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Significant association of strictures and internal fistula formation in Crohn's disease

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Abstract Intestinal inflammation in Crohn's disease (CD) may be complicated by the occurrence of strictures and fistulae. The pathogenesis of fistula formation is unknown. We therefore wanted to determine whether mechanical factors might contribute to the development of fistulae. Furthermore, we tried to define the path of internal fistulae through the muscular layer. For this purpose, surgical resection specimens from 42 consecutive patients with CD were prospectively studied. In gross examination the whole bowel was cut into circumferential cross sections 0.3 cm thick. Abnormal areas were histologically examined. Strictures were found in 38 patients (90.5%), and fistulae were observed in 27 (64.3%) patients. In 11 (40.7%) specimens fistulae were found within a stricture, in 15 (55.6%) at the proximal end, and in 1 (3.7%) no stricture was found. In 7 (25.9%) cases with fistulae, herniated mucosa was found within the muscularis propria or the subserosa. In 7 (25.9%) cases a blood vessel was identified near a fistula traversing the muscularis propria. From these findings we conclude that that mechanical factors may contribute to fistula formation. This is further supported by the fact that fistulae appear to traverse the muscular layer along piercing vessels.

Keywords Crohn's disease · Fistula formation · Vessel · Stricture

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Introduction

The occurrence of fistulae and strictures characterises the course of Crohn's disease (CD) in many patients. Although this fact has long been acknowledged, it is still not known why a subgroup of CD patients is prone to development of these complications, which are a major cause of morbidity and often lead to surgery. The observation that patients with internal fistulae often redevelop the same complication after surgical treatment has led to the suggestion that two distinct clinical types of CD exist, the perforating and the nonperforating types [4]. Patients with the perforating type are characterised by the development of fistulae, while nonperforating CD is characterised by the development of inflammation with or without strictures in the gut. However, these putative subsets still require definitive confirmation.

It has been proposed that both the formation of fistulae and the development of fissures may be caused by mechanical factors, in particular intraluminal pressure, rather than any intrinsic quality of the inflammatory process in CD. This hypothesis is supported by the observation that internal fistulae are almost exclusively found in patients with strictures [7, 8]. Other investigators claim that host factors, such as the host immune response, determine the form of CD that becomes manifest in any given individual. For example, a study of cytokine patterns in patients with perforating and nonperforating CD has shown that the latter group had elevated mRNA levels of IL-1β and IL-1 receptor antagonist, paralleling the situation in patients with leprosy, in which there are also two forms of host immune reactivity [2] which determine what type of leprosy (tuberculoid vs lepromatous) develops.

It is well known that the muscular layer is a major barrier protecting the gut from perforation: ulcers may easily form and extend as far as the submucosa, but particularly in areas not exposed to gastric acid, they rarely penetrate into and beyond the muscularis propria (e.g. in ulcerative colitis). Therefore, it is surprising that fistulae are able to pass this barrier. To the best of our knowledge

no studies have so far been directed at trying to explain how fistulae are able to do this. Hypothetically they may either traverse the muscular layer by just destroying it by means of enzymes released from inflammatory cells (e.g. collagenases) or they may use sites of minor resistance, such as areas where there are piercing vessels that can be used as a predefined path.

In the present study we tried to determine whether mechanical factors may have an important impact on fistula formation. Furthermore, we wanted to define the path fistulae take when they traverse the muscular layer.

Patients and methods

Surgical resection specimens from 42 consecutive patients with CD were prospectively studied. The series was made up of unselected patients who underwent surgery between October 1997 and December 1998 in the Department of Surgery at the University of Vienna. All operations were performed by one surgeon (H.F.) and included 21 (50%) ileocaecal resection specimens, 8 (19%) resection specimens with an ileocolonic anastomosis, 3 (7.1%) small bowel resection specimens, and 10 (23.9%) colonic resection specimens (including 6 partial colon resections, 3 ileocolonic resections, and 1 right hemicolectomy).

