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Crypts in Asymmetric Fission in Endoscopic Biopsies from German Patients With Inflammatory Bowel Disease

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Abstract. *Background/Aim: We previously found in Swedish patients with inflammatory bowel disease (IBD), crypts in symmetric fission (CSF) and in asymmetric fission (CAF). This study aimed to examine CSF and CAF in a cohort of German patients with IBD. Patients and Methods: H&E-sections from 106 IBD-patients [59 ulcerative colitis (UC) and 47 Crohn colitis (CCs)] were analysed. Results: A total of 588 crypts in fission (CF) were found; 342 (58.2%) in UC and 246 (41.8%) in CCs. Out of the 505 CAFs found, 304 (60.2%) were recorded in UC, and 201(39.8%) in CCs (p=0.15272). Conclusion: Despite that German and Swedish populations reside in disparate geographical regions with different ecological milieus, the proportions of CAF and CSF were similar, thereby suggesting that CAF and CSF develop in IBD independently of the local environmental conditions in the two regions.*

Both in humans and in rodents, the mucosa of the normal colon is built of an assemblage of mucosal invaginations called crypts (1). During the postnatal period, the colon mucosa generates new crypts through a process called crypt fission (2), also referred to as crypt branching (1, 3). In this period, crypt fission begins at its base and progresses upwards through the proliferation zone, until two identical individual crypts develop (4). However, in the normal colon in adults, crypts fission rarely occurs (1-5).

Following a classical histological description of the normal colon mucosa, it has been axiomatic to describe colon crypts as closely aligned glands in parallel, as "test tubes", vertical to the surface epithelium and the muscularis mucosae (1). However, this description applies only to well-oriented, upright crypts, often seen in sections from colectomy specimens (6), or mucosectomies (7). In contrast, endoscopic biopsies in normal donors reveal cross-cut crypts in a horizontal (tangential) plane, displaying ring-shaped cross-cut crypts, similar in outline and diameter (8, 9).

In inflammatory bowel disease (IBD) [including ulcerative colitis (UC) and Crohn's colitis (CCs)], large mucosal areas are replaced by chronic inflammation (10, 11). In these large mucosal areas, a regenerative process relentlessly ensues, including the boosting of crypt fission (6).

Despite crypt branching being mentioned in most histologic reports in patients with IBD, only but few isolated histologic studies have described the various phenotypes of crypt branching in sections from colectomy specimens (6) or from endoscopic biopsies in IBD patients (8). In well-oriented, upright crypts in IBD, the point of crypt-branching (the starting "point" of crypt fission) may be found at any level along the height of the crypt, from the top to the bottom, but in IBD biopsies, the point of crypt fission is identified by back-to-back crypts being apart by an epithelial septum (9).

In colectomy specimens and in endoscopic biopsies from Swedish patients with IBD (6), we previously found not only crypts in symmetric fission (CSF), but also crypts in asymmetric fission (CAF). Since CSF seldom occurs in the colonic mucosa of adults, and CAFs have never been recorded in the normal mucosa (1-4), the occurrence of CF, and particularly CAF in patients with IBD was considered a significant histo-biological event. Notwithstanding, it is theoretically possible that the presence of CAF in Swedish patients with IBD could be due to unknown local factors acting exclusively in the Stockholm area. To reject or confirm that possibility, we contacted pathologists working with IBD at German Universities, distant >1,000 km from the Stockholm area, to join the project.

The aim of the present study was three-fold: i) To quantify CSF and CAF in endoscopic biopsies in a cohort of German patients with IBD, ii) To investigate possible quantitative

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Key Words: Colon biopsies, inflammatory bowel disease, crypts, asymmetric fission.

