Expression of Apoptosis-related Proteins in Barrett's Metaplasia-Dysplasia-Carcinoma Sequence: A Switch to a More Resistant Phenotype

C. J. VAN DER WOUDE, MD, P. L. M. JANSEN, MD, PHD, A. T. G. M. TIEBOSCH, MD, PHD, A. BEUVING, M. HOMAN, J. H. KLEIBEUKER, MD, PHD, AND H. MOSHAGE, MD, PHD

Barrett's esophagus, or colomnar-lined esophagus (CLE), is a premalignant disorder in which the stratified squamous epithelium is replaced by metaplastic epithelium. To gain more insight into the process of carcinogenesis in CLE, we studied several factors involved in the apoptotic pathway in biopsies with gastric metaplasia (GM), intestinal metaplasia (IM), dysplasia, and/or adenocarcinoma. Immunohistochemistry was performed for Fas, Bcl-2, Bax, Bcl-xl, inducible nitric oxide synthase (iNOS), and cyclooxagenase 2 (COX-2). Fas staining was positive in the epithelium of all biopsies from patients with CLE but negative in normal gastric mucosa. Fas staining was positive in all tumor cells of the 8 cases containing adenocarcinoma. Bcl-2 was positive in lamina propria immune cells of all specimens. Bax staining was positive in the epithelium of all biopsies, including tumor cells. Bcl-xl was positive in dysplasia and tumor cells, but negative in 8 of 17 biopsies containing IM. iNOS was positive in 20 of 21 biopsies with IM and in 4 of 8 dysplasia biopsies. COX-2 was positive in 7 of 8 adenocarcinomas. We conclude that the apoptotic balance in the transformation from IM to adenocarcinoma switches to an antiapoptotic phenotype because of increased Bcl-xl expression and decreased Bax expression. Fas can be used as a marker for the differentiation of gastric mucosa and metaplasia in the esophagus. iNOS is highly positive in CLE-associated intestinal metaplasia. COX-2 is negative in nonmalignant CLE. Therefore, pharmacologic inhibition of COX-2 activity is unlikely to be effective in the preventing CLE-associated adenocarcinoma. There was no clear correlation between iNOS expression and activation of proapoptotic and antiapoptotic genes. HUM PATHOL 33:686-692. Copyright 2002, Elsevier Science (USA). All rights reserved.

Key words: **Barrett's esophagus, apoptosis, gastric metaplasia, intestinal metaplasia, dysplasia, adenocarcinoma, iNOS, COX-2, Bax, Fas, Bcl-2, Bcl-xl.**

Abbreviations: **CA, carcinoma; CLE, columnar-lined oesophagus; COX-2, cyclooxygenase 2; iNOS, inducible nitric oxide synthase; GM, gastric metaplasia; IM, intestinal metaplasia; PPI, proton pump inhibitor.**

In Barrett's esophagus, or colomnar-lined esophagus (CLE), the normal stratified squamous epithelium lining the esophagus is replaced by metaplastic columnar epithelium containing goblet cells.[1] This replacement is a risk factor for neoplastic transformation, and there is evidence for the sequential development of adenocarcinoma via intestinal metaplasia and low-grade and high-grade dysplasia.[2,3] Therefore, periodic surveillance endoscopy with multiple biopsies is recommended for CLE patients. Other modalities to evaluate the esophagus for Barrett's metaplasia and impending malignant degeneration have been investigated, but histologic examination remains the gold standard.[4-7] To simplify surveillance, new preventive treatment options are needed.[8-12]

From the Departments of Gastroenterology and Pathology, University Hospital Groningen, The Netherlands. Accepted for publication February 21, 2002.

Supported by an unrestricted grant from AstraZeneca.

Address correspondence and reprint requests to C. J. van der Woude, MD, Department of Gastroenterology, Dr Molewaterplein 40, 3015 GD Rotterdam, The Netherlands.

