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Colon mucosa of both spondyloarthritis and Crohn’s disease patients is enriched with macrophages expressing the scavenger receptor CD163

Concise report

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Abstract

Objectives: Crohn’s disease is associated with an increased number of macrophages in ileal and colonic mucosa. Data on macrophages in gut mucosa of spondyloarthritis (SpA) patients are scarce. The objective of this study was to investigate macrophages and other antigen presenting cells in gut mucosa from patients with SpA and Crohn’s disease, given the relationship between both entities.

Methods: Biopsies from patients with SpA, Crohn’s disease, ulcerative colitis and controls were immunohistochemically stained with different markers for macrophages and dendritic cells. Slides were scored semiquantitatively on a 4-point scale.

Results: SpA and Crohn’s disease were associated with large numbers of CD68+ macrophages. Both colon of SpA patients and Crohn’s disease, but not ulcerative colitis, showed increased numbers of macrophages expressing the scavenger receptor CD163.

Conclusions: The present study indicates that macrophages expressing the scavenger receptor CD163 are increased in colonic mucosa in SpA as well as in Crohn’s disease, highlighting the relationship between these entities. The increased number of CD163+ macrophages in colon mucosa of SpA patients adduces another argument for a role of macrophage scavenger receptors in this group of diseases.

Key words: spondyloarthritis; inflammatory bowel disease; macrophage; scavenger receptor
Introduction

The Th1 cytokine tumor necrosis factor-α (TNF-α) plays a pivotal role in Crohn’s disease, clearly illustrated by the effect of anti-TNF-α drugs [1]. Blocking TNF-α also leads to improvement of articular manifestations in spondyloarthritis (SpA) [2]. Together with the abundant TNF-α message in sacroiliac joint biopsy specimens from patients with ankylosing spondylitis [3], this suggests a major role for TNF-α in SpA as well.

TNF-α is primarily produced by lymphocytes, monocytes and macrophages [4]. Besides this production, macrophages play different roles in mucosal immunity, including antigen presentation and enhancement of immunoglobulin production. An increased number of mucosal macrophages in Crohn’s disease has been reported previously [5] whereas data in SpA are scarce. Studying macrophages in gut mucosa from SpA patients, however, seems worthwhile since subclinical inflammatory gut lesions, which can evolve to clinically overt Crohn’s disease, are found in 25-75% of them [6].

The present study aimed to analyze macrophages and other antigen presenting cells in gut mucosa from SpA and Crohn’s disease patients. The results were compared to those obtained in ulcerative colitis and controls. Special attention was paid for the macrophage scavenger receptor CD163, since CD163+ macrophages are increased in synovium from SpA patients compared with rheumatoid arthritis patients [7].

Methods

Spondyloarthritis patients

Ileal and colonic biopsies were obtained from twenty-one SpA patients fulfilling the European Spondyloarthritis Study Group criteria [8]. This group included ankylosing spondylitis (n=16), psoriatic arthritis (n=2), reactive arthritis (n=1), juvenile spondyloarthritis (n=1) and undifferentiated SpA (n=1). Their median age was 36.5 years (range 26-74). Six patients were taking sulphasalasin and 11 were on non-steroidal anti-inflammatory drugs. One of the patients was treated with corticosteroids. The remaining patients had no medication. As all tissue from these patients was collected during the last four years, there was no longer follow-up period. Over that time span, 1 of them developed a clinical Crohn’s like colitis, confirmed histopathologically.

Inflammatory bowel disease patients

Mucosal biopsies or samples from resection specimens were obtained from patients with Crohn’s ileitis (n=10), Crohn’s ileocolitis (n=4), Crohn’s colitis (n=6) and ulcerative colitis (n=11). The diagnosis was independently established by endoscopic, radiologic and histologic criteria. Samples were taken from areas with endoscopic or macroscopic involvement. The median age of the Crohn’s disease patients was 31 years (range 13-67), and of those with ulcerative colitis was 37 years (range 29-61). 25 IBD patients were treated with corticosteroids or other immunosuppressive drugs; 4 patients were taking only sulphasalasin. The remaining 2 patients had no medication.
Controls

Control ileal and colonic tissue was obtained from thirteen patients who underwent a segmental colectomy for colorectal carcinoma, or a colonoscopy for irritable bowel syndrome or follow-up of polyps. In cases of carcinoma, samples were taken at least 10 cm away from the tumour. The median age of these patients was 56 years (range 27-72). This study was approved by the local Ethical Committee.

