Immunohistochemical evaluation of carcinoembryonic antigen, secretory component, and epithelial IgA in ulcerative colitis with dysplasia*

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SUMMARY Immunofluorescence staining for carcinoembryonic antigen, secretory component, and epithelial IgA was evaluated semiquantitatively in routine formalin-fixed mucosal biopsy samples from five patients with ulcerative colitis who had undergone colectomy because of carcinomas. Selected areas were given fluorescence intensity scores without knowledge of whether dysplasia or reactive hyperplasia was present as judged by another observer from conventional histopathological features in adjacent sections. The two types of lesion did not differ significantly with regard to the expression of the three marker antigens. In a prospective study based on cold ethanol-fixed mucosal biopsy samples, lesions from 11 ulcerative colitis patients with dysplasia were compared blindly with lesions from six patients with reactive hyperplasia and with samples obtained endoscopically from eight normal controls. The range of disease-associated fluorescence intensity scores was wide, but staining for all markers tended to be brighter in reactive hyperplasia than in dysplasia ($P < 0.01$). In the controls, the fluorescence intensity score tended to be lower for carcinoembryonic antigen but was significantly ($P < 0.01$) higher for secretory component and epithelial IgA than in both types of lesion. Moreover, staining for secretory component and epithelial IgA in the lesions seemed to be inversely related to the grade of dysplasia and the degree of inflammation. No such trend was seen for carcinoembryonic antigen. The wide ranges of individual fluorescence scores precluded the possibility of applying carcinoembryonic antigen, secretory component, and epithelial IgA as immunohistochemical markers to differentiate between dysplasia and reactive hyperplasia in routine diagnostic work.

Ulcerative colitis predisposes towards the development of colonic carcinoma, which, according to Mottet,¹ develops in 3.1% of the patients. The incidence is especially high in extensive colitis of long duration.²⁻⁴ Consequently, some authors recommend preventive colectomy in this group of patients.^{5,6} Alternatively, attempts are made to select individuals with genuine epithelial dysplasia for closer follow-up or colectomy.^{7,8} The basis for the latter approach is the view that epithelial dysplasia represents a truly precancerous lesion^{7,9,10} and presents the possibility of obtaining multiple biopsy samples from all parts of the colon for histological evaluation. However, opinions differ about the histological identification of genuine dysplasia^{8,11-15} and its distinction from reactive hyperplasia induced by inflammation.

Although unequivocal histological criteria for precancerous lesions are lacking, some authors have reported genuine dysplasia in cases with heavily inflamed colon mucosa,^{11,13} but others have stated that reactive hyperplasia may easily be mistaken for dysplasia in such cases.⁷ A solution to this problem seemed to have been found when Isaacson,¹⁶ on the basis of immunohistochemical studies, reported that carcinoembryonic antigen was consistently present in colonic carcinoma and at the apex of dysplastic epithelial cells, whereas it was always absent from areas with reactive hyperplasia. However, this observation has, to our knowledge, not been confirmed.

Carcinoembryonic antigen is a fetal glycoprotein which may reappear during malignant development. Its presence in dysplasia is thus, in theory, compatible with precancer. Alternatively, one may look for normally occurring cell markers that are known to disappear partly or completely during malignant

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transformation, such as secretory IgA and its epithelial transport protein, the secretory component.^{17 18} We have found that both IgA and this component are decreased within the epithelium of large bowel carcinomas in parallel with histological tumour de-differentiation.¹⁹

Our present aim was to study whether immuno-histochemical localisation of the epithelial cell markers carcinoembryonic antigen, secretory component, and epithelial IgA might aid histopathological distinction between dysplasia and reactive hyperplasia in ulcerative colitis.

Methods

PATIENTS

A small retrospective group and a larger prospective group together with endoscopically normal controls were investigated.

RETROSPECTIVE GROUP

Formalin-fixed tissue blocks of colectomy specimens were obtained from the pathology department file. Five ulcerative colitis patients who had already developed carcinomas were selected (three females and two males). Their mean age was 39 years (range 23–60 years), and the mean duration of colitis symptoms was 20 years (range 11–30 years).

