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Flat Serrated Adenomas and Flat Tubular Adenomas of the Colorectal Mucosa: Differences in the Pattern of Cell Proliferation

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In the present work we have investigated the cell proliferation pattern of flat serrated adenomas and flat tubular adenomas. For this purpose tissue sections from 23 consecutive flat serrated adenomas and 22 consecutive flat tubular adenomas of the colorectal mucosa were challenged with MIB1, a monoclonal antibody directed against a proliferation-related antigen. The results (including semi-quantitative studies) demonstrated that, whereas flat serrated adenomas had a high cell proliferation at the lower part of the crypts, flat tubular adenomas had a high cell proliferation in the upper part of the crypts. In serrated adenomas with invasive adenocarcinoma, high cell proliferation was demonstrated both at the lower portion of the crypts and in the subjacent submucosa. This suggests that the cells of the lower portion of the crypts in serrated adenomas are truely neoplastic, with the capacity to evolve into invasive growth. The difference in cell proliferation betweeen the two types of flat lesions reported here is a new argument in favor of the classification of flat serrated adenomas as a novel and independent type of neoplastic change of the colorectal mucosa.

Key words: Flat neoplasia — Serrated adenoma — Colorectal mucosa

In recent years, Japanese endoscopists and pathologists have focused particular attention on the occurrence of flat neoplastic lesions (flat adenomas and flat adenocarcinomas) in the colorectal mucosa.[1–4] Their investigations have demonstrated that, in addition to the generally accepted pathway of colorectal carcinogenesis from exophytic adenomas, another no less important pathway exists, namely from flat adenomas.[5–7]

Following the colonoscopic procedure recommended by Japanese authors, flat mucosal colorectal lesions have been endoscopically detected at this hospital for the past 3 years. The histological examination of those lesions has demonstrated that flat adenomas and flat adenocarcinomas also occur in the colorectal mucosa of Swedish patients.

Recently, while examining sections from flat mucosal lesions we noticed the presence of an apparently novel histologic phenotype.[8] The novel flat lesion had scalloped epithelial infoldings in the slopes of the crypts of Lieberkhun, the basal aspect of this lesion being carpeted by dysplastic cells. We designated that lesion flat serrated adenoma[9] to distinguish it from the exophytic serrated adenoma which was described by Goldman *et al.*[10] Flat serrated adenomas differ histologically from flat tubular adenomas inasmuch as the dysplasia in the former originates at the base of the crypts and progresses upwards along the serrated slopes of the crypts, whereas in flat adenomas, the dysplastic epithelium is initially seen at the luminal aspect of the mucosa and progresses downwards along the straight slopes of the crypts. Preliminary results indicate that flat serrated adenoma may, like flat adenoma, evolve into invasive carcinoma.[8]

The possibility that the proliferation pattern in flat serrated adenomas could differ from that in flat adenomas was explored in the present work. For this purpose, the cell proliferation marker MIB1 was used.

MATERIALS AND METHODS

A total of 45 flat colorectal lesions were investigated. Sections (4 μm) were obtained from paraffin blocks and treated with the standard avidin-biotin-peroxidase complex (ABC) technique (Vector, Elite kit cat. PK-6100). Deparaffinized, rehydrated sections were pretreated for MIB1 (Immunotechn, S.A., cat. 0506), in citrate buffer (pH 6) in a microwave oven twice for 5 min. After pretreatment and rinsing, endogenous peroxidase was blocked with H_2O_2-methanol 1:5 for 30 min. Sections were then rinsed in buffer and incubated with blocking normal horse serum for 20 min. After draining of the excess serum, sections were incubated with the monoclonal primary antibody at different dilutions. The most appropriate dilution was found to be 1:150. The preparations were incubated for 12 h at 8°C. A biotinylated antimouse IgG serum was used as a secondary antibody, and was followed by the ABC. The peroxidase reaction was achieved by using diaminobenzidine tetrahydrochloride, 0.6 mg/ml, with 0.03% H_2O_2 for 6 min. Tris-phosphate-buffered saline (pH 7.6) was used for rinsing between steps. Counterstaining was not done.

Definitions Serrated adenomas were classified as flat following a modification of the criteria applied for flat

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tubular adenomas by Muto *et al.*[2] and Wolber and Owen.[11] At histology, lesions lacked an exophytic polypoid configuration and consisted of slightly elevated mucosal plaques never greater than two times the thickness of the adjacent nondysplastic mucosal segments. Serrated adenomas surpassing twice the thickness of the adjacent nondysplastic mucosa were regarded as exophytic and therefore not included in this work.

