

An Easy Method to Quantify Plasma Cells/Plasma Cell Precursors in Normal Colonic Mucosa, Collagenous Colitis and Crohn's Colitis

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Abstract. *Background: The number of round cells that should normally be present in the colonic mucosa remains disputed. Biopsies from patients with chronic diarrhoea, having a slightly increased number of round cells in the lamina propria mucosa (lpm) may be diagnosed as slight chronic colitis by some pathologists, while others may regard these biopsies as being normal. Materials and Methods: The numbers of plasma cells/plasma cell precursors (PC-PCP) were assessed in colonic biopsies from 35 patients by the aid of MUM1 immunostaining. MUM1 (multiple myeloma oncogene 1) is a member of the interferon regulatory family of transcription factors (interferon regulatory factor 4 gene, IRF4). Results: Many of the round cells considered, as lymphocytes in H&E staining were in fact MUM1-positive PCP. In 6 patients having a priori, a slightly increased number of round cells, the mean number of MUM1-labelled cells was 40.8, in 5 patients with collagenous colitis, it was 81.4 and in 12 patients with Crohn's colitis, 87.6, whereas in 12 normal individuals it was 23.3 (MUM1-positive cells in collagenous-Crohn's colitis vs. normal mucosa, $p < 0.05$). Conclusion: MUM1 was of value in recording a significant increase of PC-PCP in collagenous colitis and Crohn's colitis. The subjective impression of increased cellularity in some colonic biopsies from patients with chronic diarrhoea was substantiated in mathematical terms, by demonstrating that the number of PC-PCP was increased.*

The colonic mucosa is lined by a single layer of epithelial cells which folds downward into deep parallel crypts almost reaching the *muscularis mucosa*. This epithelial layer is supported by the *lamina propria mucosa* (lpm), a loose connective tissue traversed by smooth, elastic and muscle

fibres, vessels and nerves (1). The lpm also harbours a small number of round cells, including autochthonous T-lymphocytes and plasma cells.

In diagnostic biopsies stained with hematoxylin and eosin (H&E), the normal colorectal mucosa is usually described as containing a normal number of round cells. This empirical appreciation of normality denotes a subjective opinion based on years of experience, an estimation that unfortunately cannot be conveyed in numbers to young pathologists wondering whether there is slight inflammation or not in the mucosa of the sample they are assessing. According to Lee *et al.* (2) quantification of the types of cells normally present in the lpm of the colon is uncertain. In fact, the upper limit of round cells permitted to define a mucosa as normal has not yet been clearly established.

On the other hand, unquestionable chronic colitis is characterized, among other features, by an evident increased number of round cells, beyond normal limits (3, 4). Biopsies from patients with chronic diarrhoea having a slightly increased number of round cells in the lpm at histological examination may be diagnosed as slight chronic colitis by some pathologists, while others may regard these biopsies as normal. Robert *et al.* (4) semi-quantified the degree of cellularity in colonic biopsies into: normal (=0), or +, 1+, 2+ or 3+ increased. The number of round cells characterizing 0 (normal values) was, however, not given. Seldenrijk *et al.* (3) reported increased cellularity in colonic biopsies with chronic inflammatory bowel disease (IBD), but normal values were not specified.

Few authors have quantified the number of round cells in H&E-stained sections from the normal colorectal mucosa (5, 6). Sommers and Korelitz (5) quantified plasma cells in 500 connective tissue cells (fibroblasts, macrophages, mast cells, lymphocytes, plasma cells, eosinophils, and neutrophils) seen in H&E-stained sections from 50 control sigmoidoscopies; plasma cells accounted for 64% of the round cells in the normal mucosa. Rubio and Kock (6) recorded in normal rectal biopsies, a mean of up to 12 round cells in 10 high power fields (HPF, $\times 400$). Rectal biopsies from patients with ulcerative proctitis (7) exhibited mean numbers exceeding 13 round

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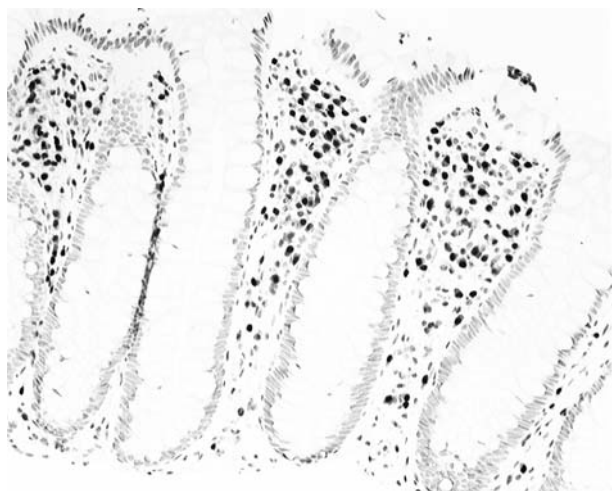


Figure 1. Normal colonic mucosa exhibiting plasma cells/plasma cell precursors stained for MUM-1 (MUM-1 immunostain, $\times 20$).