Prospective group

Multiple biopsy specimens were systematically taken at colonoscopy from 115 ulcerative colitis patients over a period of 16 months. Their mean age was 35 years (range 14–73 years), and the mean duration of

symptoms was 7.3 years (range two months–55 years). The tissue specimens were fixed in cold 70% ethanol for four hours followed by 96% ethanol overnight and further processed as detailed previously.^{20 21}

On the first examination, dysplasia was found in one or more specimens from 11 patients with a mean age of 37.6 years and a mean duration of symptoms of 12.5 years; nine had mild clinical activity of the disease and two had moderate activity.²² Endoscopic examination revealed total involvement of the large bowel; an inactive picture was seen in seven and signs of mild or moderate inflammation in four cases. Carcinoma was found in three of the patients. Additional clinicopathological information is given in Table 1.

From the same 115 patients a group of six with the histopathological diagnosis of reactive hyperplasia was selected on the first examination. Their mean age was 28 years, and the mean duration of colitis was nine years. Three had mild and three had moderate clinical activity, and all except one had total colitis. Endoscopically they presented moderate inflammation with or without ulcerations (Table 2).

Control group

Specimens of endoscopically and histologically normal colon mucosa were obtained from four men and four women (mean age, 45.3 years; range, 17–59 years) who were examined because of abdominal pain.

HISTOPATHOLOGY AND IMMUNOHISTOCHEMISTRY

Serial tissue sections were cut at 6 µm. Sections of

Table 1 Clinicopathological information about patients with dysplasia

Pat. no.	Age (yr), sex	Duration of disease (yr)	Clinical activity of disease*	Endoscopic findings†	Complications	Localisation of dysplastic area	Type of dysplasia ‡	Severity of dysplasia
1	60 F	26	Mild	Inactive/atrophy	Two carcinomas	Descending colon	Basal cell	Mild
2	22 F	10	Moderate	Inflamm./ulcerated		Coecum	Adenomatous	Mild
3	63 F	5	Mild	Inactive/atrophy		In all parts of colon	Basal cell	Mild
4	16 F	8	Mild	Mild inflamm.	Sclerosing cholangitis	In all parts of colon	Basal cell/ad.	Mild/moderate
5	22 F	10	Mild	Inactive/atrophy		In all parts of colon	Basal cell/ad.	Mild/moderate
6	36 F	18	Mild	Inactive/inflamm.		In all parts of colon	Basal cell/ad.	Mild/moderate
7	32 M	5	Mild	Inactive/inflamm.		Hepatic flexure	Basal cell	Mild
8	50 M	1	Mild	Inflamm./ulcerated		In all parts of colon	Basal cell	Mild/moderate
9	24 M	10	Mild	Inactive/inflam.	Carcinoma, sclerosing cholangitis	Transverse colon and rectum	Basal cell/ad.	Mild
10	35 M	19	Mild	Inactive/atrophy		In all parts of colon	Basal cell/Clear cell	Mild/severe
11	54 M	25	Moderate	Inflamm./ulcerated		In all parts of colon	Adenomatous	Mild

*Clinical activity according to Edwards and Truelove.²²

†All patients had total colitis.

‡Type of dysplasia according to Riddell.²⁷

Table 2 Clinicopathological information about patients with reactive hyperplasia

Pat. no.	Age (yr). sex	Duration of disease (yr)	Clinical activity of disease *	Extension of disease	Endoscopic finding
12	49 M	15	Moderate	Total colitis	Inflam./ulcerated
13	17 M	7	Mod./severe	Total colitis	Imflam./ulcerated
14	20 M	10	Mild	Total colitis	Mod. inflamm.
15	14 M	4	Mild	Total colitis	Mod. inflamm.
16	20 M	3	Moderate	Total colitis	Inflam./ulcerated
17	48 M	15	Mild	Left-sided	Mod. inflamm.

* Clinical activity according to Edwards and Truelove.²²

formalin-fixed material were treated with pronase (grade B, Calbiochem) for 15 minutes at 37°C.²¹ Paired immunofluorescence staining was performed by applying two combinations of fluorochrome conjugates with contrasting colours on adjacent sections: (1) fluorescein isothiocyanate (FITC)-labelled sheep anti-secretory component and tetramethyl-rhodamine isothiocyanate (MRITC)-labelled anti-carcinoembryonic antigen; and (2) FITC-labelled rabbit anti-IgA and MRITC-labelled sheep anti-secretory component. The preparation and characterisation of these reagents have been detailed elsewhere.^{19 23 24}

For conventional histological evaluation additional serial sections were stained with a trichrome routine method (HAS) combining haematoxylin, azofloxin, and saffron²⁵ and with alcian blue (pH 1) periodic acid Schiff (PAS).²⁶

FLUORESCENCE MICROSCOPY

The fluorescence microscope was a Leitz Orthoplan equipped with an Osram HBO 200-W lamp for rhodamine (red) and an XBO 150-W lamp for fluorescein (green) emission. A Ploem-type epilluminator provided narrow-band excitation light and selective filtration of the contrasting emission colours.

