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Leuchtenberger Bodies in Flat Adenomas of the Colorectal Mucosa: A Comparison between Japanese and Swedish Patients

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The dysplastic epithelium of flat colorectal adenomas was found to contain DNA inclusion granules, known as Leuchtenberger bodies (LB). Hematoxylin and eosin-stained sections of 263 colorectal flat neoplastic lesions (i.e., flat adenomas and flat adenocarcinomas) collected in Japan and Sweden were scrutinized for the presence of such intraepithelial inclusions. LB were recorded in 87.1% of the 263 flat colorectal neoplastic lesions. The frequency of flat colorectal lesions having LB was similar in Japan (160 of 178 or 89.8%) to that in Sweden (69 of 85 flat lesions or 81.2%), suggesting that the occurrence of LB may not be influenced by race or environment. Direct immunoperoxidase detection of nuclear DNA fragmentation and transmission electron microscopy studies indicated that the inclusions contained apoptotic material. The semiquantitative assessment of histochemically labeled apoptotic granules indicated that the number of lesions having moderate to large numbers of apoptotic granules (++/+++) was higher in flat adenomas with high-grade dysplasia, suggesting that the occurrence of these granules may be related to the degree of severity of the dysplastic process. A better knowledge of LB may improve our understanding of the possible relationship between apoptosis, host immune response and carcinogenesis in flat colorectal adenomas.

Key words: Flat colorectal adenoma — Apoptosis

Forty years ago, Leuchtenberger reported the occurrence of inclusion bodies in solitary polyps of the rectal mucosa.[1)] Subsequently similar epithelial inclusions were found in rectal polypoid tumors of familial hereditary type.[2)] Leuchtenberger *et al.* demonstrated that the bodies contained DNA and speculated that they represented intracytoplasmatic virus particles.[2)] Other authors later suggested that Leuchtenberger bodies (LB) were derived from dead lymphocytes.[3, 4)] In a previous study,[5)] we investigated the occurrence of LB in exophytic colorectal adenomas in hematoxylin and eosin (HE)-stained sections, in Feulgen-stained sections (specific for DNA) and by transmission electron microscopy (TEM). The LB were more frequently present in exophytic adenomas from patients with familial adenomatous polyposis (FAP) than in those from patients with a non-familial trait. We concluded[5)] that LB were not karyorrhectic nuclear fragments from necrotic cells, but nuclear-cytoplasmic granules sequestered into membrane-bound apoptotic bodies[6)] in the cytoplasm of dysplastic cells and of interspersed macrophages.

Earlier studies in Swedish patients had indicated that the frequency of LB increased with increasing degree of epithelial dysplasia.[6)] Since the proportion of flat adenomas with high-grade dysplasia and carcinoma is higher in Japanese patients than in Swedish patients,[7)] it was considered of interest to investigate the frequency of LB in flat colorectal neoplasias in patients from both countries.

MATERIALS AND METHODS

The materials were 263 consecutive strip biopsies carrying a flat adenoma of the colorectal mucosa: 137 were collected at Tokyo Medical and Dental University (TMDU), 41 at the Cancer Institute (CI), Tokyo and the remaining 85 at the Karolinska Institute (KI), Stockholm. The presence of LB and of lymphoid follicle aggregates underneath the adenoma, usually in the subjacent submucosa, was recorded. Ten flat adenomas were stained with Feulgen stain to identify DNA.

Definitions All adenomas reviewed in this survey had been diagnosed as flat mucosal lesions by endoscopists. All colonoscopically excised lesions which had been clinically described as pedunculated or exophytic polyps were excluded, regardless of the histological features. Adenomas were classified as flat following the criteria of Muto *et al.*[8)] and Wolber and Owen.[9)] Histologically, the lesions lacked an exophytic polypoid configuration and consisted of slightly elevated dysplastic mucosal plaques never greater than two times the thickness of the adjacent non-dysplastic mucosal segment (Fig. 1). The lesions showed, at least at the periphery, radial extension of the dysplastic epithelium in the superficial lumenal portion of the mucosa without vertical extension of the dysplastic

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epithelium to the base of the crypts. The histologic grade of epithelial dysplasia was determined by the following criteria[7]:

Low-grade dysplasia (LGD): when the dysplastic cells were present in the deeper half of the epithelium.