Gross and microscopic examination was performed by one pathologist (G.O.) according to the following protocol: following surgical excision the bowel was opened and washed, examined, and thereafter fixed in 4.5% buffered formalin for at least 24 h. During a gross examination the whole bowel was cut into circumferential cross sections 0.3 cm in thickness to reveal the structure of the bowel wall and of the mesentery and/or mesocolon. This allows detection of fistulae traversing the muscular layer and estimation of the grade of strictures. Whenever fistulae traversing the muscular layer or abnormous findings in the muscular layer – especially in areas with piercing vessels (which are grossly visible) – were observed the tissue was examined histologically. As a minimum 3 blocks were taken from areas of strictures, 4 from areas with mucosal inflammation, 1 from the ileocoecal valve, 1 from the resection margins and 1 each from normal appearing areas, from the mesentery, and the mesenteric or mesocolic lymph nodes. At least 11 blocks were taken from each case.

Sections approx. 2–3 µm in thickness were cut from the paraffin-embedded tissue and routinely stained with haematoxylin and eosin. Four further levels were cut from areas containing parts of a fistula traversing the muscularis propria. Three were stained with haematoxylin and eosin and one with an elastic Van Gieson stain (stains elastic fibres) with the aim of detecting the characteristic internal elastic lamina of arteries.

Diagnosis of CD

All cases were known from clinical and endoscopic and microscopic examinations and from follow-up to have CD prior to surgery. Standard criteria [6, 9], including focal or discontinuous inflammation and architectural changes in the lamina propria, fissural ulceration, and the occurrence of lymphoid follicles at the border of the muscular layer and the subserosal fatty tissue, were applied in resection specimens.

Definition of fistulae and strictures

Fistulae were defined as abnormal communications between the lumen of the gut and the mesentery and/or another hollow organ or the abdominal wall and skin. All lesions that had penetrated the muscularis propria were considered to be fistulae. Histologically, fistulae are composed of granulation tissue surrounding a lumen, which is mostly filled up by nuclear debris and inflammatory cells, in particular neutrophils. The term "initial" fistulae (Fig. 1) denotes cases with initial, i.e. less than 2-mm-deep, penetration of granulation tissue into the mesentery or mesocolon (in our experience this is a distinct group). Others have termed these lesions "sinus tracts".

Bowel obstruction (stricture) was diagnosed in cases of luminal narrowing and bowel wall thickening with or without prestenotic dilatation on naked eye examination. In high-grade stenosis the luminal diameter was <0.5 cm, in intermediate-grade stenosis, <1 cm and in low-grade stenosis, <2 cm. In all cases stricture was confirmed by further preoperative examinations (enteroclysis spiral computed tomography, double contrast barium enema, transabdominal ultrasound, and/or small bowel follow-through). Histologically, strictures were recognised by thickening of the muscularis mucosae and by fibrosis of the submucosa. Both are regarded as sequelae of inflammation and ulceration. Transmural inflammation without these two histological features did not present as a stenotic area on gross examination.

Statistical evaluation

Statistics were computed with the SPSS software (SPSS, Chicago, Ill.). All values are given as median (range). Statistical tests used were the Pearson test, the Chi-square test, a T-test, one-way ANOVA, and the Wilcoxon signed-rank test, as appropriate. $P \leq 0.05$ was considered significant.

