Granulomatous vasculitis and persistent measles virus infection in Crohn's disease

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The prevailing perception of Crohn's disease (CD) as a primary mucosal pathology, either as an epithelial abnormality or an aberrant response of the mucosal immune system, fails to explain many of the idiosyncracies of this condition. In the absence of a plausible pathogenic basis for skip lesions, transmural inflammation, panenteric distribution of disease and anastomotic recurrence, and the deleterious effects of smoking, many have avoided these issues in favour of more elaborate hypotheses.

It is reasonable to assume that CD is characterized by a persistent cell-mediated immune response to an, as yet, unidentified antigen or antigens (1). The capacity of cells both within the lamina propria and stimulated epithelial cells to present class II antigens (2) has, in the minds of many, reasserted the central role of the mucosa in the development of CD. The abundance of luminal antigen seems to suggest that we need go no further in identifying the source of the stimulus for persisting cellular immunity; all that remains is to isolate and characterize the antigens responsible.

Before taking this final step into the darkness of the intestinal lumen, we...
should reflect upon CD's phenotypic idiosyncracies when considering the underlying pathogenic mechanism — without a mechanism and, by extension, an appropriate source of persistent antigen, we are not in a position to seek the nature and origin of that antigen.

Clues as to the tissue localization of this putative antigen are present in CD in the form of the granuloma. This reaction localizes to persistent antigen, and in those diseases in which an etiological agent has been identified (including tuberculosis, paratuberculosis and schistosomiasis), the granuloma localizes to the causative antigen. Therefore the granuloma provides a logical starting point when considering both the mechanism and the cause of CD.

**TISSUE RELATIONSHIPS OF THE GRANULOMA IN CD**

The granuloma is an early histological feature in the evolution of intestinal inflammation in CD; these hallmark lesions occur principally in a submucosal location and can be present prior to mucosal inflammation or ulceration (3-5), an observation that argues strongly for the priority of this lesion in CD evolution. Although described previously, granulomatous vasculitis in CD has been reported as an occasional feature of no pathological significance (6,7).

For a variety of reasons, the relationship of the vasculature to focal or diffuse inflammation, such as granulomas, may be obscured in immersion-fixed, hematoxylin and eosin stained tissue sections. First, vessels collapse in the absence of an intraluminal hydrostatic pressure. Furthermore, advanced granulomatous vasculitis could be expected to destroy the vessel and to occlude the lumen of the affected vessels, obscuring the apparent vascular origin of the lesion. This is suggested by the observation that extensive vascular disruption in 'primary' granulomatous vasculitides can make it difficult to identify the vessel wherein the granuloma originated (8).

The author has recently examined 485 granulomas in 15 specimens of resected CD and shown that at least 85% of granulomas are directly involved in vascular injury (5). These studies were facilitated greatly by the combination of perfusion-fixation and immunostaining for vascular structures. Granulomas were seen to arise from within the walls of blood vessels and, in many cases, from within the vessel lumen, in intimate contact with the vascular endothelium (Figure 1), suggesting the presence of a previously unrecognized macrophage-endothelial cell interaction in CD. In addition, computerized three-dimensional reconstruction of serial sections of CD has confirmed that granulomas follow the course of the affected blood vessels, rather than coincidentally being adjacent to these vessels in a single plane of section (5). Granulomatous vasculitis — and venulitis in particular — is an integral part of the spectrum of vascular inflammation that the author believes is central to the pathogenesis of CD. Similar observations in sarcoidosis suggest that this process may be more common than was thought previously (9). The consequence of this vascular lesion in CD is likely to be ischemia of the dependent tissues. In terms of a mechanism, therefore, can this process explain some of the idiosyncrasies of CD?

**SKIP LESIONS AND MICROVASCULAR INJURY**

Discontinuous inflammation and associated ulceration are characteristic features of CD; inflamed, ulcerated mucosa may occur immediately adjacent to mucosa of normal histological appearances (10,11). In an attempt to explain this phenomenon by extrapolation of the vascular hypothesis, the author's group selectively embolized the submucosal microvascular plexus of ferret intestine (12). Microspheres (27 to 90 µm in diameter) were injected into the arterial arcade of a defined loop of intestine, causing focal inflammation and ulceration 24 to 72 h later. This model did not set out to reproduce the chronic damage of CD but it did reproduce the juxtaposition of inflamed and ulcerated mucosa alongside histologically normal mucosa. In CD, repeated episodes of focal submucosal vascular occlusion could cause the characteristic patchy mucosal damage.

