Early mucosal changes in Crohn’s disease


Abstract
Aphthoid ulceration has been regarded as an early macroscopic feature of Crohn’s disease, yet the cause of this mucosal lesion is unknown. Examination of areas of apparently normal and non-inflamed bowel in Crohn’s disease has allowed the identification of mucosal changes which occur before macroscopic and microscopic ulceration. Thirty five resection specimens from patients with Crohn’s disease were compared with 12 specimens from patients with ulcerative colitis and 13 controls. Specimens were fixed either by immersion in formalin in the routine way or by perfusion fixation with formalin at mean arterial pressure. Immunostaining for macrophages, vessel wall, and blood constituents allowed identification of small mucosal capillaries which were not apparent otherwise. In Crohn’s disease damage and rupture of these small capillaries occurred before infiltration of the lamina propria by inflammatory cells. Loss of the overlying epithelium seemed to follow this vascular damage.

(Mucosal ulceration is a common feature of many diseases of the small and large intestine. The pattern of ulceration varies in different conditions and may show characteristic appearances in certain diseases, so forming the basis of histological diagnosis. The ‘aphthoid’ ulcer, first described by Brooke in 1953,1 has been recognised for many years to be an early macroscopic feature of Crohn’s disease. More recently, the use of fibroptic endoscopic equipment has allowed the identification of subtle mucosal changes which are present before the development of aphthoid ulcers. These include patchy hyperaemia and friable mucosa, a ‘worm eaten’ mucosal pattern, and pinpoint haemorrhages the size of a single villus.4 Histological examination of these areas shows that inflammation and granulomas are present in these ‘early’ lesions4 suggesting that the disease process is already well established by this stage. Cellular inflammation also occurs before the epithelial surface erosions, described as a reliable feature of early inflammatory bowel disease by Allison et al.5 Indeed, inflammation and granulomas have been recognised in areas of macroscopically normal bowel.2 3 5 6

Early lesions, with features that have yet to be characterised, must occur before established ulceration, inflammation, and granulomas in Crohn’s disease. A search for these subtle changes in ‘normal’ areas of bowel from patients with Crohn’s disease may allow a chronological sequence of events to be postulated, which would help the understanding of the pathogenesis of Crohn’s disease. We have compared areas of bowel resected from patients with Crohn’s disease, ulcerative colitis, and controls which appeared normal macroscopically and showed no cellular inflammation histologically. Using both immersion and perfusion fixation of specimens, together with immunohistochemical staining, we have been able to visualise damage to small mucosal capillaries which is not apparent after routine processing and staining. We have found early, mucosal vascular changes in areas of apparently normal non-inflamed bowel. The areas selected for study were not ulcerated, and showed no morphologically discernible, or only minimal inflammation or fibrosis.

Methods
Small and large bowel resection specimens from 35 patients with Crohn’s disease, 12 patients with ulcerative colitis, and 13 controls were either immersion fixed in 10% formalin (13 Crohn’s disease, four ulcerative colitis, five controls) or perfusion fixed with 10% normal formalin or 4% paraformaldehyde in phosphate buffered saline at 100 mm Hg (mean arterial pressure).6 As many of the cases of ulcerative colitis were total colectomies resected for extensive disease, fewer areas of macroscopically normal bowel were available for study. Patients forming the control group included seven undergoing resection for large bowel carcinoma, one with diverticular disease of the colon, one with polypsis coli, and one who had normal bowel removed during excision of an ovarian carcinoma. Blocks of tissue were selected from macroscopically normal areas (>5 cm from tumour) and paraffin processed in the routine way. Sections (4 μm) were cut and stained with haematoxylin and eosin. Sections showing areas of microscopically non-inflamed, non-ulcerated bowel were selected and examined in detail by two independent pathologists, one of whom was kept in ignorance of the diagnosis. Occasionally, in sections included in the study there was a minimal focal surplus of lymphocytes or plasma cells, or both, which on routine histological assessment would usually be regarded as very mild changes within normal limits. Sections were discarded from this study if there was any ulceration, significant inflammation, or fibrosis. Our observations were therefore only concerned with ‘preinflammatory’ changes in non-ulcerated areas.