0046-8177/02/3307-0002$35.00/0
doi:10.1053/hupa.2002.124908

Disturbances in apoptosis are supposed to play an important role in the sequential development of dysplasia and cancer. To gain better insight into these disturbances, we studied the expression of 4 apoptosis-related proteins: Fas, Bcl-2, Bax, and Bcl-xl. We further studied the expression of 2 other closely apoptosis-related proteins: inducible nitric oxide synthase (iNOS) and cyclooxygenase 2 (COX-2). Nitric oxide, produced by iNOS, has been demonstrated to inhibit apoptosis by inhibiting caspase activity. However, chronic exposure to high levels of nitric oxide can also promote apoptosis.[13]

High COX-2 expression has been demonstrated in human colorectal adenomas and in gastric adenocarcinomas.[14,15] Inhibition of COX-2 activity promotes apoptosis and could be a promising modality for chemoprevention of these tumors, as has been shown in patients with familial adenomatous polyposis.[16,17]

MATERIALS AND METHODS

Patient Selection and Tissue Collection

We studied tissue samples taken from patients who participated in an endoscopy surveillance program between January 1998 and December 2000 at the University Hospital

TABLE 1. Immunohistochemistry Methods

Protein	Section	Antigen retrieval	Primary antibody
Fas	Paraffin	2× 15 minutes at 98°C in 1 mM EDTA, pH 6.0	Mouse monoclonal at 1:400; Upstate Biotechnology, Lake Placid, NY
Bcl-xl	Paraffin	2×15 minutes at 98°C in 0.1 M Tris HCl, pH 9.0	Mouse monoclonal at 1:100; Zymed Laboratories, South San Francisco, CA
Bax	Paraffin	MW (700 W) 8 minutes in 10 mM citrate, pH 6.0	Mouse monoclonal at 1:400; Santa Cruz Biotecnology, Santa Cruz, CA
Bcl-2	Paraffin	MW (700 W) 8 minutes in 10 mM citrate, pH 6.0	Mouse monoclonal at 1:50; Dako, Glostrup, Denmark
COX-2	Paraffin	2×15 minutes at 98°C in 1 mM EDTA, pH 6.0	Mouse monoclonal at 1:50; Transduction Laboratories,
iNOS	Paraffin	2×15 minutes at 98°C in 1 mM EDTA, pH 6.0	Mouse monoclonal at 1:50; Transduction Laboratories

Groningen. All patients used omeprazole at the time of endoscopy. Samples were stained with hematoxylin and eosin and periodic acid–Schiff. Standard histologic examination was performed on these stained samples, with attention given to the type of metaplasia, presence and degree of inflammation and dysplasia, and presence of adenocarcinoma. The most distal samples from the esophagus of each patient were used in the study. Samples from patients with esophagitis graded according to Savary and Miller[18] during endoscopy and from those with histologically active inflammation graded according to Paull et al[19] were excluded from this study. After exclusion of these samples and samples without metaplasia, dysplasia, or adenocarcinoma, the samples were histologically graded by a single pathologist. Samples were categorized as GM or specialized columnar metaplasia.[19] Dysplasia was scored as absent, indefinite-low grade, or high grade.[20] Different histologic gradations could coexist in a single sample.

Immunohistochemical Analysis

All stainings were performed on deparaffinized 4-μm-thick sections. These sections were cut from formalin-fixed, paraffin-embedded tissues. An overview of the methods is given in Table 1.

Quantitation of Immunoreactivity

The immunohistochemical sections stained with Fas, Bcl-2, Bax, Bcl-xl, iNOS, and COX-2 were scored by 3 different observers for the percentages of epithelial cells stained. In the event of differences in interpretation, the sample was scored again, and a consensus was reached. Absence of staining was scored as 0; 0 to 10% staining, as 1; 11% to 50% staining, as 2; and 51% to 100% staining, as 3. Tissue samples stained with Fas, Bcl-xl, and Bax were also scored for intensity of staining in each individual epithelial cell on a scale of 0 to 3, with 0 being negative; 1, weak; 2, moderate; and 3, strong.

Staining of tumor cells was scored in the same way.

Statistical Analysis

The relationship between grade and intensity in expression of each separate protein on the one hand and the histologic parameters on the other was evaluated using Somers' *d* test. The analysis was performed using Sigmaplot Scientific Software (SPSS, Chicago, IL). A P value <0.05 was considered significant.

RESULTS

Patients

Samples from 28 patients (20 male, 8 female) were included. The age range of these patients was 31 to 86 years (mean, 58). Six samples contained gastric metaplasia (GM), 21 contained intestinal metaplasia (IM), 8 contained indefinite for and low-grade dysplasia, and 6 contained high-grade dysplasia and carcinoma (CA). The adenocarcinoma group was expanded with archival resection material from 4 patients with CLE-associated adenocarcinoma.