Figure 1) Crohn's disease; intraluminal granuloma in a perfusion-fixed submucosal vessel. The granuloma adheres to the endothelium and there is no generalized inflammation associated with this lesion. Hematoxylin and eosin x400

**ANASTOMOTIC RECURRENCE OF CD**

The author's group believes that occult residual granulomatous vasculitis is the mechanism for anastomotic recurrence. The capacity of the bowel to heal after a focal, intramural vascular injury depends largely upon the integrity of the collateral plexus formed by the submucosal microvasculature (12). Thus, for any point in the bowel wall, submucosal collateral vessels from either side may preserve the circulation to that point, but division of the bowel before anastomosis will halve this collateral supply. Tissue perfusion at an anastomosis will be compromised further by the local pressure of a suture line. This devascularization is of little consequence when normal intestine is Anastomosed. If, however, there is a vasculitis in the macroscopically normal bowel used to make the anastomosis, the reduced capacity of the already compromised collateral circulation may be sufficient to induce focal tissue ischemia and anastomotic recurrence. Similarly, subacute obstruction of the bowel, by increasing intraluminal pressure, will cause a profound decrease in perfusion of the mucosal vasculature (13).

Osborne et al (14) recently adapted the model described by Hudson (12) to study anastomotic healing in the presence of a compromised submucosal collateral circulation. Following emboliza-
tion of the intestine, which produced a self-limiting mucosal injury, an anastomosis was created through the embolized loop of intestine 72 h following the initial procedure. At two weeks there was macroscopic and microscopic inflammation in the embo lized limb of the anastomosis with a combination of transmural inflammation, mucosal ulceration and occasional granuloma formation; these features were not seen in controls who received either anastomosis or microspheres alone, and the results suggest that the superimposition of two self-limiting ischemic insults can produce chronic inflammation at an anastomosis.

**COLONIC CD: DIVERSION OF THE FECAL STREAM AND RESTING THE GUT**

Patients with severe colonic CD often enjoy a remission following a diverting ileostomy, but relapse of the disease usually follows restoration of the fecal stream (15-17). This sequence has prompted the hypothesis that some constituent of the fecal stream is responsible for either initiating or provoking CD (17,18).

The bloodflow of the intestinal mucosa increases greatly in response to the metabolic demand placed on the mucosa by digestion and absorption (19). In CD, stenotic vascular lesions in the bowel wall would place a rate-limiting influence upon the hyperemic response to mucosal activity. This situation is analogous to intermittent claudication or exercise-induced angina – ischemia or infarction will occur when the demand for blood exceeds supply.

Fecal diversion rests the excluded bowel, helping reduce mucosal bloodflow and oxygen demand. Provided with a respite, the intestinal ischemic damage eases and healing dominates. This phenomenon may also help to explain, at least in part, the ameliorating effect of a proximal diverting ileostomy upon anastomotic recurrence (20). However, reinstition of the fecal stream is likely to induce relapse due to further ischemic damage. This may also explain the transient clinical benefit of an elemental or polymeric diet (21).

**MULTIFOCAL GASTROINTESTINAL INFARCTION AND APHTHOID ULCERATION**

It has been argued that aphthoid ulceration in CD is not a feature of mucosal ischemia (22). However, in cases of Wegener's granulomatosis or Behçet's disease, both of which are idiopathic necrotizing vasculitides involving the intestine, aphthoid ulceration of the mucosa (including the oral mucosa) is a prominent feature (23,24). It is likely that these diseases, which produce occlusive vascular inflammation in affected segments of intestine, produce focal ulceration by causing mucosal ischemia; it is interesting that anastomotic recurrence is also a feature of Behçet's disease (24). The authors group has proposed that ulceration in CD represents focal infarction of the mucosa that may progress, depending on the location and severity of the underlying vascular disease, to penetrating ulceration and fistulation (25). To accept that this damage is due to 'multifocal gastrointestinal infarction' needs a reappraisal of the concepts of infarction (22). 'Classical' intestinal infarction involves fibrosis, mucosal atrophy and hemosiderin deposition – all features of large mesenteric vessel occlusion. But this definition precludes a spectrum of tissue injury that may result from chronic damage at the level of the microvasculature of the bowel wall. Infarction of the mucosa will not be immediately apparent, not only because of the bowel's ability to shed dead tissue into the lumen, but also because of its remarkable capacity for repair and regeneration without sequelae. Mucosal ulceration may be seen in a variety of other conditions in which ischemia is implicated – for example, pseudomembranous colitis, pigbel and necrotizing enterocolitis (26-28).

These appearances are not those of classical infarction but, if infarction is defined as 'tissue necrosis due to interruption of its blood supply', these conditions qualify and so, the author suggests, does CD.

