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Macrophage-associated Chronic Diarrhea of Unknown Origin. Preliminary Observations

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Abstract. *The distribution of macrophages in the colonic mucosa was investigated in 13 patients with chronic diarrhea. Group I consisted of 5 patients; both colonoscopy and colonic biopsies were reported as normal. Group II included 5 patients with normal colonoscopy, but with collagenous colitis or lymphocytic colitis at histology. Group III consisted of 3 patients with mucosal inflammation at both colonoscopy and histology. Immunostain with CD68 (a macrophage marker) revealed, in all patients from Group I, a linear accumulation of CD68+ macrophages at the top of the lamina propria. In contrast, sections from patients in Groups II and III showed a random distribution of CD68+ macrophages. Since all three groups of patients here investigated had chronic diarrhea, it goes without saying that the linear recruitment of macrophages in Group I may not be the direct cause of chronic diarrhea, as no similar linear recruitment of macrophages was demonstrated in patients with collagenous colitis or with chronic inflammation. Although the cause(s) of chronic diarrhea in patients of Group I remain to be elucidated, it would appear that the recruitment of macrophages along the superficial aspect of the lamina propria mucosa (lpm) is a characteristic cellular response in patients with this subtype of chronic diarrhea.*

For adults on a typical Western diet, a stool weight exceeding 200g/day can generally be considered diarrheal. Diarrhea can be divided into acute if <2 weeks duration, and persistent or chronic if ≥2 weeks duration. Infectious agents cause more than 90% of acute diarrhea, whereas non-infectious causes generate most chronic diarrhea (1). Chronic diarrhea is a common symptom in patients with inflammatory bowel disease of the colon (IBD-C) showing inflammatory changes at endoscopic and at histological examinations.

In 1976, Linsdtröm (2) described one patient with chronic diarrhea and normal colonic mucosa at endoscopy. At histology, however, a thick band of collagen near the superficial epithelium was found. Linsdtröm (2) called that condition 'collagenous colitis'. In 1980, Read *et al.* (3) observed, in 8 out of 27 patients with chronic diarrhea of unknown origin, a mild increased number of inflammatory cells in colonic or rectal biopsies. Because of the normal sigmoidoscopic and barium enema examinations, those 8 cases were regarded as having 'microscopic colitis'. That denomination was later considered too ambiguous and Levinson *et al.* (4) suggested that microscopic colitis should be a term embracing cases with normal endoscopical or radiological examinations, but histological changes compatible with collagenous colitis, inflammatory disease of indeterminate type or eosinophic colitis.

In 1989, Lazenby *et al.* (5) found an increased number of intraepithelial lymphocytes in the superficial epithelium of the colon and proposed the term 'lymphocytic colitis' to identify that subgroup of patients. We subsequently detected lymphocytic colitis (*i.e.* superficial) in non-human primates with chronic diarrhea (6).

In 2000, we found an increased number of intraepithelial lymphocytes in the colonic crypts in non-human primates (7) and two years later in humans (8). Chronic diarrhea was the main symptom in both species. We called that condition 'chronic lymphocytic cryptitis'.

In 2001, we noticed, in non-human primates with chronic diarrhea (6), a microscopic condition characterized by a high number of apoptotic granules in the colonic crypts (9). In a subsequent study, the same condition was detected in patients with chronic diarrhea and normal colonoscopy (10). That phenomenon was called 'apoptotic disease of the colon'.

Most recently, we reviewed a new subset of patients with chronic diarrhea, having at colonoscopy normal colonic mucosa. Although the colonic biopsies were reported as histologically normal, a closer examination of the sections revealed that the cellular pattern of the upper part of the