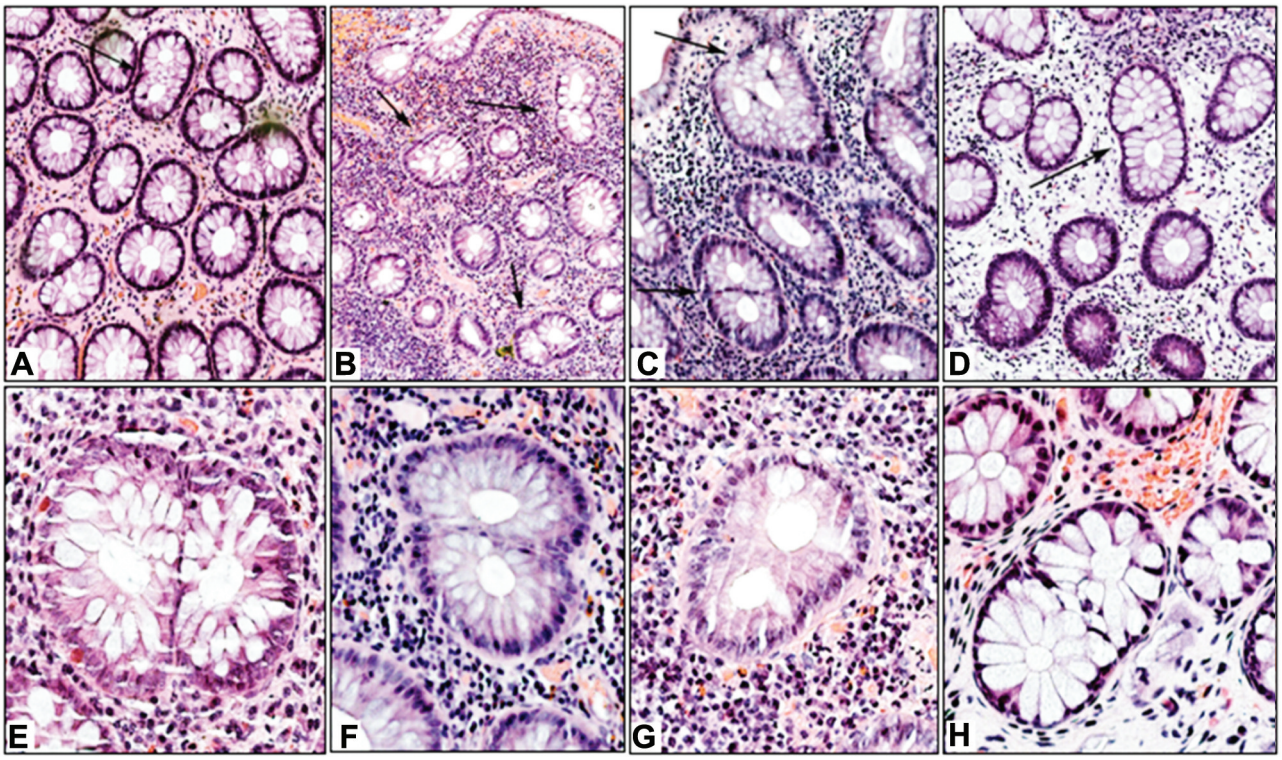

Figure 1. *Cross-cut crypts in symmetric fission found in endoscopic biopsies from German patients with inflammatory bowel disease. A and B: Cross-cut crypts in symmetric fission (at arrows) interspersed with crypts with architectural distortions (irregular contours, varying in size and shape, surrounded by chronic inflammation (H&E A original ×10, and B original ×4). C-H: Other examples of cross-cut crypts in symmetric fission; F and H disclose the point of symmetric crypt fission typified by the epithelium septum dividing the branching crypt (H&E, C and D original ×10, E-H, original ×40).*

differences in CSF and CAF in German patients with IBD, and iii) To compare the quantitative results obtained in endoscopic biopsies in German patients, to those previously reported in endoscopic biopsies in Swedish patients with IBD.

Patients and Methods

The material includes endoscopic biopsies in 106 patients with IBD without dysplasia or carcinoma, under surveillance (59 with UC and the remaining 47 with CCs) received at the Klinikum Bayreuth and Erlangen-Nuremberg University, Germany. Following the endoscopic scheme for IBD patients, biopsies were taken from 10 different colon-levels. One biopsy from an endoscopically affected area in the sigmoid colon was investigated in each of the 106 patients. Biopsies were cut into 4 μm thick sections, and stained with hematoxylin and eosin (H&E). Sections were scanned with a Hamamatsu Nanozoomer S360 scanner and made available to all three authors *via* web interface.

Definitions. In this survey, the following definitions of CSF and CAF were applied to cross-cut sections from endoscopic biopsies in patients with IBD.

CSF: Twin ring-shaped identical symmetric crypts, displaying a back-to-back setting, separated by a thin epithelial rim (Figure 1); *CAF*: Two or more back-to-back crypt-rings differing in diameter and/or shape, separated by a thin epithelial rim (Figure 2). Both CSF and CAF were encased by a thin layer of *muscularis mucosae*.