Results

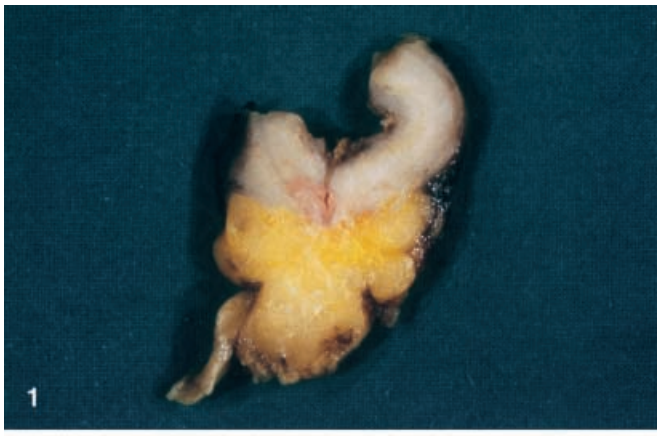
The median age of the patients was 33.5 years (range 20–78 years). Twenty-four (57.1%) patients were male, and 18 (42.9%) were female. Medication included steroids in 9 patients, azathioprine in 2 subjects, and 5-aminosalycilate in 8 individuals. Furthermore, the following drug combinations were taken: steroids and 5-aminosalycilate in 5 patients, steroids and azathioprine in 3 subjects, azathioprine and 5-aminosalycilate in 3 patients, and all three drugs in 2 individuals. No specific medication was given in 10 cases. For more clinical data see Table 1.

Strictures

Strictures were found in 38 patients (90.5%) in the resected bowel. In the group with strictures, only one ste-

Table 1 Characteristics of the patients with Crohn's disease

Duration of disease (years)	4 (0.1–19)
Duration of disease until first resection	2 (1–18)
Previous intestinal resection	13 (40%)
Time elapsed since last intestinal resection (years)	5 (2–19)
Disease parameters, median (range)	
Leukocyte count (4.0–10×10^3/mm^3)	8.9 (3.1–17.5)
Serum C-reactive protein (<0.5 mg/dl)	1.3 (0.5–11.5)
Serum orosomucoid (<120 mg/dl)	149 (184–203)
Erythrocyte count (4.2–5.8×10^3/mm^3)	4.6 (3.1–5.5)
Hemoglobin (12–17 g/dl)	13.2 (8.5–16.2)
Iron (60–150 µg/dl)	35.5 (5–111)
Platelet count (150–350×10^3/mm^3)	351 (172–752)
Subileus	12 (28.6%)



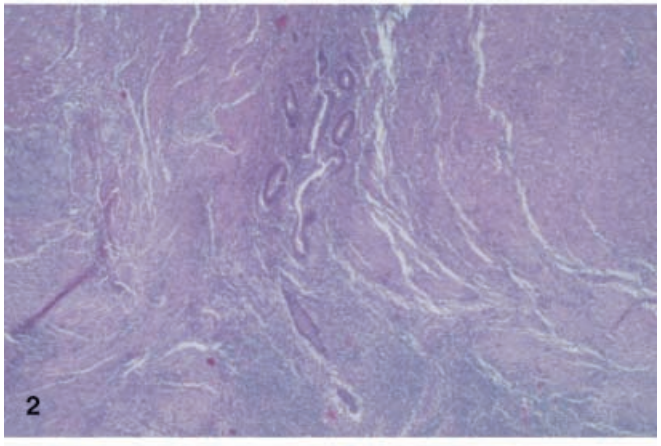


Fig. 1 Circumferential cross section of a small bowel specimen with Crohn's disease (*CD*). There is an initial fistula et the mesenteric border of the specimen

Fig. 2 Photomicrograph of the mucularis propria. Note herniated mucosa in the central part of the specimen, extending into the subserosal tissue. Hematoxylin and eosin, original magnification ×300

Fig. 3 Photomicrograph of an artery surrounded by inflammatory cells of a fistula traversing the muscular layer. *Highlighted* is the internal elastic lamina of the artery. Elastic Van Gieson stain, original magnification ×400

notic area was observed in each of 26 (61.9%) subjects, two in 8 (19%) patients, three in 3 (7.1%) cases, and, finally, six in 1 (2.4%) patient. The most severe grade of the strictures was high grade in 28 patients (73.7%), intermediate grade in 8 (21%), and low grade in 2 (5.3%).

Internal fistulae

Fistulae were observed in 27 (64.3%) patients. Nine were classified as initial fistulae, 11 were longitudinal or branching fistulae confined to the mesentery, 3 reached into the abdominal wall, 3 into the colon (2 ileocolonic, 1 duodenocolic), and 1 into the urinary bladder.