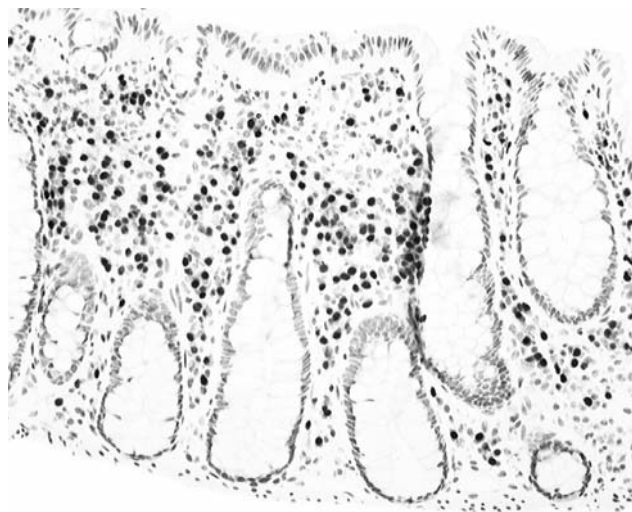


Figure 2. Colonic mucosa exhibiting, a slightly increased number of MUM-1-positive plasma cells/plasma cell precursors (cf. Figure 1) (MUM-1 immunostain, $\times 20$).

cells/10 HPF. Lee *et al.* (2) found that the percentage of plasma cells/total round cells in healthy individuals was 86.2%; in patients with chronic diarrhoea having normal histologies, 86.2%; and in those with microscopic colitis, 86.2%. Thus, plasma cells were proportionally not increased in IBD according to Lee *et al.* (2). Plasma cells were also quantified by counting the number of cells secreting immunoglobulins. Rosekrans *et al.* (8) found in IBD, a mean of 88 IgA, 134 IgG and 8 IgM immuno-stained plasma cells/mm length of lpm. Using the point-count technique, Skinner and Whitehead (9) found a mean of 896 IgA, 44 IgG and 28 IgM-positive secreting plasma cells/1000 points of lpm, while Jenkins *et al.* (10) found a mean of 257 IgA-, 41 IgG- and 98 IgM-positive cells in 3 mm of the lpm, using an image analysis technique. The numbers of IgA-, IgG- and IgM-secreting plasma cells in normal colonic mucosa were, however, not provided (10).

In light of these studies, it became apparent that an easy method that would permit to assess the number of plasma cells in colorectal biopsies should be developed. In a preliminary study, we investigated the number of plasma cells/plasma cell precursors by the aid of MUM-1 immunostaining, in 11 liver biopsies (11). MUM1 (multiple myeloma oncogene 1) is a 50-kDa protein member of the interferon regulatory family of transcription factors (interferon regulatory factor 4 gene, IRF4). MUM1 contributes to the regulation of immunoglobulin gene expression in the final step (late centrocyte) of B-cell differentiation within germinal centre light zones, initiated by centrocyte-follicular dendritic cell contact (12). MUM1 stains plasma cells, as demonstrated in colonic biopsies with chronic inflammation carrying a lymphatic follicle: while MUM1 stained plasma cells in the lpm, the lymphocytes in the follicles remained unstained (11). On the other hand, many of the cells in

the lpm regarded as lymphocytes in H&E staining, were MUM-1-positive; they were in reality, plasma cell precursors (13).

The purpose of the present investigation was to quantify MUM1-positive plasma cells/plasma cell precursors in the lpm in a cohort of colonic biopsies from healthy individuals and from patients with chronic mucosal inflammation.

Materials and Methods

Colonic biopsies from the left colon were investigated in 35 cases. Twelve were from individuals without gastrointestinal complaints, enrolled in a colonoscopy program for cancer detection with normal mucosa at histology. Six patients had chronic diarrhoea and *a priori*, a slightly increased number of round cells in the lpm under H&E staining; five had chronic watery diarrhoea and at histology, collagenous colitis; and 12 had Crohn's colitis at histology.

Sections were stained with H&E, and with MUM1 (DakoCytomation, Glostrup, Denmark).

In well-oriented sections, the numbers of MUM1-labelled cells found in the lpm between two consecutive crypts, were recorded in three high-power fields ($\times 40$ objective). By the aid of an ocular grid, the lpm was divided into two equal compartments: i) the superficial compartment and ii) the deep compartment.

Statistical analysis. Data were analyzed by the Mann-Whitney non-parametric test. Statistical significance was defined as $p < 0.05$. The Regional Ethical Committee approved the study.

Results

Normal mucosa (Figure 1). In colonic biopsies from 12 healthy individuals, a mean of 23.3 (range 6-32) MUM1-positive cells were recorded: 18.4 (range 6-27) in the superficial compartment, and 4.9 (range 0-11) in the deep compartment.



Figure 3. Colonic mucosa in collagenous colitis exhibiting an increased number of MUM-1-positive plasma cells/plasma cell precursors (MUM-1 immunostain, $\times 10$).