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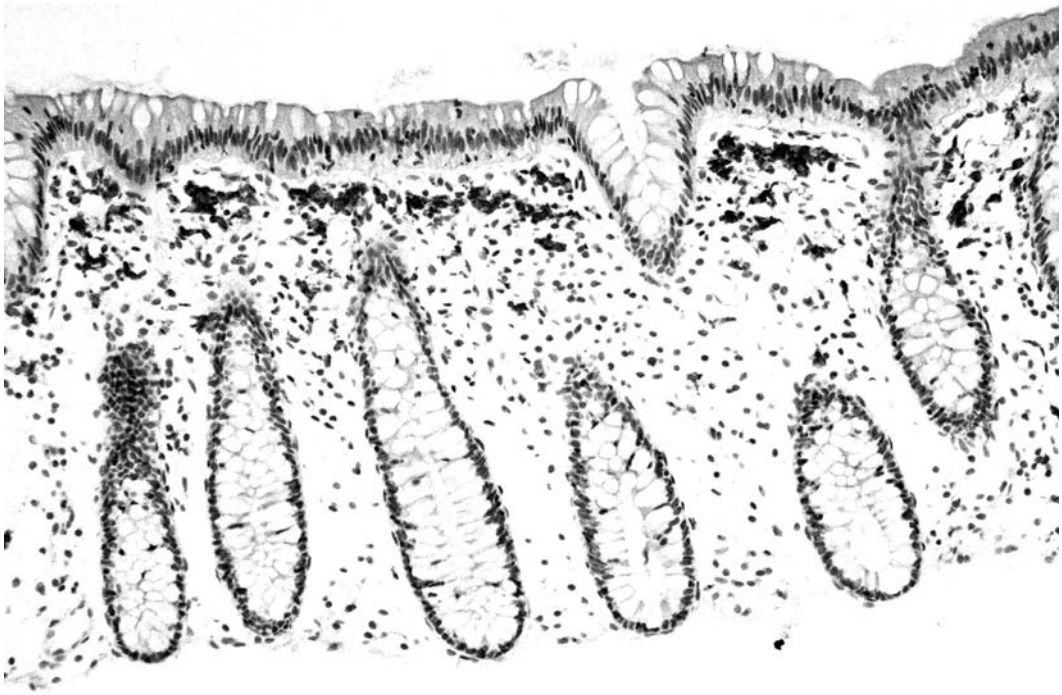

Figure 1. *Colon biopsy reported as normal in hematoxylin-eosin-stained section. Patient with chronic diarrhea and normal mucosa at colonoscopy. Note the linear distribution of macrophages at the top of the lpm following CD68 immunostain (200x).*