The epithelium covering the slopes of the crypts of Lieberkuhn showed scalloped infoldings and the dysplastic cells were usually found at the bottom of the crypts.[8] Depending upon the degree of dysplasia within the epithelium, flat serrated neoplasias were divided into those with: 1) low-grade dysplasia, when the dysplastic cells were present in the deeper half of the epithelium and 2) high-grade dysplasia, when the dysplastic cells were even found in the upper half of the epithelium. Invasive flat serrated adenocarcinomas were considered to be those flat serrated lesions having glands in the submucosa furnished with neoplastic cells.

Semi-quantitative studies of MIB1 were carried out as follows. With the aid of an ocular micrometric scale, the

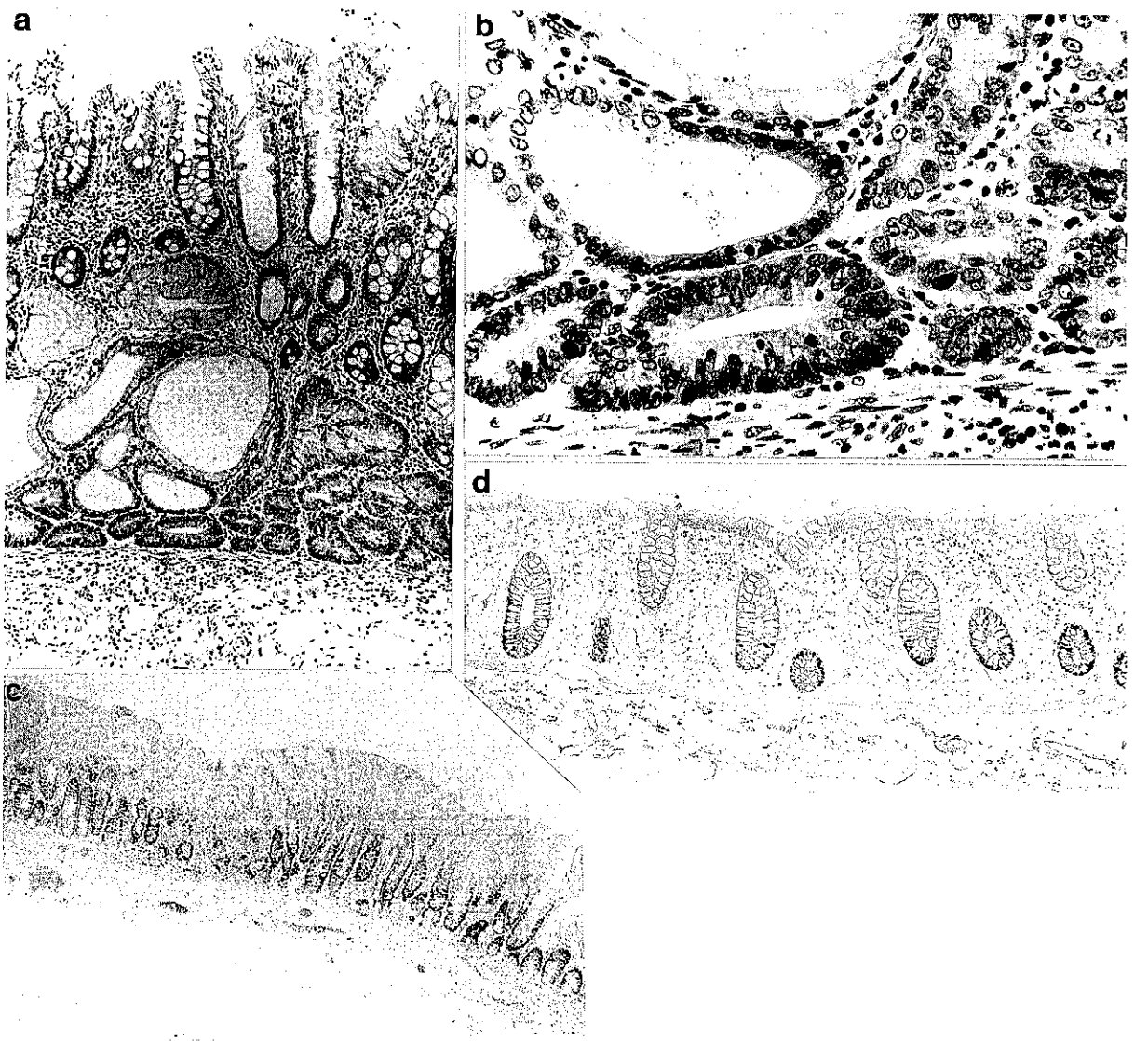

Fig. 1. a) Flat serrated adenoma of the colon. Note serrated structures in the upper part of the crypts (hematoxylin and eosin, ×100). b) Detail from a) showing dysplastic cells in the lower part of the crypts (hematoxylin and eosin, ×250). c) Flat serrated adenoma incubated with MIB1, showing proliferation in the lower portion of the crypts. Note unlabeled epithelium on top (MIB1, ×40). d) MIB1 in normal colonic mucosa showing slight labeling at the basal aspect of the crypts (MIB1, ×65).

epithelium was divided into two halves: a lower mid-basal portion (LMBP) and an upper mid-superficial portion (UMSP). The occurrence of cells expressing MIB1 in each of the two compartments was graded as +, ++, or +++, corresponding to slightly, moderately or intensely positive staining, respectively.

