn disease.

*Results were expressed as mean ± SD and number of patient.

**Result were expressed as median (min-max).

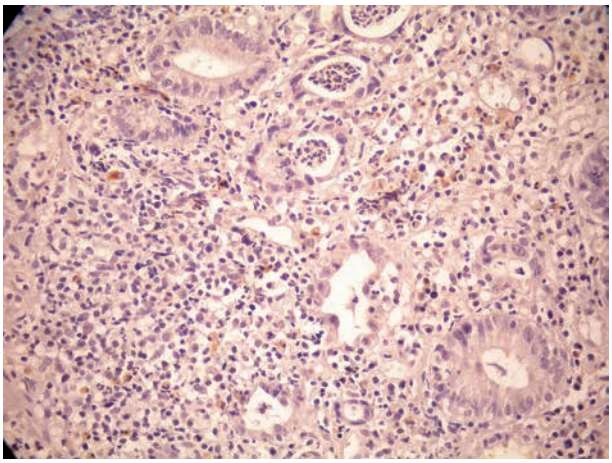

Fig. 3. — IgG4 (+) plasma cells with strong cytoplasmic staining (arrow) ; more than 10 cells per high power field for ulcerative colitis (400× biotin-streptavidin).

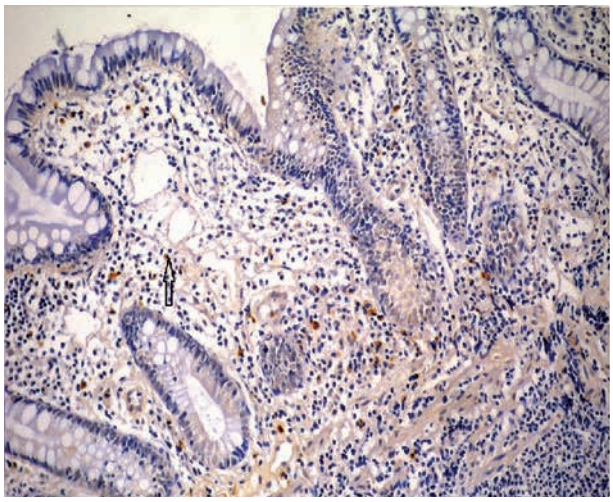

Fig. 4. — IgG4 (+) plasma cells with strong cytoplasmic staining (arrow) ; more than 10 cells per high power field for Crohn's disease (200× biotin-streptavidin).

(min-max) for non-normally distributed data. Linear regression analysis was performed to determine the effect of independent variables on a dependent variable. Significance was set at $p < 0.05$.

Results

Demographic and clinical data are presented in Table 2. Seventy-two cases of IBD were enrolled, 55 cases of UC (33 males and 22 females) and 17 of CD (10 males and 7 females). Patients' ages ranged from 17 to 80 years. The mean age of the UC group was 48.9 ± 15.6 years and that of the CD group 50.7 ± 12.5 years. There were no significant differences in terms of age and sex distribution between the two groups. Although there were no significant differences in terms of Hb, WBC or CRP levels between the groups, the CD group had significantly higher PLT counts and sedimentation measurements (p:0.013 and p:0.044, respectively) compared to the UC patients (Table 2).

The prevalence of IgG4-positive PCs was significantly higher in UC patients compared to those with CD (52.7% and 17.6%, respectively, p :0.01) (Table 3) (Figs. 3 and 4). The majority of CD cases (23.5%) were not stained with IgG4 (Fig. 5).

The prevalence of IgG4-positive PCs increased with endoscopic and histopathological activity, as shown in graphic 1. At linear regression analysis, histopathological activity was identified as the independent factor affecting IgG4-positivity (t:3,525 ; p:0.001)

Subgroup analysis was performed among the UC patients based on IgG4-positivity. Demographic and clinical data for IgG4-positive and -negative UC patients are shown in table 5. There were no significant differences in terms of age or sex distribution. IgG4-positive UC patients had significantly higher WBC and sedimentation values and significantly lower Hb values. IgG4-positive UC patients tended to have pancolitis and higher levels of moderate and severe histopathological activities, while IgG4-negative UC patients tended to have left-sided

Table 3. — **Prevalance of IG4 positivity in UC and CD patients**

Parameters	UC	CD	P value*
IG4 (-) (n, %)	26 (47, 3%)	14 (82, 4%)	0.01
IG4 (+) (n, %)	29 (52, 7%)	3 (17, 6%)	

UC, Ulcerative colitis ; CD, Crohn disease ; IG4, Immunglobuline G4.

*Comparison between groups was calculated by chi-square test.

colitis and higher levels of inactive and mild histopathological activities. IgG4 positive colitis also had higher levels of severe endoscopic activity (Table 5).