High-grade dysplasia (HGD): when the dysplastic cells were found in the superficial half of the epithelium in at least three tubules or crypts, the normal configuration of the crypts of Lieberkhun being retained.

Intramucosal carcinoma: had the same dysplastic alterations as HGD but the crypts were no longer parallel: there was a distortion of the structure of the mucosa with molding and budding of the crypts.

Invasive adenocarcinoma: when neoplastic cells were found in the submucosal tissues.

Apoptosis: HE- (Fig. 2) or Feulgen-positive granules (Fig. 3) or granules revealed by direct immunoperoxidase detection of DNA nick-end extension[6] (Fig. 4). The granules were usually found in the basal aspect of the cytoplasm of dysplastic cells or in interspersed macrophages. Some of the granules had a hematoxylin- or Feulgen-stained core surrounded by an eosinophilic cytoplasmic rim. The latter structures could be readily discerned at TEM (Fig. 5).

Specific labeling of nuclear DNA fragmentation Sections from 20 flat adenomas were tested for apoptotic material. For this purpose, the immunoperoxidase detection of digoxigenin-labeled genomic DNA was assessed in thin sections of formalin-fixed tissues using the ApopTag *in*

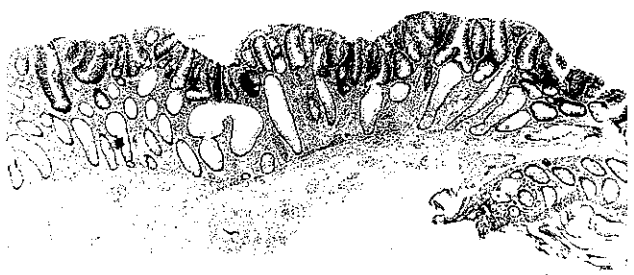

Fig. 1. Flat adenoma of the human colon (hematoxylin and eosin, ×25).

Fig. 3. Feulgen-stained flat adenoma of the colon demonstrating that the granules are Feulgen-positive (i.e., DNA) (hematoxylin and eosin, ×180).

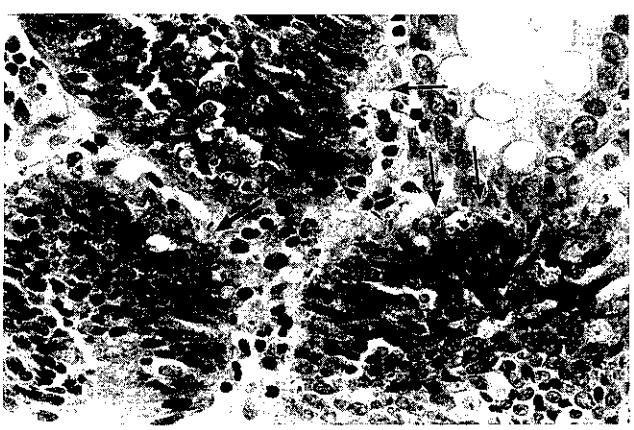

Fig. 2. Detail of a flat adenoma showing intraepithelial Leuchtenberger bodies at the basal aspect of the dysplastic epithelium (hematoxylin and eosin, ×300).

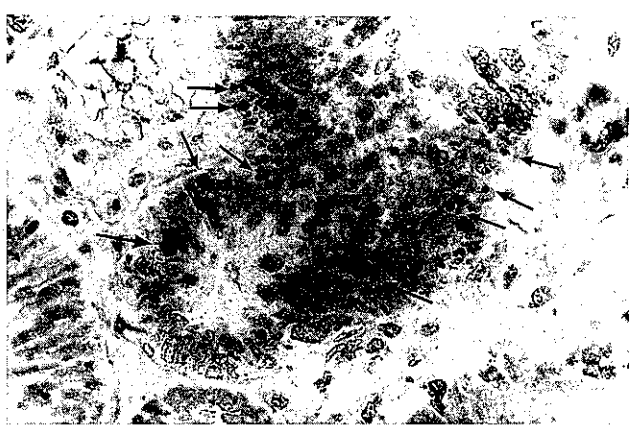

Fig. 4. Immunohistochemical staining to demonstrate apoptosis-positive granules (ApopTag *in situ* without counterstain, ×300).

situ Apoptosis Detection Kit (Oncor, Gaithersburg, MD). Reactions were checked in the entire lesion using a ×10 ocular and a ×40 objective.