Ethical approval. Ethical approval was obtained from the ethics committee of Friedrich-Alexander University, Erlangen-Nuremberg, Germany, for validation of the Distribution, Chronicity. Activity (DCA)-IBD-score (study number: 175_20 Bc).

Statistical analysis. The non-parametric Mann-Whitney *U*-test was applied to compare difference between groups, using the Social Science Statistics Program Statistical significance was defined as $p<0.05$.

Results

Table I shows that the number of CFs recorded in the colon biopsies of the 106 patients with IBD was 588 (5.5 CF/biopsy). Out of these, 342 CFs (58.2%) were recorded in UC, and the remaining 246 (41.8%), in CCs. The difference in CF between UC and CCs was non-significant (p-value=0.70394).

Out of the 83 crypts recorded in CSF, 38 CSFs (45.8%, 0.6/biopsy) were found in UCs, and 45 (54.2%, 0.9/biopsy) in CCs, and out of the 505 CAFs detected, 304 CAFs (60.2%,

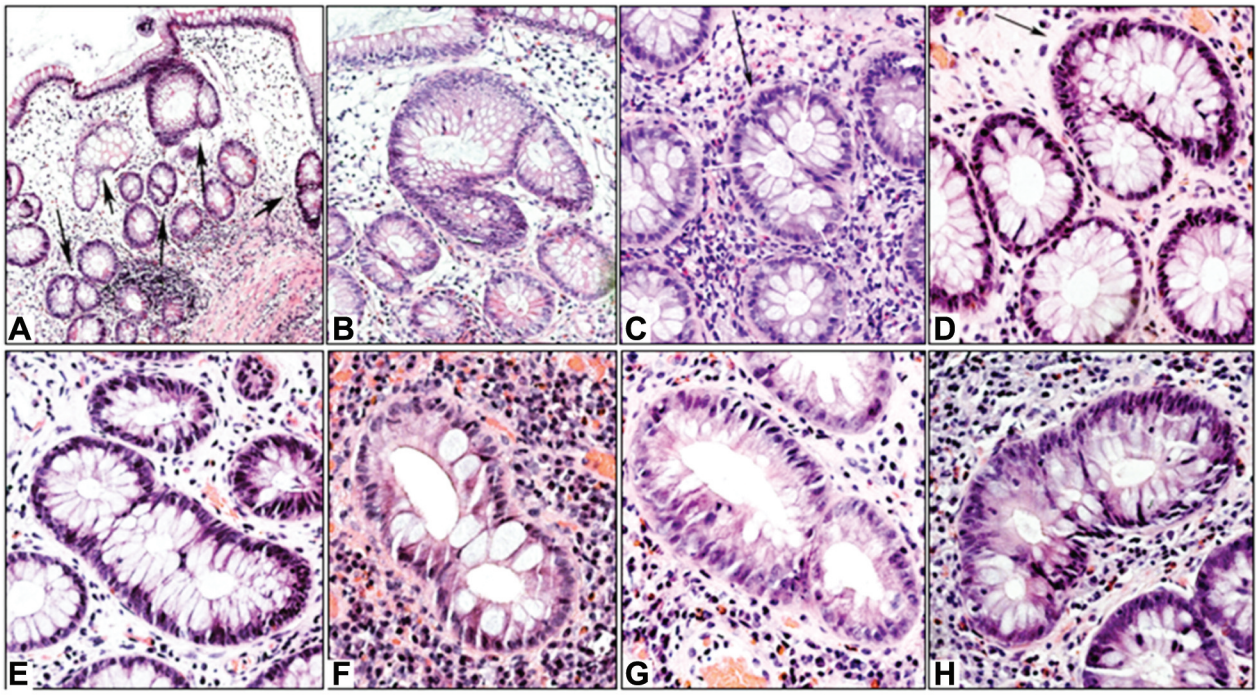

Figure 2. *Cross-cut crypts in asymmetric fission found in endoscopic biopsies from German patients with inflammatory bowel disease. A: Cross-cut crypts in asymmetric fission near the surface epithelium of the colon (H&E original × 4), B-H: Other examples of cross-cut crypts in asymmetric fission. Note the asymmetry in the lumen of the branching crypts; other crypts show more than two lumina (H&E B-H original ×20).*