Discussion

This study determined a significant increase in the prevalence of IgG4-positive PCs in colonic pinch biopsies of patients with UC compared to patients with CD. IgG4-positive UC patients also had findings of more severe disease compared to IgG4-negative UC patients. On the basis of these results, we conclude that IgG4 may help determine the disease type and severity in IBD in the light of the different immunopathogenetic mechanisms involved.

There is increasing evidence that a disturbed balance in the T cell regulatory system is involved in the pathogenesis of IBD (14). T lymphocytes have different subtypes. Among these, T-helper (Th) 1 and Th2 are the best-defined subtypes. Th1-mediated lesions are marked by transmural cellular infiltration sometimes associated with granuloma. Additionally, whereas epithelial cell layer changes are clearly present, these are not a dominant feature. A similar histopathology is observed in CD, which also appears to be Th1-mediated. Th2-mediated inflammation is characterized by a more superficial colonic inflammation and epithelial hyperplasia, somewhat similar to UC (15). Although UC has not been clearly shown to be a Th2-mediated inflammation, Th2-mediated colitis models resembling UC have been described (16). Increased production of some Th2 cytokine such as IL-5 and IL-13 has also been observed in lamina propria mononuclear cell isolates from UC patients compared to CD patients (16,17).

IgG4 switching is also dependent on Th2 response. Excessive Th2 responses triggered by TLR ligands together with activation of Tregs create abnormal immunological environments leading to enhanced IgG4 production. In addition, IL-13 promotes proliferation of B cells and class switching to IgG4 and IgE (18). Expression of Th2 cytokines (IL-4, IL-5, and IL-13) has also been shown to be up-regulated in the affected tissues of patients with IgG4-related disease (18). Increased IgG4 levels have been shown in sera and colonic mucosa of UC patients (10,20,21), supporting the idea that UC and excessive IgG4 production have a similar immunopathogenesis.

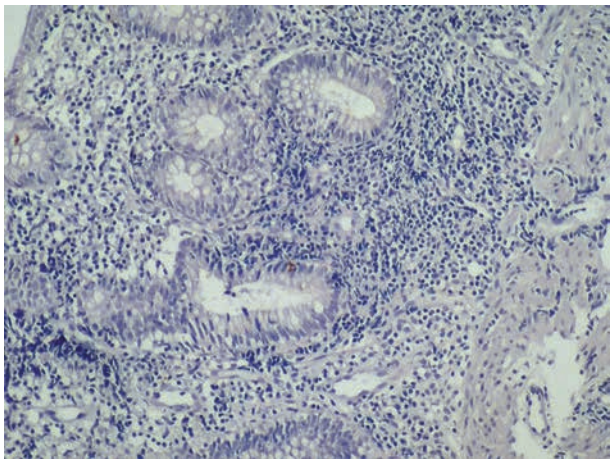

Fig. 5. — Immunonostaining for IgG4 revealing no positive staining plasma cells in inactive ulcerative colitis (400× biotin-streptavidin).

Although colonic mucosa in both UC and CD have been shown to contain high numbers of IgG-producing PCs (3), the distribution of IgG subtypes in the colonic lamina propria varies. Significantly higher levels of IgGl have been reported in UC compared to CD. Conversely, a significantly higher level of IgG2 immunocytes has been reported in CD (4). Recent studies have reported significantly increased IgG4-containing PCs in lamina propria isolates from UC patients compared to CD, as in our study (10). However, it is unclear whether IgG4 is involved in the pathology of UC.

A specific IgG subclass response may suggest specific stimulation by a particular antigen (22). IgG4 response has been demonstrated in chronic antigen stimulation and chronic allergic disease. High levels of specific IgG4 are frequently observed in subjects who are sensitive to food antigens (23). The high level of IgG4-positive PCs in UC suggests that chronic allergic stimulation by luminal or colonic epithelial cell antigens plays a role in the pathogenesis of UC (24). In contrast, Arato *et al*. suggested that colorectal IgG3 and IgG4 responses might be characteristic of early changes in UC (21). There are some etiological differences between UC and CD. An etiological role for commensal enteric bacteria has therefore been suggested in the pathogenesis of CD, but this represents a less obvious influence in UC, because Th1-mediated colitis is also seen in infective colitis (25). Additionally, CD responds to metronidazole, ciprofloxacin or broad spectrum antibiotics, although these agents are not effective in the majority of cases of UC (26). These etiological differences may be supported by IgG4 positivity and Th2-related immune response, which is also seen in allergic reactions (27), suggesting the possibility of an allergic stimulus in the etiopathogenesis of UC.