Semiquantitative assessment of apoptotic granules The presence of occasional apoptotic (i.e., ApopTag-positive) granules was subjectively graded as (+), (++) or (+++). Absence of such bodies was recorded as 0.

Fig. 5. Transmission electron microscopy of a flat adenoma of the colon. Note intraepithelial granules at the basal aspect of the epithelium and macrophages with cellular debris (transmission electron microscopy, ×3000).

Other techniques Two flat adenomas (from a Swedish patient showing LB in HE-stained sections) were processed for TEM, as described elsewhere for exophytic colonic adenomas.[5] Adjacent sections were challenged with epithelial immunohistochemical markers (cytokeratin MNF 116 and epithelial membrane antigen EMA).

Statistical analysis Differences were measured using Fisher's exact test. Probability values of $P<0.05$ were regarded as statistically significant.

RESULTS

Histologic findings The results recorded in 263 flat neoplasia of the colorectal mucosa are shown in Table I. Of the 178 flat neoplastic lesions reviewed in Japan, 57 (32.0%) had LGD, 101 (56.7%) had HGD, 5 (2.8%) were intramucosal carcinomas and the remaining 15 (8.4%) were invasive carcinomas. Of the 85 flat neoplastic lesions in Sweden, 50 (58.8%) were flat adenomas with LGD, 32 (37.6%) showed HGD and the remaining 3 were invasive carcinomas (3.5%).

LB in flat adenomas The results in Table I show that of the 263 flat neoplasias investigated, 229 or 87.1% had LB. The percent of flat lesions with LB was somewhat lower in the KI material, but the difference from the Japanese material was non-significant ($P<0.6$). Table I also shows that the percent of flat adenomas with LGD and with HGD having LB was similar at the 3 institutions. The difference between the occurrence of LB in LGD on the one hand and of HGD-carcinoma on the other was significant ($P<0.01$). The percent of flat adenomas with intramucosal carcinoma having LB was similar at the 2 Japanese centers. The difference between the occurrence of those granules in LGD and in invasive carcinoma was, however, non-significant. Of the cases

Table I. The Percent of Lesions with Leuchtenberger Bodies (LB) in 263 Flat Colorectal Neoplasias : in 178 Japanese and 85 Swedish Patients

Histology	No. of lesions with LB bodies			
	TDMU	CI	KI	Total
Low-grade dysplasia	41/50	6/7	38/50	85/107 (79.45%)
High-grade dysplasia	73/78	23/23	29/32	125/133* (93.9%)
Intramucosal carcinoma	3/3	2/2	—	5/5 (100.0%)
Invasive adenocarcinoma	6/6	6/9	2/3	14/18 (77.8%)
All	123/137 (89.8%)	37/41 (90.2%)	69/85 (81.2%)	229/263 (87.1%)

TDMU, Tokyo Medical and Dental University; CI, Cancer Institute; KI, Karolinska Institute.
* The number of flat lesions with high-grade dysplasia and LB was significantly higher ($P<0.01$) than that of flat lesions with low-grade dysplasia and LB.

Fig. 6. Flat adenoma of the colon. Note central, subjacent lymphoid aggregates (hematoxylin and eosin, ×18).

with invasive carcinoma, the percent of cases having LB was similar at CI and KI. At TMDU the percent of cases having invasive carcinoma and LB was significantly higher ($P<0.01$) than at the other 2 institutions.

Detection of DNA nick-end labeling This method demonstrated the occurrence of apoptotic granules (Fig. 4) in the basal aspect of the dysplastic epithelium, in the extracellular tissue surrounding the basal aspect of the dysplastic epithelium and in interspersed macrophages. The nuclear envelope in some cells also appeared labeled (Fig. 6). The number of apoptotic granules was semiquantitatively estimated in the 20 flat neoplastic lesions from Swedish patients (Table II). From the 12 flat adenomas without (0) or with only occasional (+) apoptotic granules, 3 (25.0%) had HGD. From the 8 flat adenomas with moderate (++) or with high numbers (+++) of apoptotic granules, 6 (75.0%) had HGD. The difference was significant ($P<0.001$).

