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LETTER TO THE EDITOR
ΓΡΑΜΜΑ ΠΡΟΣ ΤΟΝ ΕΚΔΟΤΗ

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Inflammatory "storm" in inflammed bowel mucosa
An immunohistochemical study in patients with inflammatory bowel disease

Dysregulation of the mucosal immune system is one of the main causes of inflammatory bowel disease (IBD). Direct macrophage induced toxicity on the gut mucosa causes chronic inflammation.[1] Human leucocyte antigen G (HLA-G) is a non-classical major histocompatibility complex (MHC) molecule, and its expression interferes with the function of natural killer (NK) and T-cells, leading to immunological tolerance. Neutrophilic infiltration is a hallmark of the histopathology of ulcerative colitis (UC). The release of myeloperoxidase (MPO) from neutrophils acts as mediator of active colitis and tissue damage.[2] Cluster of differentiation (CD) 68+ macrophages, another element of the active inflammation in IBD, are downregulated after administration of infliximab.[3] Our short preliminary study aims to explore the expression of these three factors in the setting of acute inflammation in moderate to severe CD terminal ileitis and UC through immunohistochemistry.

The study was approved by the Ethical Committee of the Aristotle University of Thessaloniki. Histological specimens from 20 patients, specifically 13 with histologically confirmed UC colitis, 5 with confirmed CD terminal ileitis, and two control subjects, were processed using immunohistochemistry. The mean age of the patients was 38 years. All 18 patients with IBD had clinical and endoscopic features of suspected IBD with active inflammation, they were not on treatment, and had undergone endoscopy between January 2019 and December 2020. Two healthy cases were used as control subjects, from whom biopsies were obtained on routine surveillance endoscopy. Three different monoclonal antibodies were used in the immunohistochemistry testing, against MPO (dilution 1/1,000, Dako), HLA-G (clone MEM-G/2, Santa Cruz Biotechnology, Inc, USA) and CD-68 (clone 514H12, Leica Microsystems, UK). The material was processed anonymously by two different pathologists. The medical history was retrieved from the medical orders enclosed with the specimens. The Nancy histological index and Naini-Cortina score were used for histological evaluation. Most of the cases were rated as moderate to high severity, with Nancy score >2 and Naini-Cortina score >4. Intensity of staining was recorded as negative (0), weak (1+), moderate (2+) and strong (3+).

The results were processed with the Statistical Package for Social Sciences (SPSS), version 25.0. Correlation was checked using Spearman rank analysis.

Figures 1 and 2 show strong staining for MPO, demonstrated in a case of UC, and figures 3 and 4 show moderate staining for CD-68 in a case of terminal ileitis. The immunohistological scoring of all cases is presented in table 1. No statistically significant correlation was found between both MPO and CD-68 expression and histology score in UC or CD was found ($p>0.05$). MPO expression (figures 1, 2) and CD-68 expression (figures 3, 4) were significantly increased in the relevant IBD cases compared with the control subjects (tab. 1).

Previous studies have identified a positive correlation between downregulation of MPO and CD-68 and severity of inflammation following infliximab treatment between active IBD and controls.[3] In our study, HLA-G expression was absent in all cases, which is the first study to show absent expression of HLA-G in inflamed intestinal mucosa in patients with active UC and CD. Previous immunohistochemical studies showed that HLA-G is highly expressed in the inflamed mucosa in both UC and CD, and is strongly

Key words

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Table 1. Immunohistochemical expression of myeloperoxidase (MPO), human leucocyte antigen G (HLA-G) and cluster of differentiation (CD-68) in active cases of inflammatory bowel disease.

No	Age	Gender	Biopsy site	Clinical diagnosis	Pathological diagnosis	Nancy index (UC) and Naini and Cortina Index (CD)	MPO (+)	HLAG (+)	CD-68 (+)
1	57	M	Terminal ileum	Control	Normal	N/A	0	0	0
2	45	M	Colon	Control	Normal	N/A	0	0	0
3	30	F	Colon	UC	UC	3	2	0	2
4	42	M	Colon	UC	UC	3	2	0	2
5	55	F	Colon	UC	UC	4	3	0	1
6	49	M	Colon	UC	UC	4	3	0	1
7	19	M	Sigmoid	UC	UC	3	3	0	2
8	60	F	Colon	UC	UC	3	2	0	3
9	24	F	Sigmoid	UC	UC	2	2	0	2
10	59	M	Colon	UC	UC	4	1	0	1
11	37	F	Terminal ileum and duodenum	CD	CD	4	2	0	1
12	28	F	Terminal ileum	CD	CD	10	2	0	2
13	51	M	Terminal ileum	CD	CD	7	3	0	1
14	38	M	Terminal ileum	CD	CD	6	2	0	3
15	18	F	Terminal ileum	CD	CD	4	1	0	2
16	22	F	Terminal ileum	CD	CD		2	0	1
17	27	F	Left colon	UC	UC	2	1	0	2
18	24	F	Rectum	UC	UC	2	3	0	1
19	46	M	Caecum	UC	UC	3	3	0	2
20	32	M	Terminal ileum/colon	UC	UC	3	1	0	1
21	39	M	Colon	UC	UC	3	2	0	2