Independent opinions were noted, and differences were resolved by consensus and by arbitration by a third histopathologist. 112 sections from patients with Crohn’s disease (68 large bowel, 44 small bowel), 56 sections from patients with ulcerative colitis (52 large bowel, four small bowel), and 90 sections from controls.
The diagnoses of Crohn’s disease and ulcerative colitis were established using the usual clinical, radiological, and histological criteria. Twenty nine of the 35 cases of Crohn’s disease contained granulomas. No difference in the features described below was noted between those specimens which had been fixed by immersion in formalin and those which had been perfused with formalin at mean arterial pressure. Those appearances which were seen before the appearance of inflammatory cells in the surrounding lamina propria (macrophage accumulation and ‘pre-inflammation’) have been separated from those changes which were identified in the presence of polymorphs, lymphocytes, and plasma cells (early inflammation).

**MACROPHAGE ACCUMULATION**

The first mucosal change identified in both Crohn’s disease and ulcerative colitis, and present before a definable mucosal ‘lesion’ could be recognised, was the diffuse accumulation of large eosinophilic macrophages within the lamina propria. These cells were larger in size and number than in controls. They were seen most clearly in large bowel specimens, where they had abundant eosinophilic cytoplasm and often formed a thick, continuous band, predominantly beneath the surface epithelium (Fig 1). The band occupied up to one third of the lamina propria. Smaller cells, with less cytoplasm, were also scattered in the remaining lamina propria and, to a lesser extent, in the submucosa. In small bowel specimens the cells were mainly localised in the tips of villi. They showed immunostaining for KP1 and were occasionally seen adherent to the endothelium of vessel walls within the superficial lamina propria (Fig 2). A second cell type, a dendritic cell immunostaining for factor XIIIa, was also diffusely increased in the non-inflamed lamina propria of patients with Crohn’s disease and ulcerative colitis compared with controls. These cells were found predominantly beneath the KP1 macrophages in the lower two thirds of the lamina propria (Fig 3) and submucosa.

**PRE-INFLAMMATION**

Pre-inflammation changes were identified in areas of bowel which were macroscopically normal. Microscopically, these areas showed normal numbers of mucosal plasma cells and no polymorphs or lymphocyte aggregates – that is, no histological inflammation. The findings are presented in the table. The first focal lesions identified were small haemorrhages at all levels of the mucosa. These were defined as extra-

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**TABLE**  
Summary of early microscopic mucosal changes in Crohn’s disease

<table>
<thead>
<tr>
<th></th>
<th>No of cases</th>
<th>No of cases perfused fixed</th>
<th>Haemorrhages (slides) (%)</th>
<th>Haemorrhages (cases) (%)</th>
<th>Summum lesions (slides) (%)</th>
<th>Summum lesions (cases) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crohn’s disease</td>
<td>35/112</td>
<td>22</td>
<td>13 (12)</td>
<td>9 (26)</td>
<td>8 (7)</td>
<td>6 (17)</td>
</tr>
<tr>
<td>Ulcerative colitis</td>
<td>12/56</td>
<td>8</td>
<td>3 (5)</td>
<td>2 (17)</td>
<td>nil</td>
<td>nil</td>
</tr>
<tr>
<td>Control</td>
<td>13/90</td>
<td>8</td>
<td>nil</td>
<td>nil</td>
<td>nil</td>
<td>nil</td>
</tr>
</tbody>
</table>
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Figure 4: Rupture of small capillaries with haemorrhage into the lamina propria. A plug is present within the tip of the capillary (arrow). (Haematoxylin and eosin, original magnification ×365.)

Figure 5: Immunostaining for fibrinogen shows trails of fibrillary material extending from damaged capillaries within the mucosa towards the surface epithelium. (Haematoxylin and eosin, original magnification ×146.)

Figure 6: Disruption of the basement membrane of a small superficial mucosal capillary shown by immunostaining with collagen IV (original magnification ×365.)

Figure 7: Eruptions of fibrin and epithelial cells from the mucosal surface form 'summit' lesions in Crohn's disease. There is no surrounding inflammation in the lamina propria. (Haematoxylin and eosin, original magnification ×365.)