Subjacent lymphoid follicle satellites. Of the 178 flat neoplastic lesions in Japanese, 70 (39.3%) demonstrated subjacent discrete lymph follicle aggregates (Fig. 6). Similar subjacent lymphoid structures were found in 39 (45.9%) of the 85 flat neoplastic lesions in Swedish patients. In Japanese patients, discrete lymph follicle aggregates were present in 56 (45.5%) of the 123 flat neoplastic colorectal lesions having LB. On the other hand, lymph follicle aggregates were also present in 14 (19.2%) of the 73 lesions lacking LB. In Swedish patients, discrete lymph follicle aggregates were found in 37 (53.6%) of the 69 flat neoplastic colorectal lesions having LB. On the other hand, lymph follicle aggregates were also present in 2 (12.5%) of the 16 lesions lacking LB. The difference between the lymphoid follicle aggregates-LB combination and the lymphoid follicle aggregates-absence of LB combination was significant ($P<0.01$).

Table II. Semiquantitative Assessment of Immunohistochemically (ApopTag) Stained Apoptotic Granules in 20 Flat Colorectal Neoplasias in Swedish Patients with Either Low-grade Dysplasia (LGD) or High-grade Dysplasia (HGD)

Histology	Apoptotic granules				
	0	+	++	+++	Total
LGD	3	6	1	1	11
HGD	1	2	2	4	9
Total	4	8	3	5	20

DISCUSSION

We found that 87.1% of the flat colorectal adenomas from Japanese and Swedish patients contained LB. The frequency of flat neoplastic lesions with such granules was similar in both ethnic groups. Consequently, the occurrence of that phenomenon appears not to be influenced by race or environment in the two disparate geographic regions investigated.

All the morphologic prerequisites of apoptosis were present in the epithelium of many flat adenomas, such as hematoxylin- and Feulgen-positive granules and specific labeling of nuclear DNA fragments (apoptotic granules) of various sizes, as well as membrane-bound (TEM) cytoplasmic remnants in interspersed macrophages.[6] The presence of labeling in the nuclear envelope in some adenomatous cells was considered as an indication that those cells were engaged in the process of apoptosis.[10]

It is the general concensus that LGD in exophytic adenomas antedates HGD.[11] Assuming that the same pathway is followed in flat adenomas, it would appear that the apoptotic granules increased in frequency with increasing "biological age" (i.e., degree of epithelial dysplasia) of the flat adenomas, since flat adenomas with HGD contained more apoptotic granules than flat adenomas with LGD. These results are consistent with those reported by Arai and Kino[12] in exophytic colorectal adenomas; they found that apoptosis was more frequent in adenomas with severe atypia than in those with mild atypia.

Yamagata *et al.*[13] and Minamoto *et al.*[14] have reported that the lower expression of K-*ras* mutation in flat colorectal adenomas correlated poorly with the degree of cellular atypia. Thus, the genetic alterations reported by those authors[13, 14] appear to be unrelated to the phenomenon of apoptosis reported here.

The origin of the apoptotic granules remains enigmatic. Recent studies on cell proliferation in flat adenomas[15, 16] have shown the absence of karyorrhexis in dysplastic cells in flat adenomas. Though LB were negative for immunohistochemical epithelial markers, an epithelial origin of the apoptotic granules can not be totally

rejected. Interestingly, the apoptotic granules (LB) accumulated in the cytoplasm of dysplastic cells, usually in the basal aspect of the nuclei. If those granules had originated in epithelial cells, a more haphazard distribution within the cytoplasm might be expected.

It has been suggested that the LB derive from intraepithelial lymphocytes.[3–5] In this respect it should be mentioned that intraepithelial lymphocytes were found in some adenomas lacking apoptotic granules. Moreover, the positive correlation between the occurrence of LB and satellite lymphoid tissues in many flat tubular adenomas appears to substantiate that possibility. Nevertheless, subjacent lymphoid aggregates may only mirror a reaction to the apoptotic events occurring in the dysplastic epithelium higher up. Thus, we can not say whether the apoptotic granules were derived from dysplastic adenomatous cells or from intraepithelial lymphocytes.

Recently, Pasricha *et al.*[17] found that treatment with Sulindac induced the regression of exophytic adenomas in patients with familial adenomatous polyposis. The regression was a consequence of increased apoptosis and not reduced cell proliferation. These results in exophytic adenomas indicate that apoptosis may be an important biologic phenomenon in colorectal neoplasias. Increased knowledge on LB should improve our understanding of the possible relationship between apoptosis, host immune response and carcinogenesis in flat colorectal adenomas.