F: Feminine, M: Masculine, N/A: Not applicable, UC: Ulcerative colitis, CD: Cluster of differentiation terminal ileitis

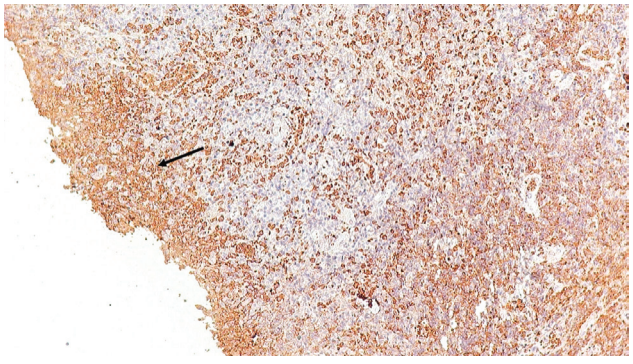

Figure 1. Ulcerative colitis (pancolitis): Biopsy of the colon (×100): Strong (+++) staining for myeloperoxidase (MPO).

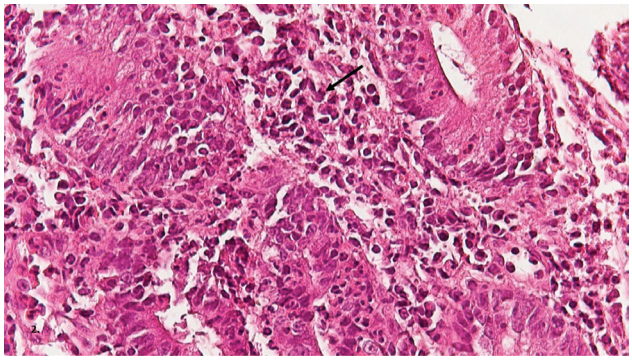

Figure 2. Ulcerative colitis (pancolitis): Biopsy of the colon (×400): Strong (+++) staining for myeloperoxidase (MPO).

expressed in intestinal epithelial cells (IECs), suggesting a protective role for these cells in such pathology.[1,4]

Limitations of this study were the small number of patients, and the possible bias of choice of specimens of moderate to severe CD terminal ileitis and UC colitis, prior to treatment. The two most important outcomes were the absence of linear statistically significant correlation between CD-68 and MPO expression and the histological score in

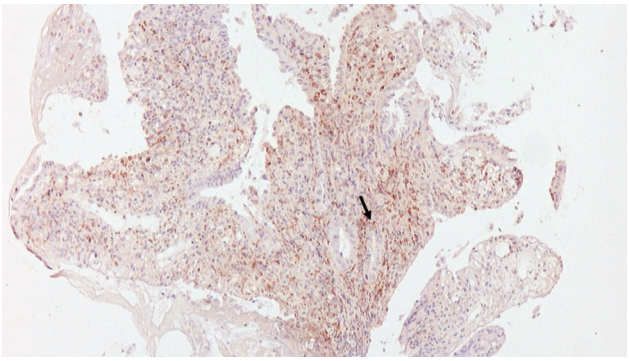

Figure 3. Cluster of differentiation (CD) terminal ileitis: Biopsy of the terminal ileum (×100): Moderate (++) staining for CD-68.

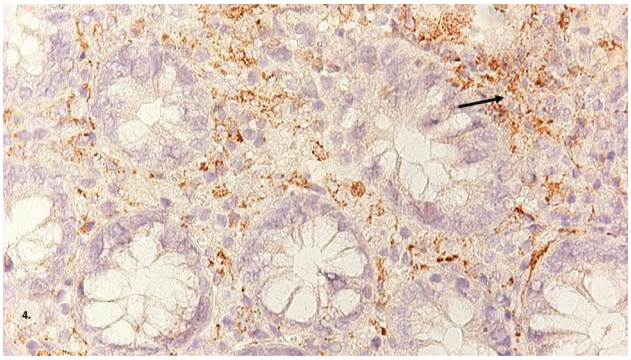

Figure 4. Cluster of differentiation (CD) terminal ileitis: Biopsy of the terminal ileum (×400): Moderate (++) staining for CD-68.

active disease, not clearly emphasized by previous bibliography,[5] and the absence of HLA-G expression that can possibly indicate a chronic rather than an acute response in the inflammatory process. Further research will explore the trend of these inflammatory markers in the future.

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ΠΕΡΙΛΗΨΗ

Ανοσοϊστοχημική μελέτη της «καταιγίδας» της φλεγμονής στο εντερικό επιθήλιο ασθενών με ιδιοπαθείς φλεγμονώδεις νόσους του εντέρου (ΙΦΝΕ)

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Λέξεις ευρετηρίου: Ανοσοϊστοχημεία, CD-68, HLA-G, ΙΦΝΕ, Μυελοϋπεροξειδάση

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