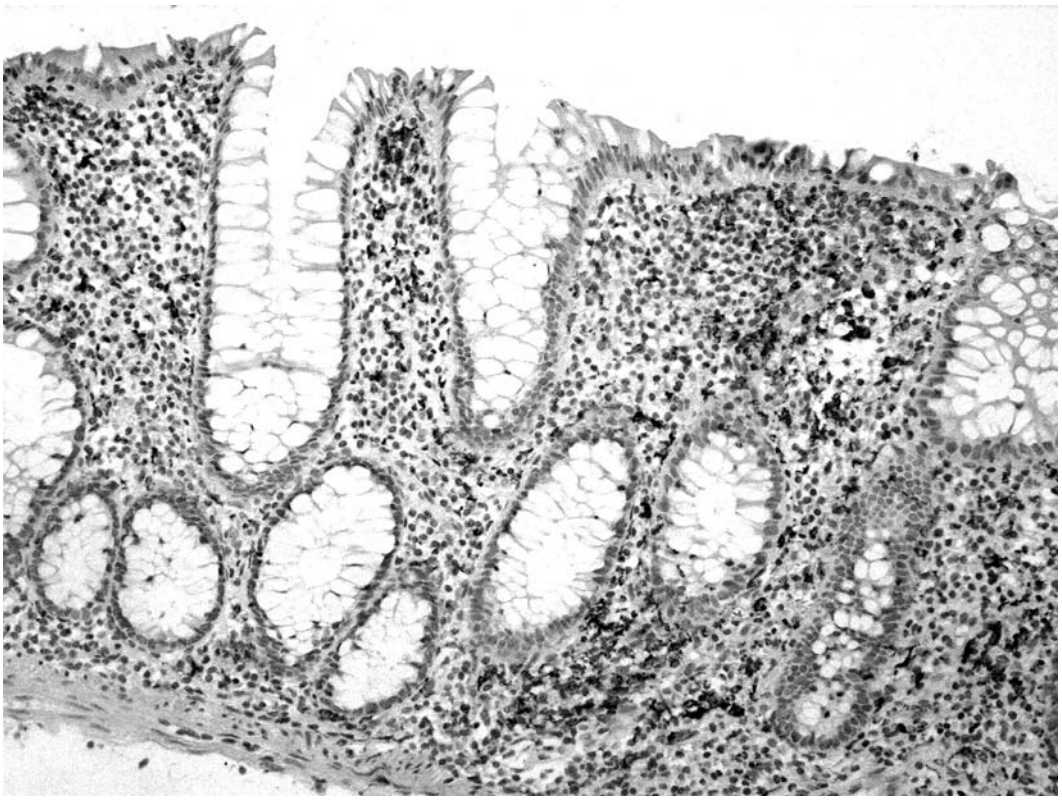

Figure 2. *Colon biopsy showing chronic inflammation. Patient with chronic diarrhea and Crohn's disease. Note the random distribution of macrophages within the lpm following CD68 immunostain (200x).*

lamina propria mucosa (lpm) of the colon was not completely normal.

We have now identified the cell responsible for that abnormal pattern present in the *lpm*. The results of those observations are reported in this preliminary communication

Materials and Methods

Thirteen patients with chronic diarrhea were investigated. Ten patients had normal colonoscopies and the remaining 3 inflammatory changes at endoscopy. Colonic sections were stained with hematoxylin and eosin and with CD 68 (Dako Cytomation, Glostrup, Denmark). CD68 is a LPG glycosylated lysosomal membrane protein that is expressed in the cytoplasmic granules in macrophages and other mononuclear cells with phagocytic properties.

The distribution of CD 68-positive cells was divided into linear superficial (*i.e.* underneath the luminal epithelium) and random (*i.e.* randomly distributed within the thickness of the *lpm*).

Results

Group I: Consisted of 5 patients showing at colonoscopy a normal mucosa, but at histology an abnormal cellular pattern in the upper aspect of the *lpm*. Three of the patients were males and the remaining 2 females. The mean age was 48 years (range 32-58 years). Bacteriological studies of the feces revealed no unusual pathological bacteria.

The immunostain showed an accumulation of CD68+ cells underneath the superficial epithelium in a linear fashion in the most superficial aspect of the *lpm* (Figure 1).

Group II: Included 5 patients showing normal mucosa at colonoscopy, but collagenous colitis (3 patients) or lymphocytic colitis (2 patients) at histology. Three of the patients were males and the remaining 2 females. The mean age was 42 years (range 28-60 years). The immunostain showed a random distribution of CD68+ cells within the *lpm*.

Group III: Consisted of 3 patients showing mucosal inflammation at colonoscopical and histological examinations. Two were diagnosed as Crohn's colitis and the remaining one as ulcerative colitis. The immunostain showed a random distribution of CD68+ cells within the *lpm* (Figure 2).

Discussion

The results of this preliminary communication indicate that some patients with chronic diarrhea having a normal colonoscopy may show, at histology, a linear accumulation of CD 68+ macrophages in the superficial aspect of the *lpm*. In contrast, patients with chronic diarrhea and normal colonoscopy having collagen colitis or with abnormal histological features (IBD-C) had randomly distributed CD 68+ macrophages within the thickness of the *lpm*. Rationally, it appears safe to assume that the CD 68+ macrophages in patients with macrophage-associated chronic diarrhea (M-ACD) had been summoned to the most superficial layers of the *lpm* by a factor present within the lumen of the organ. This phenomenon was not due to the occurrence of pathological bacteria in the feces.

Since all 3 groups of patients here investigated had the same cardinal clinical symptom, it must be stated that the recruitment of macrophages in M-ACD may not be the direct cause of chronic diarrhea, as no linear recruitment of macrophages was demonstrated in patients with collagenous colitis or with IBD-C. Although the cause(s) responsible for chronic diarrhea in patients with M-ACD remains to be elucidated, it is apparent that the recruitment of macrophages along the superficial aspect of the *lpm* appears to be a characteristic cellular response in patients with M-ACD.

Studies with transmission electron microscopy have been initiated in this department, with the purpose of exploring whether the macrophages recruited at the top of the *lpm* in M-ACD differ from those haphazardly distributed in the *lpm* in other colonic diseases with chronic diarrhea such as collagenous colitis or IBD-C.

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