Immunohistochemistry

Frozen sections of ileal and colonic mucosa from all patients and controls were fixed in aceton and incubated with antibodies against CD68 and CD163. Moreover, we performed stainings for CD14, CD80, CD83, CD86 and HLA-DR on ileal and colonic mucosa from all controls and all patients suffering from ulcerative colitis, Crohn’s ileitis and Crohn’s ileocolitis, and from 10 SpA and 5 Crohn’s colitis patients. The antibodies we used are presented in Table 1.

Immunostaining was visualised using New Fuchsin (for CD68) or 3-amino-9-ethylcarbazole (for the other markers), both obtained from Dako Corporation (Carpintera, USA). Parallel sections were incubated with irrelevant isotype and concentration matched antibodies as negative control.

Table 1. Antibodies used in this study.

<table>
<thead>
<tr>
<th>Antibody</th>
<th>CD Group</th>
<th>Major specificity in normal lymphoid tissues</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>TUK4 (IgG2a)</td>
<td>CD14</td>
<td>Monocytes</td>
<td>DAKO</td>
</tr>
<tr>
<td>EBM11 (IgG1)</td>
<td>CD68</td>
<td>Monocytes and macrophages</td>
<td>DAKO</td>
</tr>
<tr>
<td>N-20 (polyclonal IgG)</td>
<td>CD80</td>
<td>B7-1 (antigen presenting cells)</td>
<td>Santa Cruz Biotechnology</td>
</tr>
<tr>
<td>HB15A (IgG2b)</td>
<td>CD83</td>
<td>Dendritic cells</td>
<td>Immunotech</td>
</tr>
<tr>
<td>BU63 (IgG1)</td>
<td>CD86</td>
<td>B7-2 (resting monocytes and dendritic cells)</td>
<td>DAKO</td>
</tr>
<tr>
<td>Ber-MAC3(IgG1)</td>
<td>CD163</td>
<td>Activated monocytes, macrophages</td>
<td>DAKO</td>
</tr>
<tr>
<td>TAL.1B5 (IgG1)</td>
<td>_</td>
<td>HLA-DR, alpha-chain (monocytes, macrophages, dendritic cells, B cells)</td>
<td>DAKO</td>
</tr>
</tbody>
</table>

Evaluation of immunohistochemical staining

The number of positive cells in the lamina propria was analyzed using magnification x100 and coded by two independent observers, blinded for diagnosis and clinical data. A grading scale from 0 to 3 was applied, ranging from absent to numerous stained cells. As some histological markers are more abundant than others in gut tissue, the scoring system was calibrated for each marker separately by examining a representative number of samples. Thus, the sensitivity of the scoring system was different for each marker, allowing comparison of a specific marker between the different patient groups, but not comparison of different markers in one group. The analysis included all areas of the biopsies and a global score was given for each parameter.
The scores obtained by both observers were concordant for more than 90%. In case of discordant scores, which differed by maximal one point, the mean of the two scores was used.
Statistical analysis

Patient groups were compared with controls; P-values were calculated with the Mann-Whitney U test. P<0.05 was considered statistically significant. Since the data were not compared between multiple groups and since the analysis of each marker was considered as an individual comparison between two groups, Bonferroni corrections for multiple comparisons were not applied in this exploratory study.

Results

Increased number of CD163+ macrophages in both Crohn’s colitis and colon mucosa from SpA patients.

Control ileum and colon showed a subepithelial band of CD68+ macrophages. In inflammatory cases multitudinous CD68+ cells were found in the ulcer bases; they were especially numerous in transmural inflammation in Crohn’s disease. CD68+ cells were increased in ileal (P<0.02) and colonic (P<0.01) Crohn’s disease and also in colon from SpA patients (P<0.005).