By examining macroscopically normal, noninflamed areas of bowel in patients with CD, Sankey and colleagues...
(29) identified subtle mucosal changes that would normally be obscured in areas of active inflammation. Using a combination of perfusion-fixation at mean arterial pressure and immunohistochemical techniques, she was able to examine small mucosal capillaries in detail. Focal, early mucosal changes appear to be associated with damage to small capillaries. This was manifest as disruption of the capillary basement membrane or the vascular endothelium with hemorrhage and trails of fibrinogen in the surrounding lamina propria. These were very much more common in patients with CD (50%) than ulcerative colitis (15%) or in controls (15%). This vascular damage preceded both the focal accumulation of inflammatory cells and necrosis of the overlying epithelium and cannot, therefore, be a consequence of inflammation.

The mucosal changes described by Sankey et al occurred at all levels of the mucosa, both in areas close to, and distant from, Peys's patches. The relation between these mucosal changes and the classical 'aphthoid' ulcers occurring over Peys's patches is unclear. The centres of lymphoid follicles are supplied by end arterioles. It has been suggested that as a consequence, they are particularly prone to ischemia (30); thus, Peys's patches may provide a favoured site for this kind of ulceration. Alternatively, many 'aphthoid' ulcers overlie lymphoid aggregates rather than true follicles; it is possible that progression and extension of the early features that were described would be compatible with the ultimate development of an 'aphthoid' ulcer.

SYSTEMIC THROMBOGENESIS, SMOKING AND CD

Independent risk factors for systemic thrombogenesis and an increased incidence of thrombotic disease include smoking, elevated plasma levels of lipo-protein(a) (31) and factor VIIc (32).

The author's group recently showed that only six of 49 patients with CD did not have one or more of these additional risk factors for thrombosis (unpublished data). It is tempting to postulate that a primary mesenteric microvascular injury and a systemic thrombotic tendency combine to precipitate clinically active CD.

MESENTERIC MICROVASCULAR ENDOThEliUM AND MEASLES VIRUS

The observation of granulomatous vasculitis in CD, and the possibility that many of the idiosyncrasies of this condition might be explained by a compromised intramural circulation, focused attention upon the mesenteric microvasculature. Vascular endothelium plays a central role in both immunity and hemostasis (33); furthermore, it can harbour both productive and persistent viral infections (34,35). There was sufficient epidemiological evidence of an infectious etiology for CD to progress this hypothesis in search of a source for persistent antigen. Since CD is neither vertically nor horizontally transmissible, it is likely that the putative infection is nonproductive and therefore cell-associated. If so, is it possible that the microvascular endothelium of the gut is the source of this infection and, thus, the persistent antigenic stimulus that drives cellular immunity in CD?

It was hypothesized that measles virus could be the etiological agent in CD. Measles virus-infected lymphocytes 'home' to the lymphoid tissues of the gut during the primary viremia; subsequently, the virus infects the microvascular endothelium of these tissues and induces vasculitis - secondary transmural lymphoid follicles develop in the gut wall and there is necrosis of the overlying epithelium (36), all of which are characteristic features of CD (3,4,11). Thus, measles can produce Koplik's spots throughout the gut that are similar to the aphthoid ulcers characteristic of CD (36-38). Giant cell synctia at the centre of secondary lymphoid follicles in the intestine are a prominent feature of measles virus infection (36). Hadfield (3) reported similar changes in his classical histological description of CD. Acute endothelial cell infection by measles virus occurs principally in the submucosal microvasculature throughout the entire length of the gastrointestinal tract (36), the lesional topography of CD (3,5).

Recent detailed analysis of 24 cases of intestine affected by CD using a combination ultrastructure, immunohistochemistry (Figure 2) and in situ hybridization (Figure 3) suggests that measles virus may play an etiological role in this condition (38,39), and that intestinal microvascular endothelial cells may be the principal reservoir of infection (infected cells were often observed specifically in foci of granulomatous vascular injury). A profound cellular immune response to apparently low levels of endothelial cell-associated virus, with the consequent vascular compromise that is seen, might account for the extensive tissue injury in CD. Identical intranuclear paramyxovirus inclusions in CD have been observed recently by others (40). There is an urgent need to confirm these observations at a molecular level and provide amplifiers for viral sequencing studies.

A possible role for measles virus in CD has received recent support from epidemiological studies undertaken in Sweden: Ekbom et al (41) observed that perinatal measles virus infection was a strong risk factor for the development of CD later in life, a phenomenon that exhibits similarities to subacute sclerosing panencephalitis.

CONCLUSIONS

In early nonulcerated CD there is evidence of granulomatous and lymphocytic vasculitis: the combination of this lesion with either a systemic prothrombotic environment or mechanical devascularization of the intestine, before anastomosis, may induce mucosal ischemia and ulceration. We hypothesised that the mesenteric microvascular endothelium may harbour the antigenic stimulus for vasculitis in CD, and early morphological and epidemiological data suggest that this stimulus may be persistent measles virus infection. A great deal more work needs to be undertaken to confirm or refute these early etiological observations before any firm conclusions can be reached.
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REFERENCES

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