In 26 (96.3%) of these patients a stricture was found. In 11 (42.3%) patients fistulae were observed within a stricture and in 15 (57.7%) at the proximal end of the stricture. The association of fistulae and strictures was significant ($P<0.02$). In 1 patient a duodenocolic fistula originating in the duodenum was observed, but no stricture could be found.

In 7 (25.9%) of the 27 cases with fistulae, herniated mucosa (Fig. 2) was found within the muscularis propria or the subserosal fatty tissue. The possibility that the herniated mucosa might be an artefact from preparation or sectioning was ruled out. That it might have been misplaced epithelium from previous resections was considered unlikely, as the displaced epithelium was not found near anastomotic regions.

In 7 (25.9%) cases a vessel (Fig. 3) was identified side by side a fistula traversing the muscularis propria. The vessels displayed a high-grade reactive vasculitis and were partly destroyed. Mostly identification was only possible through demonstration of the internal elastic lamina by the elastic-Van Gieson stain. In 6 (22.2%) more cases a paravascular path of fistulae through the muscular layer was suggested, but not proven, by the following features: observation of vessel-like structures, which did not contain an internal elastic lamina and/or observation of vessels above and/or below but not within the area where the fistula passed through the muscular layer.

In most cases only very narrow tracts less than 1 mm in diameter were found to pass into the bowel wall. This was also observed in cases with branching fistulae in the mesentery.

Comparison with clinical data

There was no significant correlation of the occurrence of fistulae with onset of disease, duration of disease, medication (e.g. steroid use), and laboratory investigations, with the exception of the platelet count, which was $434\times10^3/mm^3$ ($235–752\times10^3/mm^3$) in patients with fistulae, and $294\times10^3/mm^3$ ($172–500\times10^3/mm^3$) in the remainder ($P<0.003$). Female patients were overrepresented in the group that had developed fistulae (14 female patients (77% of all female patients), whereas male

patients were comparably distributed in both groups (13 male patients (54% of all male patients)). The difference was statistically significant ($P<0.001$).

Discussion

In the present study we have shown that the development of internal fistulae (patients with perianal fistulae were not analysed) is significantly associated with luminal narrowing of the bowel. Furthermore, we have shown that fistulae appear to pass through the muscularis propria, at least in a subgroup of patients, along piercing mesenteric vessels. These locations seem to represent a site of minor mechanical resistance to intraluminal pressure.

The muscular layer itself is a major barrier for penetrating ulcers or fissures. Fissural ulcers often reach to the muscularis propria but are not able to traverse it (personal unpublished observation). Although fissural ulcers might be able to degrade the muscular layer and to traverse the bowel wall thereafter, they are more likely to follow sites of minor mechanical resistance, such as areas with vessels piercing the muscular layer. Piercing vessels are up to 1 mm in diameter, including the surrounding connective tissue, and can readily be observed macroscopically in circumferential cross sections. Therefore, they appear to be large enough to be used as a predefined path. Indeed, we have shown here that in some cases, in particular with initial fistulation, fistulae traverse the muscular layer via these sites of minor resistance, as was confirmed by the presence of at least parts of an artery in the fistulous tract. These arteries were destroyed or severely damaged in all these cases, so that only parts of them could be identified. This may be one reason why vessels can no longer be observed as the disease progresses.

We do not know whether intraluminal pressure alone guides the fistulae along the arteries. Potentially, arteries are able to contribute to the inflammatory reaction. This notion is supported by the fact that the endothelium may contribute to the cytokine network of the intestinal mucosa with the ability to respond to locally generated cytokines and to produce potent inflammatory mediators [10]. Also, transmural inflammation itself, inducing muscle abnormalities and alterations of the intrinsic innervation, could play a part.