Table I. *The number of colon crypts in asymmetric and in symmetric fission in endoscopic biopsies from 106 German patients with inflammatory bowel disease (IBD): 59 with ulcerative colitis and in 47 with Crohn colitis.*

	No. of crypts in asymmetric and in symmetric fission in 59 endoscopic biopsies in patients with ulcerative colitis			No. of crypts in asymmetric and in symmetric fission in 47 endoscopic biopsies in patients with Crohn colitis		
	No. of asymmetric crypt in fission	No. of symmetric crypt in fission	Total	No. of asymmetric crypt in fission	No. of symmetric crypt in fission	Total
Total	304	38	342	201	45	246
Mean	5.2	0.6	2.9	4.3	0.9	2.6
Range	0-12	0-3	0-12	0-15	0-3	0-15

Table II. *The number of colon crypts in asymmetric and in symmetric fission in endoscopic biopsies from 80 Swedish patients with inflammatory bowel disease: in 40 patients with ulcerative colitis and in 40 with Crohn colitis.*

	No. of crypts in asymmetric and in symmetric fission in 40 endoscopic biopsies in patients with ulcerative colitis			No. of crypts in asymmetric and in symmetric fission in 40 endoscopic biopsies in patients with Crohn colitis			Total
	No. of asymmetric crypt in fission	No. of symmetric crypt in fission	Total	No. of asymmetric crypt in fission	No. of symmetric crypt in fission	Total	
Total	166	30	196	130	27	157	353
Mean	3.8	0.8	4.9	3.3	0.7	3.9	4.4
Range	0-7	0-2	1-7	0-7	0-2	1-7	0-7

5.2/biopsy) were found in UCs, and 201 (39.8%, 4.3/biopsy) in CCs (Table I). The frequency between CAFs in UC and CAFs in CC was non-significant (p-value=0.15272).

Discussion

The results of this investigation showed that the majority of the biopsies sampled from endoscopically affected areas in the sigmoid colon of German patients with IBD, harbored CFs, most of them being CAFs. The relatively high frequency of CF in colon biopsies in IBD contrasts with the rare occurrence of CF in the normal colon of adults and with the total absence of CAF in healthy individuals (1-4).

CSFs are the results of a homeostatic compensatory mechanism of boosted crypt production in mucosal areas previously occupied by chronic inflammation (2, 9). Although the possible significance of CAF could not be assessed from these studies, their occurrence in the colonic mucosa of IBD patients appears to suggest a genuine pathologic aberration in cryptogenesis.

In the light of these results, one crucial question appears to be relevant: Are CAFs in IBD patients generated by the protracted active chronic inflammation? In this context, studies carried out on sigmoid colon specimens removed for protracted severe diverticulitis or adenocarcinomas (that also exhibited severe diverticulitis) by Goldstein and Ahmad (12) disclosed that despite ongoing severe chronic inflammation, the colonic crypts retained their normal shape. In fact, neither CF nor CAF were found. Their findings (12), strongly suggest that the ongoing chronic inflammation per se does not encourage the development of CF; hence, causes other than chronic inflammation might be responsible for the evolution of CF (including CAF) in German patients with IBD.

In the Swedish study (9), one unselected endoscopic biopsy from endoscopic affected areas in the colon was also investigated in the 80 IBD patients (40 with UC and 40 with CCs). Table II shows that out of 196 CFs in Swedish UC patients, 84.7% were CAFs, and the remaining 15.3% were CSFs. Table II also shows that out of the 353 CFs in Swedish CCs patients, 83.9% were CAFs and the remaining 16.1%, CSF, p=00634).

Thus, despite that German and Swedish populations dwell in disparate geographical areas, and are being subjected to different ecological factors, the proportions of CSF and CAF are similar, strongly suggesting that CF and in particular CAF, develop in IBD independently of the local environmental conditions in the two regions.

In conclusion, the present findings in German patients with IBD, were similar to those previously reported in endoscopic biopsies in Swedish patients with IBD (9). It is not inconceivable that the occurrence of CSF and CAF in German and Swedish IBD patients may be similar to that of IBD patients in other countries.

Conflicts of Interest

The Authors have no conflicts of interest to declare regarding this study.

Authors' Contributions

CAR was responsible for the concept and design of the study, the review of the scanned sections, the analysis and interpretation of data, and wrote the original draft. CL-S and MV scanned sections with a Nanozoomer S360, making them available for review *via* web interface. They also revised the manuscript.

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