In controls, CD163+ macrophages were also localized in the superficial lamina propria of the colon (Fig. 1A) and in ileal villous tips, but they were less numerous than CD68+ cells. Scarce CD163+ cells were found in the deeper layers of the lamina propria. Crohn’s colitis revealed increased numbers of CD163+ cells throughout the mucosa (P<0.01) (Fig. 1B). In colon from SpA patients, increased numbers of CD163+ cells were detected as well (P<0.002) (Fig. 1C). This contrasts with the normal findings in ulcerative colitis (Fig. 1D). Results concerning the scores for CD68 and CD163 in colon are presented in Figure 2.

The number of dendritic cells and HLA-DR positive inflammatory cells is unaltered in mucosa of inflammatory bowel disease or spondyloarthritis.

CD83+ cells were present in T-cell zones of lymphoid follicles in both normal and inflamed bowel mucosa. Although outside the follicles fewer CD83+ cells were found, clusters of positive cells were present in IBD but not in controls and SpA patients.

CD80+ cells were scarce. CD86+ cells occurred scattered throughout the lamina propria and could be found in the dome area overlying lymphoid follicles; in follicles, however, they were scarce or absent. There was no increase in patient groups.

In all cases, HLA-DR+ inflammatory cells were found in the lamina propria. Significant differences between patients and controls were absent.

Discussion

We demonstrated an increased number of CD163+ macrophages in colon from SpA as well as Crohn’s disease patients, but not in ulcerative colitis. The number of CD163+ cells was not related to the patients’ age or medication (data not shown).

The transmembrane protein CD163 is a member of the scavenger receptor cysteine-rich (SRCR) superfamily. The SRCR-proteins are involved in the development of the immune system and in the regulation of the immune response. Although the precise function of CD163
is unknown, a role in the regulation of the immune response of macrophages is supposed. Comparison of CD163− and CD163+ swine monocytes/macrophages showed that the latter are more mature [9]. CD163+ cells induce a greater allogeneic response on T lymphocytes. LPS-stimulated CD163− and CD163+ macrophages both produce the proinflammatory cytokines IL-1 and TNF-α, but anti-inflammatory IL-10 is only detected on the former population [9]. An increase of CD163+ macrophages in colon mucosa in SpA as well as in Crohn’s disease raises the possibility that these cells can induce a similar dysregulation of the cytokine balance in the colon in both disorders. Previously we described also for T cells common characteristics in the cytokine profile [10,11]. These observations, together with similar alterations in adhesion molecule expression [12,13], fit in the concept that some SpA patients have subclinical Crohn’s disease.

The CD163 protein scavenges haemoglobin by mediating endocytosis of haptoglobin-haemoglobin complexes [14]. Haeme-containing compounds are potentially a valuable source of iron for invading microorganisms; although bacteria have evolved several strategies to overcome iron shortage, increased scavenging of haemoglobin-haptoglobin complexes might alter the composition of the bacterial gut flora.

In conclusion, the present study indicates that CD163+ macrophages are increased in colonic mucosa in SpA as well as in Crohn’s disease, highlighting the relationship between these entities. Since the macrophage receptor with collagenous structure (MARCO) is also susceptible to modulation in SpA [15], we adduce another argument for a role of macrophage scavenger receptors in this group of diseases. However, CD163 is the first scavenger receptor shown to be altered in colon mucosa from SpA patients.

Acknowledgments and affiliations

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Figure legends

Figure 1. Immunohistochemical staining for CD163 (peroxidase method). (A) Macrophages weakly positive for CD163 in the superficial lamina propria of control colon. (B) Increase in number of CD163⁺ cells in Crohn’s colitis. (C) Non-inflamed colonic mucosa from a spondyloarthritis patient showing a similar increase in number of activated macrophages. (D) No increase in number of CD163⁺ cells in ulcerative colitis.

Figure 2. Box-and-whisker plots representing the semiquantitative scores for CD68 and CD163 in the colonic lamina propria of spondyloarthritis (SpA), Crohn’s disease (CD) and ulcerative colitis (UC) patients versus controls. P-values were calculated with the Mann-Whitney U test.
References

Figure 1.
Figure 2

**CD 68**

- Controls: 0.5
- SpA: 2.3
- CD: 2.9
- UC: 1.7

Statistical significance:
- $P < 0.001$
- $P < 0.005$

**CD 163**

- Controls: 0.8
- SpA: 2.5
- CD: 2.6
- UC: 2.3

Statistical significance:
- $P < 0.002$
- $P < 0.01$