Slight chronic colitis (Figure 2). In biopsies from six cases with *a priori* slight chronic inflammation, a mean of 40.8 (range 35-53) MUM1-positive cells were recorded: 29.8 (range 21-40) in the superficial compartment and 11.0 (range 9-14) in the deep compartment.

Collagenous colitis (Figure 3). In five cases with collagenous colitis, 81.4 (range 58-104) MUM1-positive cells were found: 39.2 (range 31-45) in the superficial compartment, and 42.2 (range 25-59) in the deep compartment.

Crohn's colitis (Figure 4). In 12 cases with Crohn's colitis, a mean of 87.6 (range 51-160) MUM1-positive cells were found: 47.1 (range 33-78) in the superficial compartment, and 40.6 (range 18-82) in the deep compartment.

The difference between the number of MUM1-positive cells in the colonic mucosa as a whole, as well as in the superficial and deep compartments, were significantly higher in collagenous colitis and Crohn's colitis compared to normal colonic mucosa ($p < 0.05$). On the other hand, despite the number of MUM1-positive cells being higher in colonic biopsies, regarded *a priori* as having slight chronic inflammation than in those regarded as normal, the difference was non-significant.

Discussion

A method for counting plasma cells/plasma cell precursors with MUM1 immunostaining is presented. The method is straightforward and easy to perform. Notably, many cells recorded as lymphocytes in H&E staining were in reality, plasma cell precursors. Hence, the assessment of the lymphocyte/plasma cell ratio based on H&E staining should be regarded as unreliable. This investigation showed that the numbers of plasma cells/plasma cell precursors in colonic

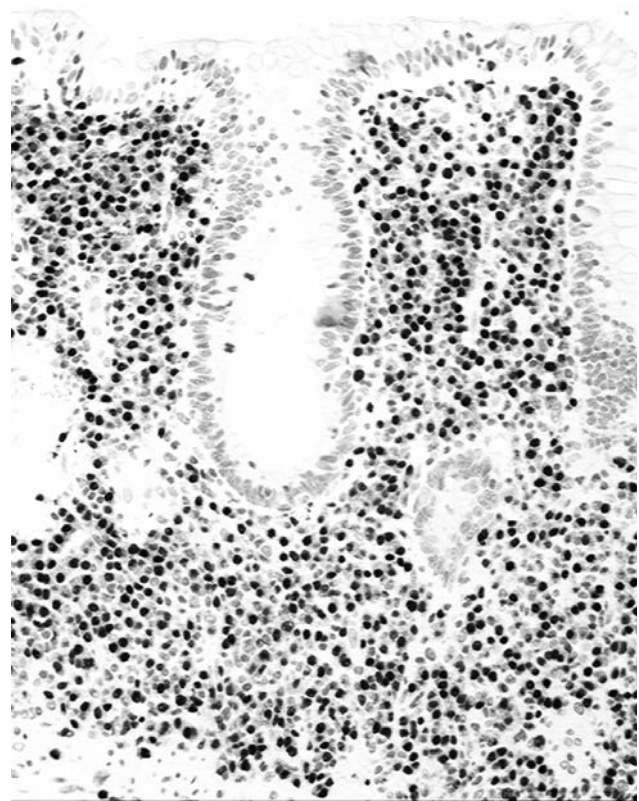


Figure 4. Colonic mucosa in Crohn's colitis exhibiting an increased number of MUM-1-positive plasma cells/plasma cell precursors (MUM-1 immunostain, $\times 20$).

biopsies from patients with collagenous colitis and with Crohn's colitis were significantly higher in the superficial and deep compartments of the lpm than in healthy individuals or those with slight chronic colitis.

In diagnostic sections from biopsies exhibiting manifested chronic colitis, many plasma cells are often found underneath the base of the crypts. It is recognized that subcryptal plasma cell infiltration is the single most reliable histological parameter to diagnose chronic mucosal inflammation. Difficulties in interpreting chronic mucosal inflammation may arise in biopsies having a discrete increase of round cells in the lpm without subcryptal plasma cell infiltration. In the present survey it was found that sections from patients with chronic diarrhoea, diagnosed *a priori* as having slight chronic inflammation, had a higher proportion of MUM1-positive cells in the lpm than did sections from healthy individuals, despite the absence of subcryptal plasma cell infiltration. Although the difference was non-significant, the subjective impression based on experience of increased cellularity was substantiated in mathematical terms by demonstrating increased numbers of MUM1-positive cells in biopsies diagnosed *a priori* as having slight chronic inflammation.

In the introduction it was postulated that: “the empirical appreciation of normality denotes a subjective opinion based on years of experience, an estimation that unfortunately cannot be conveyed in numbers to young pathologists wondering whether there is slight inflammation or not in the sample they are assessing”. In the light of the current results, this assertion should perhaps be re-evaluated.

Further studies with a greater number of cases will be necessary before the upper limit of plasma cells/plasma cell precursors can be definitively established in colorectal biopsies from healthy asymptomatic individuals.

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