Vasated red blood cells associated with surrounding homogeneous or fibrillary material (Fig 4) which were found to be positive for fibrinogen after immunostaining. Simple extravasation of red blood cells, occurring as a result of handling or sectioning of the bowel by the pathologist and identified by morphological traumatic artefact, was not associated with similar trails of fibrin. These small haemorrhages were present in specimens which had been fixed by either immersion or perfusion. They were seen more commonly in patients with Crohn's disease (13/112 (12%)) of all sections examined (9/35 (26%) patients), and were identified in 3/56 (5%) of sections from patients with ulcerative colitis (2/12 (17%) patients) and in none of the 90 control sections. Immunostaining for fibrinogen showed trails of fibrillary material originating from ruptured capillaries and extending towards the surface epithelium (Fig 5). Some capillaries in these areas showed discontinuous collagen IV (Fig 6) or QBEnd10 immunostaining, although this could not be shown at every focus of haemorrhage.

The overlying epithelium remained intact in these 'early' haemorrhagic lesions, but sometimes showed necrosis of individual cells with nuclear pyknosis. In a few cases of Crohn's disease (8/112 (7%) of all sections examined; six patients) a small focus of the overlying epithelium, with a plume of fibrin, was seen erupting into the lumen of the bowel as a micro ulceration or 'summit' lesion (Fig 7). No similar 'summit' lesions were identified in areas of normal bowel from patients with ulcerative colitis or controls. Loose accumulations of eosinophilic, fibrillary
material were seen in the superficial lamina propria. In places this material formed 'fibrinoid' plugs partly occluding the lumen of damaged capillaries particularly at the point of rupture (Fig 4). This material did not immunostain with fibrinogen, but did immunostain with factor XIIIa and PGIIIa (Fig 8). In these areas of haemorrhage there were no focal increases in KP1 positive macrophages or dendritic cells immunostaining for factor XIIIa, either within the lamina propria or the underlying submucosa.

**EARLY INFLAMMATION**

Histological evidence of cellular inflammation was uncommon in normal areas of bowel from patients with ulcerative colitis and controls. In a small number of sections from patients with ulcerative colitis (8/56) there were small scattered collections of chronic inflammatory cells, mainly lymphocytes and plasma cells, within the lamina propria. Three sections from the controls showed single, isolated, dilated glands with minimal cryptitis in otherwise normal mucosa. In patients with Crohn's disease the first mucosal inflammatory change was macrophage and lymphocyte collections within the lamina propria. These were present loosely within the interstitium, around areas of capillary damage in some cases or at the base of otherwise normal glands. These aggregates occurred at any level within the mucosa, giving the lamina propria a patchy appearance. The overlying epithelium was generally intact, particularly if the collections of macrophages were deep. Remaining microscopic evidence of haemorrhage was not always present in these deep lesions; however, more superficial collections were occasionally associated with trails of fibrinogen, extravasated red blood cells or 'summit' lesions. With increasing numbers of inflammatory cells the infiltrate showed some organisation and early invasion of gland epithelium at the base of glands, particularly by lymphocytes and macrophages (Fig 9).
vascular polymorphs were present in a few areas; some were present in the lamina propria admixed with the other inflammatory cells. In places these lesions occurred over lymphoid aggregates (Fig 10A) or near to Peyer’s patches (Fig 10B). Dendritic cells immunostaining for factor XIIIa, and usually scattered in the mid zone of the lamina propria, were increased in number around these areas of inflammation.

**Discussion**

Attempts to define a chronological order of events from histological material is always fraught with difficulties. It is only possible to infer a sequence by examination of many sections and multiple levels, and then to imagine spatial and temporal dimensions. These interpretations, therefore, must be regarded as subjective. With these reservations in mind, we propose a sequence of superficial mucosal changes which occur in Crohn’s disease before the development of ‘aphthoid ulceration’ of the overlying mucosa. It is uncertain whether the lesions described are an essential precursor of overt Crohn’s disease or a parallel feature of it.

The term ‘aphthoid’ ulceration has been used by pathologists to describe varying degrees of mucosal ulceration associated with a lymphocytic infiltrate. 

Brooke first used this term for the earliest macroscopic appearance over Crohn’s disease but the first description of mucosal ulceration as an early microscopic change in Crohn’s disease was by Lockhart-Mummery and Morson who reported ulceration of lymphoid follicles and Peyer’s patches in the terminal ileum. Later, these focal microscopic lesions were also given the name ‘aphthoid’ ulcers and the term expanded to include ulceration overlying focal accumulations of lymphocytes in the basal part of the mucous membrane. This paper seeks to examine the mucosal changes which occur before the presence of ‘aphthoid’ ulceration. For this study, we use ‘aphthoid’ ulcer to mean a microscopic ulcer with associated active inflammation, overlying an area of chronic inflammation, lymphoid aggregate or Peyer’s patch.