Immunohistochemistry (Tables 2 and 3)

Fas Staining in Epithelium and Tumor Cells

Three of 21 tissue samples containing IM, 2 of 8 samples containing dysplasia, and 1 of 10 samples containing CA were not stained, because of a lack of material. Fas staining was present in the epithelium of all tissue samples, including GM (Fig 1A). Tumor cells were all positive (Fig 1B). Fas staining of normal gastric mucosa in patients with GM and in controls was negative (Fig 1C).

The correlation between staining grade and sequence from IM to CA was significant: $r = 0.527$ ($\pm$ 0.126), $P = 0.01$. The correlation between staining

TABLE 2. Staining Intensity of Fas, Bax, and Bcl-xl Expression

	Fas*				Bax†				Bcl-xl§			
Intensity	GM	IM	D	CA	GM	IM	D	CA	GM‖	IM	D	CA
0	0	1	0	0	0	0	0	1		8	0	1
1	0	4	2	0	1	4	4	6		8	1	0
2	3	9	2	3	1	7	2	2		1	4	3
3	3	4	2	6	3	9	2	0		0	3	6

*A significant difference from intestinal metaplasia to cancer.

†A significant difference from intestinal metaplasia to cancer.

§A significant negative difference from intestinal metaplasia to cancer.

‖Not scored.

TABLE 3. Staining Grade of iNOS, Fas, Bax, and Bcl-xl Expression

	iNOS*				Fas†				Bax§				Bcl-xl‖			
Grade	GM	IM	D	CA	GM	IM	D	CA	GM	IM	D	CA	GM#	IM	D	CA
0	6	1	4	6	0	1	0	0	0	0	0	1		8	0	1
1	0	3	4	0	0	5	2	0	0	3	0	0		7	1	0
2	0	14	0	0	2	9	1	0	2	3	0	0		2	3	2
3	0	3	0	0	4	3	3	9	3	14	8	8		0	4	7

*Significant negative difference from intestinal metaplasia to cancer.
†Significant difference from intestinal metaplasia to cancer.
§Not significant difference from intestinal metaplasia to cancer.
‖Significant difference from intestinal metaplasia to cancer.
#Not scored.

intensity and sequence from IM to CA was also significant: $r = 0.329$ ($\pm$ 0.126), $P < 0.001$.

Bcl-2 Staining in Epithelium and Tumor Cells

Bcl-2 staining was negative in epithelium of CLE and was also not present in tumor cells. Lamina propria immune cells showed positive staining (Fig 1D).

Bax Staining in Epithelium and Tumor Cells

Because of a lack of material, 1 tissue sample of the GM, 1 of the IM group, and 1 of the CA group was excluded from the Bax staining series. In all groups, epithelial cells stained positive (Fig 1E). In tumor cells, Bax staining was also clearly positive (Fig 1F).

The correlation between staining grade and sequence from IM to CA was not significant: $r = 0.302$ ($\pm$ 0.197), $P = 0.141$. The correlation between staining intensity in each individual epithelial cell and sequence from IM to CA was significant: $r = -0.443$ ($\pm$ 0.101), $P = 0.001$.

Bcl-xl Staining in Epithelium and Tumor Cells

Because of a lack of material, 4 tissue samples of the IM group were excluded from the Bcl-xl staining series. Staining in dysplasia and tumor cells (Fig 1G) was mostly positive with a strong intensity. The correlation between staining grade and sequence from IM to CA was significant: $r = 0.600$ ($\pm$ 0.085), $P < 0.001$. The correlation between staining intensity and sequence was also significant: $r = 0.600$ ($\pm$ 0.088), $P < 0.001$.

iNOS Staining in Epithelium and Tumor Cells

iNOS staining was intensely positive in the epithelium of IM (Fig 1H). Epithelial staining was positive in 4 of 8 samples containing dysplasia (Fig 1I). GM and tumor cells were negative for iNOS. The correlation between staining grade and sequence from IM to CA was significant: $r = -0.678$ ($\pm$ 0.084), $P < 0.001$.

COX-2 Staining in Epithelium and Tumor Cells

COX-2 expression was not present in the epithelium of CLE and associated dysplasia. Lamina propria immune cells and myofibroblasts showed positive staining (Fig 1J). In the adenocarcinoma group, tumor cells, but not normal epithelium, were positive for COX-2 in 9 of 10 samples. In these biopsies, however, only a minority of tumor cells stained positive (Fig 1K).