EVALUATION

Epithelial staining for carcinoembryonic antigen, secretory component, and secretory IgA was scored by the same observer throughout the study. A semi-quantitative scale ranging from 0 to 3 was used as detailed previously;¹⁹ a score of 3 indicated high fluorescence intensity and 0 negligible or no staining.

Routine staining was evaluated blindly by another observer. Dysplasia was typed and graded as mild, moderate, or severe according to Riddell.²⁷ The degree of inflammation was also evaluated when present.

REPRODUCIBILITY

More than eight weeks after the fluorescence scoring, 25 sections were selected at random and the staining intensity was re-evaluated blindly by the same observer. Excluding identical scores (34 of 75), the

one-sided sign test²⁸ showed no systematic difference between the two series of results ($P>0.05$). Also 18 routine sections were re-examined blindly after six weeks, resulting in identical diagnosis in 16 cases ($P>0.2$ according to the one-sided sign test).

TITRATION CONTROL

Selected tissue blocks (four with dysplasia and eight with reactive hyperplasia) that had received comparable fluorescence scores were re-examined by subjecting serial sections to staining with increasing dilutions of the anti-carcinoembryonic antigen and anti-secretory component conjugates. The end-points giving detectable fluorescence were recorded in each case for both components.

STATISTICAL EVALUATION

Comparisons between groups with regard to fluorescence scores and histological grades were based on the Mann-Whitney U test.²⁸ Reproducibility was evaluated by the sign test.²⁸

Results

RETROSPECTIVE STUDY

Thirteen tissue blocks with dysplasia and 12 with reactive hyperplasia were found in this group. The lesions did not differ significantly with regard to staining for the three epithelial markers (Table 3); carcinoembryonic antigen was negative in three dysplastic and five hyperplastic lesions.

PROSPECTIVE STUDY

Histopathology

All tissue blocks from patients with dysplasia or reactive hyperplasia and the control blocks were

Table 3 Scores (median and observed range) of fluorescence staining obtained in retrospective study of formalin-fixed colectomy specimens from five ulcerative colitis patients who had developed carcinoma

Type of lesion	CEA	SC	IgA
Dysplasia (n=13)*	1.0 (0-1)	0.8 (0-2)	0.5 (0-2)
Hyperplasia (n=12)	0.5 (0-1)	0.5 (0-2)	0.5 (0-2)

*n: number of lesions studied.

Fig. 1 Histology and immunohistochemical staining of mucosa from endoscopically normal colon. (a) HAS-stained section shows normal histology. (b) Adjacent section stained for carcinoembryonic antigen shows red fluorescence restricted to an apical rim. (c) Same section as in (b) shows green fluorescence for sc in a normal pattern. (d) Adjacent section stained for IgA shows a normal pattern similar to that observed for secretory component. ($\times 275$.)

Fig. 2 Histology and immunohistological staining of colonic mucosa with mild basal cell dysplasia. (a) HAS-stained section shows goblet cell depletion and unclear pleomorphism, occasional prominent nucleoli, and loss of nuclear polarity. (b) Adjacent section stained for carcinoembryonic antigen shows red fluorescence in the glycocalyx region and partly in the cytoplasm of the epithelial cells, especially in the lower part of the crypt. (c) Same section as (b) stained green for secretory component shows that the crypt epithelium lacks this component. (d) Adjacent section stained for IgA is also negative in the epithelium, but several IgA-producing immunocytes are present in the lamina propria. $\times 275$.