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REFERENCES

1) Leuchtenberger, C. Cytoplasmic "inclusion bodies" containing desoxyribose nucleic acid (DNA) in cells of human rectal polyps. *Lab. Invest.*, **3**, 132–142 (1954).
2) Leuchtenberger, C., Leuchtenberger, R. and Lieb, E. Studies of the cytoplasmatic inclusions containing desoxyribose nucleic acid (DNA) in human rectal polypoid tumors including the familial hereditary type. *Acta Genet.*, **6**, 291–297 (1956)
3) Fisher, E. R. and Sharkey, D. A. The ultrastructure of colonic polyps and cancer with special reference to the epithelial inclusion bodies of Leuchtenberger. *Cancer*, **15**, 160–170 (1962).
4) Walb, D. and Sandritter, W. Inclusion bodies in rectal polyps. *Arch. Pathol.*, **78**, 104–107 (1964).
5) Rubio, C. A., Alm, T., Aly, A. and Poppen, B. Intraepithelial bodies in colorectal adenomas : Leuchtenberger bodies revisited. *Dis. Colon Rectum*, **34**, 47–50 (1991).
6) Stewart, B. W. Mechanisms of apoptosis : integration of genetic, biochemical and cellular indicators. *J. Natl. Cancer Inst.*, **86**, 1286–1295 (1994).
7) Rubio, C. A., Kumagai, J., Kanamori, M., Yanagisawa, A., Nakamura, K. and Kato, Y. Flat adenomas and flat adenocarcinomas of the colorectal mucosa in Japanese and Swedish patients : a comparative histologic study. *Dis. Colon Rectum*, **38**, 1075–1079 (1995).
8) Muto, T., Kamiya, T., Sawada, T., Konishi, F., Sugihara, K., Kubota, Y., Adachi, M., Agawa, S., Saito, Y., Morioka, Y. and Tanprayoon, T. Small "flat adenoma" of the bowel with special reference to its clinicopathological features. *Dis. Colon Rectum*, **28**, 847–851 (1985).
9) Wolber, R. and Owen, D. A. Flat adenomas of the colon. *Hum. Pathol.*, **22**, 70–75 (1991).
10) Gavrieli, Y., Sherman, Y. and Ben-Sasson, S. A. Identification of programmed cell death *in situ* via specific labeling of nuclear DNA fragmentation. *J. Cell Biol.*, **119**, 493–501 (1992).
11) Muto, T., Bussey, H. J. and Morson, B. C. The evolution of cancer of the colon and rectum. *Cancer*, **36**, 2251–2270 (1975).
12) Arai, T. and Kino, I. Role of apoptosis in modulation of the growth of human colorectal tubular and villous adenomas. *J. Pathol.*, **176**, 37–44 (1995).
13) Yamagata, S., Muto, T., Uchida, Y., Masaki, T., Sawada, T., Tsuno, N. and Hirooka, T. Lower incidence of K-*ras* codon 12 mutation in flat colorectal adenomas than in polypoid adenomas. *Jpn. J. Cancer Res.*, **85**, 147–151 (1994).
14) Minamoto, T., Sawaguchi, K., Mai, M., Yamashita, N., Sugimura, T. and Esumi, H. Infrequent K-*ras* activation in superficial-type (flat) colorectal adenomas and adenocarcinomas. *Cancer Res.*, **54**, 2841–2844 (1994).
15) Rubio, C. A. and Rodensjö, M. Flat serrated adenomas and flat tubular adenomas of the colorectal mucosa: differences in the pattern of cell proliferation. *Jpn. J. Cancer Res.*, **86**, 756–760 (1995).
16) Rubio, C. S. and Rodensjö, M. p53 Overexpression in flat serrated adenomas and flat tubular adenomas of the colorectal mucosa. *J. Cancer Res. Clin. Oncol.*, **121**, 571–576 (1995).
17) Pasricha, P., Bedi, A., O'Connor, K., Rashid, A., Akhtar, A., Zahurac, M., Piantadosi, S., Hamilton, S. and Giardello, M. The effect of Sulindac on colorectal proliferation and apoptosis in familial adenomatous polyposis. *Gastroenterology*, **109**, 994–998 (1995).