DISCUSSION

In CLE, iNOS is highly expressed in IM and in 50% of samples containing dysplasia, but not in CLE-associated adenocarcinoma. All of our samples containing high-grade dysplasia were positive for iNOS, as reported by Wilson et al.[21] However, in contrast to these authors, we did not observe iNOS expression in CLE-associated adenocarcinomas. Nitric oxide, the product of iNOS, is able to inhibit apoptosis in low concentrations, due in part to inhibition of caspase activity.[13] In high concentrations, it can induce apoptosis. We could not detect apoptosis in CLE IM using staining for caspase-cleaved cytokeratin 18 (cytodeath) (data not shown). Whether this means that iNOS inhibits apoptosis in CLE IM remains to be established, because apoptosis was also absent in iNOS-negative CLE dysplasia.

The role of apoptosis in the sequence of IM to adenocarcinoma is not clear.[22,23] Our results suggest that Bcl-2 is not involved in the carcinogenesis of CLE, because only lamina propria immune cells, not the epithelium, showed positive staining. Bax, a proapoptotic member of the Bcl-2 family, was positive in all samples. Although no significant differences in staining grade were observed among the different groups, there was a significant negative correlation between the intensity of Bax staining in each individual epithelial cell and the transformation of IM to adenocarcinoma. According to these observations, the epithelial cells transform into less Bax-positive cells and thus more apoptosis-resistant cells. These results contrast with previous reports[24] that found a positive association between progression to adenocarcinoma in CLE and Bax expression.

Members of the Bcl-2 family play an important role in the regulation of apoptosis. This family contains proapoptotic members (Bax, Bid, Bad, and Bak) and antiapoptotic members (Bcl-2 and Bcl-xl). Bcl-2 proteins regulate the permeability of the mitochondrial membrane. Increased mitochondrial permeability al-