Similarly to etiological and immunopathogenetic differences between CD and UC, differences in expression of immunological markers may also be expected. Thus,

Table 4. — **Demographic and clinical data in IG4 positive and negative UC patients**

Parameters	IG4 (-) UC	IG4 (+) UC	P value
Age (year)	49.7 ± 11.8	48.3 ± 18.6	0.759
Male (n, %) Female (n, %)	15 (57, 7%) 11 (42, 3%)	18 (62, 1%) 11 (37, 9%)	0.478
Hb (gr/dl)	13.4 ± 1.6	12.3 ± 1.8	0.024
WBC (/mm3)	7500 ± 2800	10900 ± 4000	0.001
PLT (/mm3)	271885 ± 79506	303172 ± 88391	0.175
Sedim (mm/h)	22.3 ± 7.7	45.2 ± 22.5	< 0.001
CRP	1.2 (0,12-75)	4.1 (0.3-26.5)	0.556
Left Sided Colitis (n, %) Pancolitis (n, %)	20 (76, 9%) 11 (42, 3%)	13 (54.2%) 11 (45.8%)	0.028
Histopathologic Activity Absent (n, %) Mild (n, %) Moderate (n, %) Severe (n, %)	 17 (65, 4%) 5 (19, 2%) 4 (15, 4%) 0	 2 (6, 9%) 3 (10, 3%) 13 (44, 8%) 11 (37, 9%)	 < 0.001
Endoscopic Activity Inactive colitis (n, %) Mild (n, %) Severe (n, %)	 4 (15, 4%) 15 (57, 7%) 7 (23, 3%)	 0 5 (17, 2%) 24 (92, 8%)	 < 0.001

Hb, Hemoglobine ; WBC, white blood cell count ; PLT, Platelet count ; CRP, C-Reactive Protein ; Sedim, Sedimentation ; UC, Ulcerative colitis ; IG4, Immunglobuline G4.

*Results were expressed as mean±SD and number of patient, **Result were expressed as median (min-max).

in the light of our results and those of previous studies (10,13) it may be concluded that immunohistochemical IgG4 staining may assist in differentiating between UC and CD.

Another aspect of this study that IgG4-positive UC patients had findings of more severe disease. It is unclear whether IgG4-containing PCs aggravate the disease severity or whether the increase in IgG4-containing PCs is a result of the increased activity. IgG4 may enhance the release of chemical mediators from mucosal mast cells, which contributes to the intestinal inflammation in UC, because IgG4 functions together with IgE in allergic states. In support of this view, Senju *et al*. reported an increase in IgG4-containing cells with increasing IgE-containing cells and tissue histamine levels in active colonic mucosa in cases of UC (28). IgG subclasses also increase in tissue destruction, either through a protective effect or excessive immune response. Previous studies have also found that the prevalence of IgG4 positivity is correlated with the histological severity of UC (10, 13). Our study supports these previous findings. It may be expected that as pathologic activation increases, IgG production increases. In addition, the prevalence of IgG4 positivity in this study increased with increased EAI. The prevalence of IgG4 may reflect the disease activity.

UC is a heterogeneous disease in terms of site of involvement, race, region, etc. The lack of IgG4-positive PCs in a substantial minority of cases of UC may reflect the immunological non-homogeneity of this disease. Although UC has been suspected to be a part of IgG4-RD (6), the diagnosis of IgG4-related disease requires both a characteristic histopathological profile and elevated numbers of IgG4-positive plasma cells (29). UC lacks both storiform fibrosis and phlebitis, and therefore does not fit the profile of IgG4-related disease. Nevertheless, UC is associated with elevated levels of IgG4-positive plasma cells, suggesting that there may be a IgG4-related subtype of UC with severe clinical and pathological outcomes.

Similarly, more severe clinical outcomes have been reported in IgG4-related PSC and pouchitis (20,30,31).

There are a number of limitations of this study. First, it involved patients who were pretreatment or receiving treatment, as advised by Hartman in 2014 in a letter (32). Since, data were collected from medical records, and no exact information about treatment on biopsy time was available. Thus the effect of treatment on IgG4 positivity could not be assessed. In addition, detailed data concerning the pattern of disease such as sclerosing, penetrating or fistulizing and the site of involvement such as the ileal, colonic or ileocolonic were not available for all patients with CD. These parameters were therefore not included in the analysis. It is also worth to note that a lower number of IgG4+ PLCs in inactive or slightly active UC, or even CD, may indicate that the inflammatory infiltrate is less dense or patchy. Multiple biopsy samples in each case should be studied in order to overcome this problem.

In conclusion, this study shows that immunohistochemical IgG4 staining may help differentiate between UC and CD. IgG4 positivity contributes to predicting disease severity. Whether UC has subtypes related to IgG4 is a subject requiring further investigation. Further clinical and multicenter studies are needed to determine this.

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