RESULTS

The reasons for colonoscopy in the present series were: altered bowel habits, abdominal pain, previous colorectal polyps or carcinoma, positive blood in the stools and family cancer history. The results of the localization, size and endoscopic findings in 54 flat serrated adenomas have been reported elsewhere.[12] Of the 45 flat neoplastic lesions reported here, 23 were flat serrated adenomas and 22 flat tubular adenomas. Fifteen of the 23 flat serrated neoplasias occurred in males (i.e. 65.6%) and the remaining 8 in females. The majority of the flat serrated neoplasias were located in the colon sigmoideum and rectum (21/23=91.3%). The mean size of the detected lesions was 3.5 mm (range 2–12 mm). The pit pattern demonstrated round, rather large and stellate pits, similar to the pattern of hyperplastic polyps.[12]

Topographic distribution of cell proliferation in flat adenomas and in flat serrated adenomas In flat serrated adenomas the bulk of proliferation occurred in the lower portion of the crypts (Fig. 1) in all 23 lesions investigated. In contrast, the cell proliferation in the 22 flat tubular adenomas occurred mainly in the superficial part of the dysplastic lesions (Fig. 2). Invasive adenocarcinomas arising in flat serrated adenomas were present in 2 of the 23 specimens. Fig. 3 shows that MIB1 was intensively positive in areas of adenocarcinoma invading the submucosa. That figure also shows that despite inva-

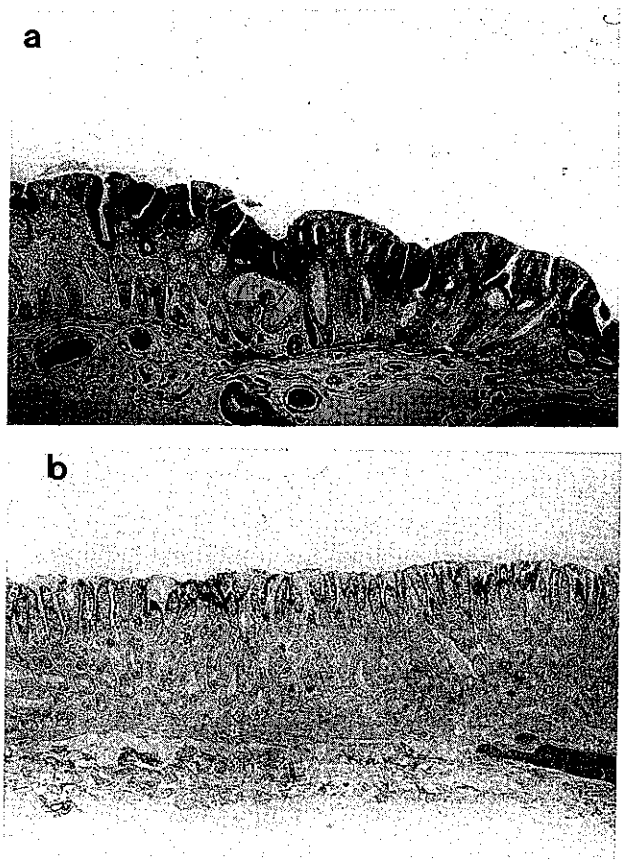

Fig. 2. a) Flat tubular adenoma of the colon stained with hematoxylin and eosin (×40). b) Flat tubular adenoma of the colon incubated with MIB1. Note proliferation in the upper part of the crypts (MIB1, ×40).