By examining macroscopically normal, non-inflamed areas of bowel in patients with known Crohn’s disease we have identified subtle mucosal changes which would normally have been obscured by active inflammation. Using a combination of perfusion fixation at mean arterial pressure and immunohistochemical techniques we have been able to examine small mucosal capillaries in detail. Focal, early mucosal changes seem to be associated with damage to small capillaries. This was shown in our tissues by disruption of the capillary basement membranes (collagen IV immunostaining) or the vascular endothelium (QHEnd10) with haemorrhage and trails of fibrinogen in the surrounding lamina propria. This damage was seen in the absence of local accumulation of inflammatory cells and necrosis of the overlying epithelium.

One of the earliest features identified in Crohn’s disease and ulcerative colitis was an increase in the numbers of eosinophilic macrophages beneath the surface epithelium of the lamina propria. The location of these cells, adherent to the endothelium of capillaries within the superficial lamina propria, suggested that they had migrated actively to this site. Similar cells have been described in areas of inflamed mucosa from patients with inflammatory bowel disease. 

Gionchetti et al. examined macrophages in biopsy specimens from patients with Crohn’s disease which were taken from areas with minimal abnormality (recognised only with a magnifying colonoscope); they compared them with biopsy specimens from areas of apparently normal bowel from the same patients, reporting an increase in macrophages staining for RFD9 (an epithelioid macrophage marker) in the diseased areas. Microscopically, however, these areas were already histologically inflamed. They did not compare their normal Crohn’s disease biopsy specimens with control (non-Crohn’s disease) material. Donnellan suggested that these cells were increased in patients with either ulcerative colitis who had been receiving steroid treatment or in patients with obstruction. All of our patients with ulcerative colitis and nearly all those with Crohn’s disease were taking, or had been treated with, steroids before their bowel resections. The seven patients with Crohn’s disease who had not taken steroids showed a slight decrease in eosinophilic macrophages compared with the remaining Crohn’s disease cases, but the numbers were still higher compared with controls (unpublished data). In each of these cases, section had been performed for strictures or symptoms or pseudo-obstruction. The single control patient who presented with obstruction showed no increase in these cells. It remains possible that steroid treatment directly increased the numbers of eosinophilic macrophages in the lamina propria and was responsible for the generalised increase which we found. Alternatively, the increase may reflect a generalised, systemic effect of inflammation elsewhere or a reaction to morphologically inconspicuous mucosal damage.

Small intramucosal haemorrhages were the first focal changes within the mucosa. These often occurred in areas of intact epithelium and were more common in patients with Crohn’s disease (26%) than ulcerative colitis (17%) or controls (0%). Further evidence of vascular damage in the form of ‘summit’ lesions, with plumes of fibrin and loss of superficial epithelial cells were only identified in patients with Crohn’s disease (17%). Early vascular damage was shown clearly by disruption of the collagen IV basement membrane capillaries, and extravasation of red blood cells and fibrinogen into the surrounding lamina propria. This microvascular damage occurred before (that is, was present without) inflammation or epithelial erosion. It is therefore unlikely to be a secondary consequence of either mucosal oedema or erosion or ulceration. The mechanism of this vascular damage is unclear: adhesion, migration, and accumulation of eosinophilic macrophages in a diffuse, sub-epithelial distribution usually occurred before this damage, while focal accumulation of similar macrophages and lymphocytes within the lamina propria seemed to follow it. These features were not seen in all cases of Crohn’s disease, and this
may reflect the patchy nature of the initial disease process and shows one of the difficulties of identifying such early changes.

Dourmashkin et al reported ‘patchy epithelial necrosis in the absence of acute inflammation’ as an early feature of Crohn’s disease in two of seven rectal biopsy specimens,\(^{26}\) and this is supported by our findings. They also noted extravasated red blood cells in the lamina propria and epithelium, and interpreted this feature as an artefact of the biopsy technique. Our sections were obtained from carefully handled resection specimens and are unlikely to have suffered similar direct trauma. Our control specimens did not show these features, and so they cannot be attributed to trauma alone.