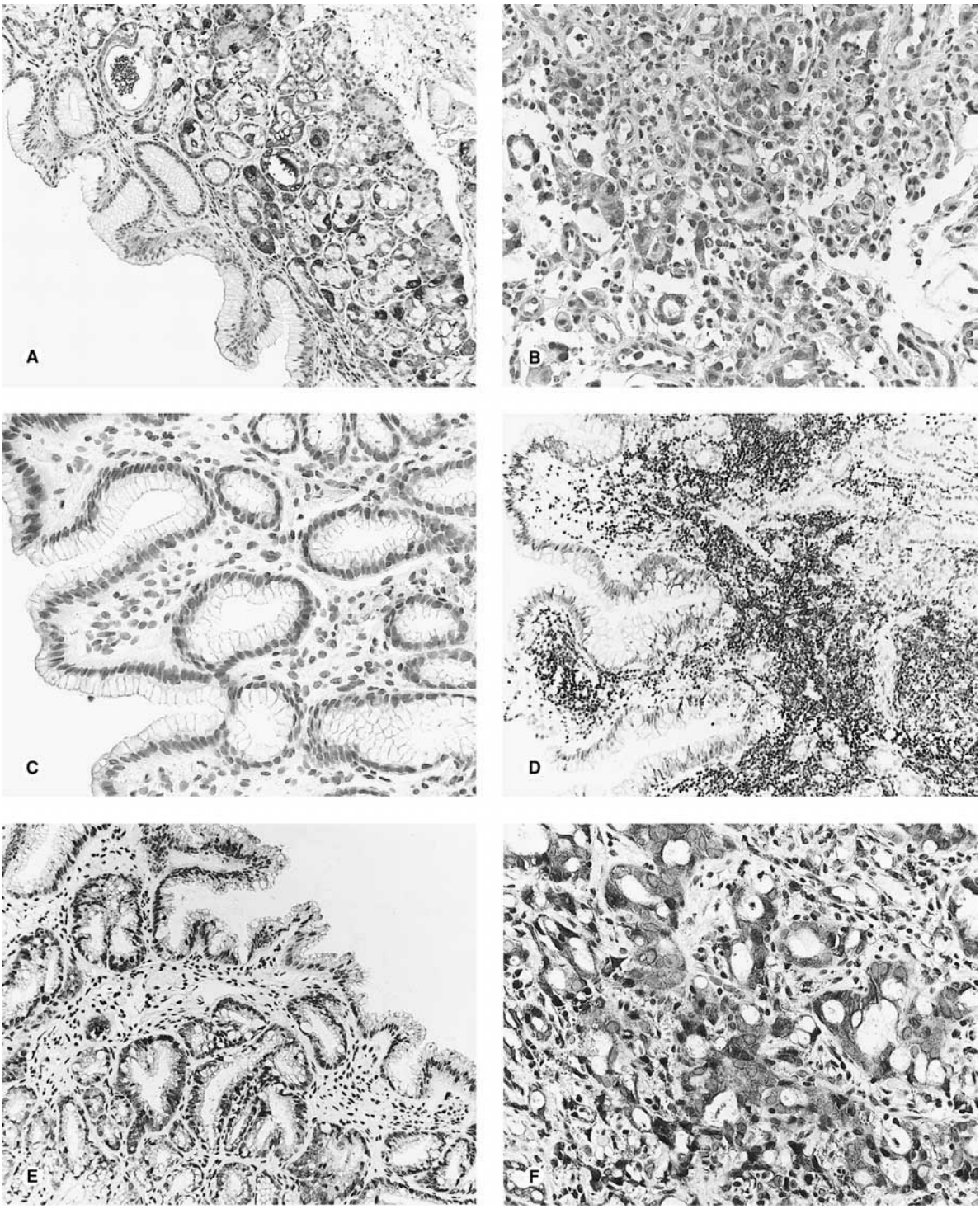

FIGURE 1. Immunohistochemistry results. (A) Fas staining is clearly positive in epithelium of GM. (B) Fas staining is positive in tumor cells. (C) Fas staining in normal gastric mucosa is negative. (D) Bcl-2 staining only positive in lamina propria immune cells. (E) Bax staining is positive in the epithelium of all groups; shown here is IM. (F) Bax positivity in tumor cells.

lows leakage of cytochrome C from mitochondria into cytoplasm, triggering caspase activation and apoptosis. Proapoptotic Bcl-2 proteins increase mitochondrial membrane permeability, whereas antiapoptotic members antagonize the effects of proapoptotic Bcl-2 proteins.[25] The continuous expression of Bax could be triggered by overexpression of mutated p53 (proapoptotic) found in earlier reports.[26,27]

Bcl-xl, an antiapoptotic Bcl-2 family member, was highly positive in dysplasia and tumor cells but not in IM. The increase in Bcl-xl grade and intensity of staining in the transformation from IM to CLE-