The highly significant association of fistula formation with stricture development has also been found by others [3, 8, 13], whereas some groups did not disclose such an interrelationship [5, 11]. The different results may be explained as follows. Owing to a referral bias it may be that only patients presenting with symptoms associated with stricture formation were referred to our institution. Indeed, most of our specimens comprised some elements of stricturing. Thus, the association of fistulae and stricturing might have been a mere coincidence. Militating against such a referral bias is the fact that fistula formation poses an even greater therapeutic challenge to the treating physician than strictures alone. Therefore, cases with fistulae are more likely to be sent to a tertiary referral centre (which our institute is). The possibility that only patients with strictures were operated on because of signs of bowel obstruction, whereas patients with fistulae only were treated conservatively is refuted by the fact that our policy is to treat fistulae by surgery. Screening of all our CD patients [1] by ultrasound and radiology has ruled out such a selection bias. Furthermore, the fact that most fistulae developed at the proximal end or at least within the proximal third of strictures militates against a mere chance coincidence of fistulae and strictures.

Host factors might also potentially influence the association of fistulous disease and strictures. A study population particularly prone to fistula development may only need a small increase in luminal pressure to develop this complication. In this situation pathologic changes of the bowel wall leading to this increased luminal pressure, such as submucosal fibrosis of the ileocaecal valve, might be mild and be overseen by the pathologist. Furthermore, specific therapy that can lead to a decrease in submucosal oedema, and consequently to a decrease in luminal narrowing, may mask the view that increased luminal pressure might have led to fistula formation.

A further explanation for the different findings recorded in the literature may also be explained by different pathological work-up and/or varying definitions of fistulae and strictures. Pathological work-up may be difficult in cases with adhesions of the bowel walls or in specimens with an inflammatory pseudotumour. Both findings are observed particularly in patients with fistulation and may lead to an underestimation of the prevalence of strictures. With respect to the definition of fistulae, we defined fistulae as tracts lined by granulation tissue with central accumulation of detritus and granulocytes that reach beyond the muscular layer (which distinguishes fistulae from fissural ulcers). The “classic” definition of fistulae as tracts connecting two hollow organs does not seem to fit in CD, for various reasons. Most importantly, most fistulae are confined to the mesentery or mesocolon either as longitudinal or as branching tracts. The clinical problems of mesenteric/mesocolic fistulae are similar to those of cases with fistulae according to the classic definition; they include the formation of inflammatory pseudotumours, abscesses, and the development of fever and sepsis. Of course, patients with initial fistulae, which according to our definition means tracts that do not extend more than 1–2 mm into the mesentery, do not seem to manifest these clinical problems and are usually only recognised after histological examination of the resection specimen (personal observations). They may represent either a rudimentary disease or an early stage that may or may not result in more severe disease.

Three observations support the significance of increased intraluminal pressure in fistula formation:

1. The development of fistulae at the proximal end or at least within the proximal third of strictures. In these

areas the pressure exerted on the bowel wall is particularly high. In the case of a merely coincidental development of fistulae and strictures a proportion of cases would presumably have developed fistulae at the aboral end of strictures, which was not the case in our study.

2. The development of fistulae almost exclusively at the mesenteric border. This is the area where the largest vessels can be found. Probably larger vessels are less resistant to intraluminal pressure owing to a broader rim of surrounding loose connective tissue. Of course fistulae can, although they very rarely do, traverse the bowel wall at the antimesenteric border, which results in free or sealed perforation of the gut [4, 12]. Interestingly enough, in the rare cases described in the literature an increased intraluminal pressure proximal to an obstruction was considered an important underlying pathomechanism [4, 12].
3. The fact that mucosa may be translocated into the submucosa, muscularis propria or even subserosa supports the notion that pathologic intraluminal pressure may be operative in these patients.

In conclusion, our data support the notion that fistula formation is at least partially dependent on an increased luminal pressure, as suggested by the occurrence of fistulae within or close to strictures in most cases. At least in a subgroup of patients fistulae penetrate the muscular layer along piercing vessels, that is to say at a site of minor resistance to intraluminal pressure.

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