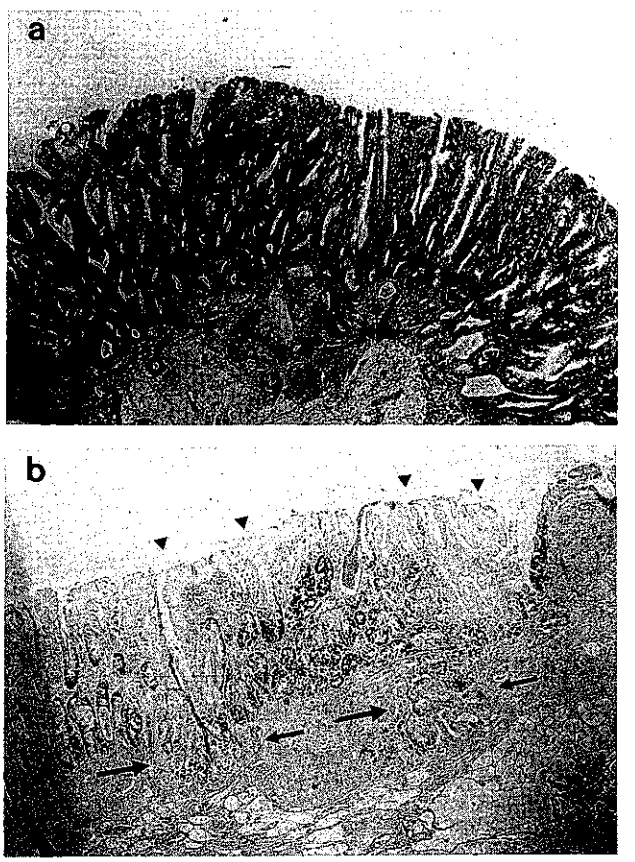

Fig. 3. a) Flat serrated adenoma of the colon with cell clusters invading the submucosal layer at arrows (hematoxylin and eosin, ×40). b) Flat serrated adenoma of the colon with invasive carcinoma incubated with MIB1. Proliferation is seen at the lower portion of the adenoma and in the two invading areas in the submucosa at the arrows (MIB1, ×40). The upper part of the crypts contains only occasional proliferating cells (arrowheads).

sive growth, the upper part of the crypts of the serrated adenomas had only occasional proliferating cells.

Results of semiquantitative studies are shown in Table I. It is seen that in flat serrated adenomas MIB1 was intensively positive (+++) in the LMBP in all 23 lesions and that in 2 of them MIB1 was slightly positive (+) even in the UMSP. In flat tubular adenomas the reverse was found: MIB1 was intensively positive (+++) in the UMSP in all 22 adenomas and only slightly positive (+) in the LMBP in 2 of them. The table also shows that in the normal colorectal mucosa of 12 control cases, the MIB1 was slightly to moderately positive (+~++) in the LMBP, whereas the UMSP was usually unlabeled. The difference in LMBP-MIB1 expression between flat tubular adenomas and flat serrated adenomas and normal colorectal mucosa was significant ($P<0.001$) as judged by use of the non-parametric Wilcoxon signed-rank test.

Table I. MIB1 Expression in the Upper-mid Superficial Portion of the Crypts (UMSP) and in the Lower-mid Basal Portion of the Crypts (LMBP) in Flat Serrated Adenomas and Flat Tubular Adenomas

Cases	MIB1 expression	Flat serrated adenoma (n=23)	Flat tubular adenoma (n=22)	Colorectal mucosa of normal donors (n=12)
UMSP	−	21	0	0
	+	2	0	0
	++	0	0	0
	+++	0	22	0
LMBP	−	0	20	0
	+	0	2	12
	++	0	0	0
	+++	23	0	0

DISCUSSION

Cell proliferation has been investigated for many years to gain knowledge on the growth fraction of tissues.[13–15] Ki-67 monoclonal antibody has been recently tested as a proliferation-associated marker.[16, 17] The drawback of Ki-67 is that it requires unfixed material and frozen sections have to be examined.

Using recombinant parts of the Ki-67 molecule as immunogens new monoclonal antibodies have been raised. One of them, MIB1,[18, 19] was found to be useful for studying the growth fraction in formalin-fixed tissues. Thus, MIB1 can be applied to paraffinized archival material to study cell proliferation. In previous publications we reported on histomorphological differences between flat tubular adenomas and flat serrated adenomas of the colorectal mucosa.[8, 9] In the present work, a difference in the pattern of cell proliferation was demonstrated between the two lesions. Whereas flat serrated adenomas showed intense cell proliferation in the lower portion of the crypts of Lieberkhun, flat adenomas proliferated mainly in the upper portion of the crypts. In serrated adenomas with invasive adenocarcinoma, intense cell labeling was detected not only in the lower portion of the crypts but also in the subjacent submucosa. This finding suggests that the cells of the base of the crypts in serrated adenomas may acquire the capability of independent growth. In summary, the results demonstrate that there are differences between the proliferation pattern of flat serrated adenomas on the one hand and that of flat tubular adenomas on the other. This difference in cell proliferation is a new argument in favor of the classification of flat serrated adenomas[8] as a novel and independent phenotype of neoplastic lesion of the colorectal mucosa.

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