Some mucosal capillaries were associated with intraluminal plugs of factor XIIIa positive material, usually close to the point of rupture. Similar material was sometimes present just beneath the surface epithelium in areas of capillary damage. Factor XIIIa is a protein found in plasma and platelets which is activated by thrombin to form factor XIIIa.\(^{27}\) This is a transamidase enzyme which, in the presence of calcium, catalyses the covalent cross linking between the \(\gamma\) and \(\alpha\) chains of fibrin to form stabilised fibrin – that is, the final event in the coagulation of blood. The presence of factor XIIIa immunostaining of the intravascular eosinophilic plugs in our sections suggests that this material represents either platelet thrombi or cross linked fibrin. It is only possible to speculate if these plugs represent true microvascular thrombi, or an attempt at haemostasis and coagulation following capillary haemorrhage. Intravascular microthrombi have been identified before in Crohn’s disease,\(^{28,29}\) although they are not widely recognised as a feature of this disease.\(^{30,31}\) Microthrombi have been described in association with ischaemic bowel conditions, both in experimental animal models and human disease.\(^{32,33}\)

Comparison with the histological changes seen in other conditions known, or thought to have, an ischaemic cause shows some similarities. McGovern and Goulston\(^{34}\) described the main criteria for the diagnosis of ischaemic enterocolitis: mucosal haemorrhage, necrosis, and ulceration (all shown in our study); thrombosis and a moderate infiltrate of polymorphs were also notable features. Microthrombi have been identified in association with mucosal necrosis and ulceration in diverse ischaemic conditions where large vessel occlusion is not present. These include other reports of ischaemic enterocolitis,\(^{35}\) pseudomembranous colitis,\(^{36}\) and haemolytic-uraemic syndrome,\(^{37,38}\) paroxysmal nocturnal haemoglobinuria,\(^{39}\) and ano-rectal ulceration associated with ergotamine suppositories.\(^{40}\)

Models of experimental ischaemia offer the chance to examine the earliest features of ischaemia, something rarely possible in the human situation. Whitehead described early features: dilatation and disruption of superficial capillaries, haemorrhage, oedema with fibrin exudation, and eosinophilic necrosis of the epithelium.\(^{41}\) A polymorphonuclear leucocyte response did not appear until 12–24 h. These features are similar to the observations in this paper.

Pseudomembranous colitis is thought by some to have an ischaemic pathogenesis.\(^{42}\) The foci of micro-ulceration or epithelial loss seen in our lesions closely resembled the ‘summit’ lesions described by Price and Davies in their type I lesions of pseudomembranous colitis.\(^{43}\) They also described one case of Crohn’s disease in which similar lesions were seen, but significant differences in the degree of surrounding inflammation led them to believe that these lesions were not identical. In our cases, however, vascular changes without inflammation were identified as well as lesions where vessel damage and inflammation coincided.

The mucosal changes identified here occurred at all levels of the mucosa, both in areas close to, and distant from Peyer’s patches. The relation between these mucosal changes and the classic aphthoid ulcers occurring over Peyer’s patches is

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**Figure 11:** Proposed early mucosal changes in Crohn’s disease. Small mucosal capillary close to base of crypt. (A) Endothelial insult and capillary disruption: haemorrhage into lamina propria. (B) Fibrinoid plug occluding damaged capillary: macrophages attracted to area of relative ischaemia. (C) Lymphocytes attracted to damaged area: invasion of base of crypt by lymphocytes (cryptitis).
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unclear. The centres of lymphoid follicles are supplied by end arterioles and it has been suggested that, as a consequence, they are particularly prone to ischaemia; thus, Peyer’s patches may provide a preferred site for this kind of ulceration. Alternatively, many aphthoid ulcers overlie lymphoid aggregates rather than true follicles and such ‘advanced’ inflammatory lesions were not present in the areas of ‘normal’ bowel examined in this study. Progression and extension, however, of the early features we have described in this paper would be compatible with the ultimate development of an aphthoid ulcer.

The cause of mucosal changes and aphthoid ulcers in Crohn’s disease has been debated for many years with some authors suggesting that their early presence lends support to a luminal cause for Crohn’s disease. Others believe that mucosal ulceration is a later feature of the disease and is a consequence of the thickening, fibrosis, and inflammation of the submucosa. Our results suggest that capillary damage may play a role in the mucosal changes seen in Crohn’s disease (Fig 11). Repeated microvascular insults, associated with chronic inflammation, could explain many of the clinical features of Crohn’s disease.

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