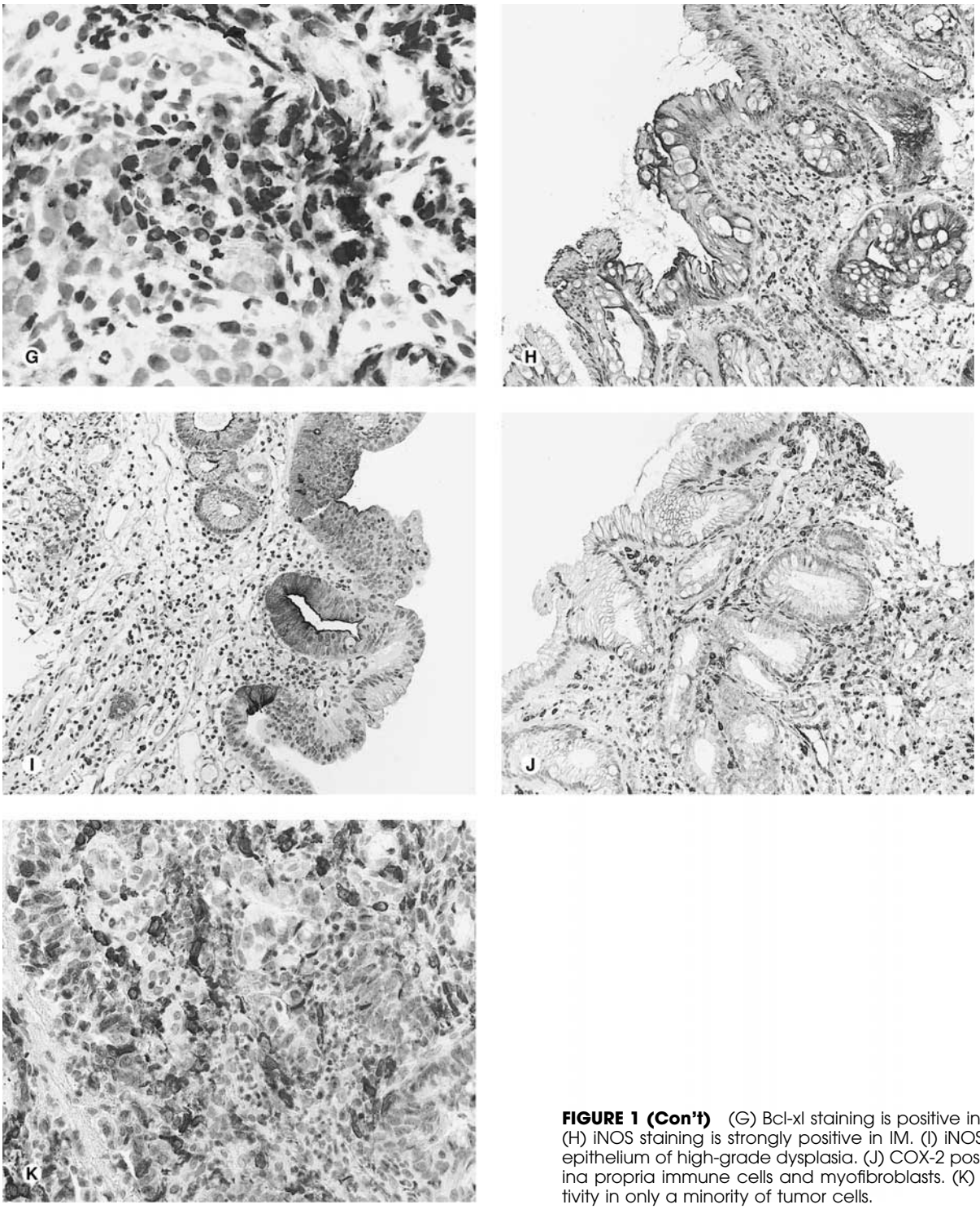

FIGURE 1 (Con't) (G) Bcl-xl staining is positive in tumor cells. (H) iNOS staining is strongly positive in IM. (I) iNOS positivity in epithelium of high-grade dysplasia. (J) COX-2 positivity in lamina propria immune cells and myofibroblasts. (K) COX-2 positivity in only a minority of tumor cells.

associated adenocarcinoma was significant. The reciprocal changes in the expression of Bax and Bcl-xl in the sequence from IM to adenocarcinoma indicate that these cells become increasingly more resistant to apoptotic cell death, giving these cells a survival and proliferation advantage.

Fas is a member of the tumor necrosis factor receptor superfamily. Activation of this receptor by its ligand activates caspase 8 and the apoptotic signal transduction pathway. Fas-expressing cells are vulnerable to Fas ligand–induced cell death. Fas ligand is predominantly expressed by lymphocytes, but can also be expressed by other cells. Therefore, Fas-mediated cell death can occur only when Fas ligand–positive cells are in close proximity to Fas-positive target cells.[28] Fas was present not only in CLE IM, but also in GM of the esophagus. Decreased Fas expression has been reported in CLE. However, in this study Fas staining of

goblet cells was investigated.[29] Previously, Fas expression was not found in normal gastric mucosa,[30] and we confirmed these results. Therefore, Fas expression can be used to differentiate between normal gastric mucosa and GM in the esophagus. The expression of Fas ligand has been reported during malignant transformation of Barrett's metaplasia.[31] However, the simultaneous expression of Fas and Fas ligand does not necessarily lead to apoptotic cell death. Various antiapoptotic mechanisms may exist in Fas/Fas ligand coexpressing cells that protect these cells against apoptosis.[32]

Most CLO-associated adenocarcinomas were COX-2 positive but only in a minority of tumor cells. In IM and dysplasia, COX-2 staining was negative, and only lamina propria immune cells showed COX-2 expression. This contrasts with previous reports,[33,34] although other reports support our findings.[35] Pharmacologic inhibition of COX-2 activity has proven effective in reducing colonic polyp formation in humans. COX-2 staining in this study was negative in the precancerous state in CLE. Our results do not support a role for COX-2 inhibition in the prevention or treatment of Barrett's dysplasia and cancer, and a recent report from Tsibouris et al[36] found no differences in cancer occurence in CLE in the presence or absence of nonsteroidal anti-inflammatory drugs.

All patients in our study were using proton-pump inhibitors (PPI). Peters et al[37] reported that high-dose PPI treatment resulted in partial endoscopic regression of CLE,[37] and effective PPI treatment decreased proliferation in an earlier study.[38] However other factors, such as duodenogastroesophageal reflux, may contribute to the development of CLE. Therefore, the effects of PPI treatment on proliferation in Barrett's esophagus remains unclear. Likewise, nothing is known about the effect of PPI treatment on apoptosis in Barrett's esophagus. Considering the regression of metaplasia and decreased proliferation in Barrett's esophagus of patients using PPIs, a proapoptotic effect of PPI could be hypothesized, but data are lacking.

In conclusion, the apoptotic balance in the transformation from IM to adenocarcinoma switches to an antiapoptotic phenotype due to increased Bcl-xl expression and decreased Bax expression. Most Barrett's esophagus–associated adenocarcinomas are COX-2 positive but in only a minority of tumor cells. COX-2 is not positive in nonmalignant Barrett's esophagus. Therefore, pharmacologic inhibition of COX-2 activity is unlikely to be effective in preventing Barrett's esophagus–associated adenocarcinomas. iNOS is highly positive in IM, and Fas expression can be used as a marker for differentating between normal gastric mucosa and GM in the esophagus.

Acknowledgment: We thank B. Hansen for her help with statistical analysis.

REFERENCES

1. Weinstein WM, Ippoliti AF: The diagnosis of Barrett's esophagus: Goblets, goblets, goblets. Gastrointest Endosc 44:91-95, 1996

2. Levine DS, Haggitt RC, Irvine S, et al: Natural history of high-grade dysplasia in Barrett's esophagus. Gastroenterology 110: A550, 1996

3. Hameeteman W, Tytgat GNJ, Houthoff HJ, et al: Barrett's esophagus:development of dysplasia and adenocarcinoma. Gastroenterology 96:1249-1256, 1989

4. Adrain AL, Ter HC, Cassidy MJ, et al: High-resolution endoluminal sonography is a sensitive modality for the identification of Barrett's metaplasia. Gastrointest Endosc 46:147-151, 1997

5. Falk GW, Chittajallu R, Goldblum JR, et al: Surveillance of patients with Barrett's esophagus for dysplasia and cancer with balloon cytology. Gastroenterology 112:1787-1797, 1997

6. Peuchmaur M, Potet F, Goldfain D: Mucin histochemistry of the columnar epithelium of the oesophagus (Barrett's oesophagus): a prospective biopsy study. J Clin Pathol 37:607-610, 1984

7. Reid BJ, Haggitt RC, Rubin CE, et al: Barrett's esophagus. Correlation between flow cytometry and histology in detection of patients at risk for adenocarcinoma. Gastroenterology 93:1-11, 1987

8. Neumann CS, Iqbal TH, Cooper BT: Long term omeprazole treatment of patients with Barrett's oesophagus. Aliment Pharmacol Ther 9:451-454, 1995

9. Van Laethem JL, Cremer M, Peny MO, et al: Eradication of Barrett's mucosa with argon plasma coagulation and acid suppression: Immediate and mid term results. Gut 43:747-751, 1998

10. Edwards JE, Gable DR, Lentsch BS, et al: The rationale for esophagectomy as the optimal therapy for Barrett's esophagus with high-grade dysplasia. Ann Surg 5:585-591, 1996

11. Ell C, May A, Gossner L, et al: Endoscopic mucosal resection of early cancer and high-grade dysplasia in Barrett's esophagus. Gastroenterology 118:670-677, 2000

12. Barr H, Shepherd NA, Dix A, et al: Eradication of high-grade dysplasia in columnar-lined (Barrett's) oesophagus by photodynamic therapy with endogenously generated protoporphyrin IX. The Lancet 348:584-585, 1996

13. Haendeler J, Zeiher AM, Dimmeler S: Nitric oxide and apoptosis. Vitam Horm 57:49-77, 1999

14. Eberhart CE, Coffey RJ, Radhika A, et al: Up-regulation of cyclooxygenase 2 gene expression in human colo-rectal adenomas and adenocarcinomas. Gastroenterology 107:1183-1188, 1994

15. Ristimäki A, Honkanen N, Jänkälä H, et al: Expression of cyclooxygenase-2 in human gastric carcinoma. Cancer Res 57:1276-1280, 1997

16. Giardiello FM: Sulindac and polyp regression. Cancer Metastasis Rev 13:279-283, 1994

17. Van Stolk R, Stoner G, Hayton WL, et al: Phase I trial of exisulind (sulindac sulfone, FGN-1) as a chemopreventive agent in patients with familial adenomatous polyposis. Clin Cancer Res 6:78-89, 2000

18. Savary M, Miller G: The Esophagus Handbook and Atlas of of Endoscopy. Solothurn, Switzerland, Verlag Grassman AG, 1978

19. Paull A, Trier JS, Dalton MD, et al: The histologic spectrum of Barrett's esophagus. N Engl J Med 295:476-480, 1976

20. Riddell RH, Goldman H, Ransohoff DF, et al: Dysplasia in inflammatory bowel disease: Standardized classification with provisional clinical application. HUM PATHOL 14:166-178, 1983

21. Wilson KT, Fu S, Ramanujam KS, et al: Increased expression of inducible nitric oxide synthase and cyclooxygenase-2 in Barrett's esophagus and associated adenocarcinomas. Cancer Res 58:2929-2934, 1998

22. Whittles CE, Biddlestone LR, Burton A, et al: Apoptotic and proliferative activity in the neoplastic progression of Barrett's oesophagus: A comparative study. J Pathol 187:535-540, 1999

23. Soslow RA, Remotti H, Baergen RN, et al: Suppression of apoptosis does not foster neoplastic growth in Barrett's esophagus. Mod Pathol 12:239-250, 1999

24. Woodward TA, Klingler PD, Genko PV, et al: Barrett's esophagus, apoptosis and cell cycle regulation: Correlation of p53 with Bax, Bcl-2, and p21 protein expression. Anticancer Res 20:2427-2432, 2000

25. Gross A, McDonnell JM, Korsmeyer SJ: Bcl-2 family members and the mitochondria in apoptosis. Genes Dev 13:1899-1911, 1999

26. Younes M, Ertan A, Lechago LV, et al: p53 Protein accumulation is a specific marker of malignant potential in Barrett's metaplasia. Dig Dis Sci 42:697-701, 1997

27. Miyashita T, Reed JC: Tumor suppressor p53 is a direct

transcriptional activator of the human bax gene. Cell 80:293-299, 1995

28. Waring P, Mullbacher A: Cell death induced by the Fas/Fas ligand pathway and its role in pathology. Immunol Cell Biol 77:312-317, 1999

29. Younes M, Lechago J, Ertan A, et al: Decreased expression of Fas (CD95/APO1) associated with goblet cell metaplasia in Barrett's esophagus HUM PATHOL 31:434-438, 2000

30. Vollmers HP, Dammrich J, Hensel F, et al: Differential expression of apoptosis receptors on diffuse and intestinal type stomach carcinoma Cancer 79:433-440, 1997

31. Younes M, Schwartz MR, Finnie D, et al: Overexpression of Fas ligand during malignant transformation in the large bowel and in Barrett's metaplasia of the esophagus. HUM PATHOL 30:1309-1313, 1999

32. Pinkoski MJ, Brunner T, Green DR, et al: Fas and Fas ligand in gut and liver. Am J Physiol 278:G354-G366, 2000

33. Shirvani VN, Ouatu-Lascar R, Kaur BS, et al: Cyclooxygenase 2 expression in Barrett's esophagus and adenocarcinoma. Ex vivo induction by bile salts and acid exposure. Gastroenterology 118:487-496, 2000

34. Morris CD, Armstrong GR, Bigley G, et al: Cyclooxygenase-2 expression in the Barrett's metaplasia-dysplasia-adenocarcinoma sequence. Am J Gastroenterol 96:990-996, 2001

35. Garewal HS, Ramsey L, Sampliner RE, et al: Post-ablation biomarker abnormalities in Barrett's esophagus (BE): Are we increasing the cancer risk? Gastroenterology 120:A79, 2001

36. Tsibouris P, Hendrickse MT, Zissis M, et al: NSAIDs do not protect patients with Barrett oesophagus from adenocarcinoma development. Gastroenterology 120:A3022, 2001

37. Peters FTM, Ganesh S, Kuipers EJ, et al: Endoscopic regression of Barrett's oesophagus during omeprazole treatment: A randomized double blind study. Gut 45:489-494, 1999

38. Quatu-Lascar R, Fitzgerald RC, Triadafilopoulous G: Differentiation and proliferation in Barrett's esophagus and the effects of acid suppression. Gastroenterology 117:327-335, 1999