Fig. 3 Histology and immunohistochemical staining of colonic mucosa with reactive epithelial hyperplasia. (a) H&E-stained section shows goblet cell depletion, crypt epithelium destruction, and some enlarged nuclei with prominent nucleoli. (b) Adjacent section stained for carcinoembryonic antigen shows red fluorescence as a thick luminal rim with intercellular extension, especially at the bottom of the crypt, and also positive deposits in the crypt lumen. (c) Same section as (b) stained for secretory component shows green fluorescence diffusely distributed in the crypt epithelium and only slightly intensified apically. (d) Adjacent section shows virtual lack of intracellular IgA in the epithelium, but intercellular staining is seen in the upper part of the crypt, probably representing passive leakage of IgA. Also the crypt lumen contains IgA and there are several IgA-producing immunocytes in the lamina propria. $\times 275$.

randomised and re-examined blindly by conventional histology with the inclusion of adjacent sections subjected to paired immunohistochemical staining. In the 11 patients with dysplasia, dysplastic lesions were found in 58 sections and reactive hyperplasia in 48. Seven of these patients had dysplastic changes in all parts of the large bowel, whereas, in the remaining four, dysplasia was restricted to one or two segments. The dysplastic lesions were of the basal cell or adenomatous type, with the exception of a single tissue block containing clear cell dysplasia. Severe dysplasia was found in only one block (Table 1).

Immunohistochemistry

Carcinoembryonic antigens Staining for carcinoembryonic antigens was in most normal controls confined to a tiny luminal rim lining the apical part of the columnar epithelial cells, although some intercellular extension of the fluorescence was often seen. The goblet cells were always negative (Fig. 1).

The range for carcinoembryonic antigen staining was much wider in the ulcerative colitis patients than in the controls. When bright staining was seen in the pathological areas, it appeared as a thick border in the glycocalyx region of the columnar epithelial cells (Figs. 2 and 3), and also cytoplasmatic fluorescence occurred occasionally.

In eight of the tissue blocks with dysplasia, the

fluorescence was more intense than in the controls. However, this was also so in 29 blocks from patients with only hyperplasia. Staining of hyperplastic lesions in patients with dysplasia (hyperplasia associated with dysplasia) showed, on the average, intermediate intensity (Fig. 4). Despite the wide individual ranges, the median carcinoembryonic antigen score in the group with only hyperplasia was significantly higher ($p < 0.01$) than that of normal controls (Fig. 4).

The carcinoembryonic antigen staining intensity did not generally seem to be related to the grade of dysplasia (Fig. 5) or the degree of inflammation (Fig. 6). In patients with only reactive hyperplasia, however, the intensity tended to increase with increased degree of inflammation (data not shown).

To substantiate that carcinoembryonic antigen was not underestimated in dysplasia, four such lesions and eight hyperplastic lesions matched with regard to fluorescence scores (ranging from 1 to 2) were subjected to comparative titration tests using twofold dilutions of the conjugate. The end-point for detectable staining (1:16 to 1:32) from the original working dilution) did not depend on the type of lesion.

SC and epithelial IgA When present, both secretory component and IgA were distributed in the epithelium of the ulcerative colitis lesions in a pattern similar to that seen in the normal controls, although the cytoplasmic staining sometimes appeared to be more

Fig. 4 Scores given for immunofluorescence staining obtained in colonic mucosa that was endoscopically normal (Δ) or showed different types of ulcerative colitis lesion: epithelial dysplasia ($\bullet$) or hyperplastic lesions ($\circ$) from colons with (middle left) or without dysplasia (middle right). The dotted lines connect median scores for each category. The carcinoembryonic antigen median is significantly lower for both dysplastic and normal mucosa than for hyperplasia ($P < 0.01$). Secretory component and IgA medians are significantly higher for normal mucosa than for the three categories of lesions and also higher for hyperplasia than for dysplasia ($P < 0.01$).

Fig. 5 Scores given for immunofluorescence staining obtained in dysplastic ulcerative colitis lesions of various severity. The dotted lines connect the median scores for the mild and moderate grades. The carcinoembryonic antigen medians did not differ, whereas the secretory component and IgA medians were significantly higher in mild than in moderate dysplasia ($P < 0.01$).

Fig. 6 Scores given for immunofluorescence staining obtained in dysplastic ulcerative colitis lesions associated with different degrees of inflammation. The medians are connected by dotted lines, and did not differ for carcinoembryonic antigen scores. The secretory component and IgA medians were significantly lower ($P < 0.01$) when dysplasia was associated with inflammation (+ and ++).

diffuse in the lesions. The staining was confined to the columnar epithelial cells and intensified apically, especially for IgA. However, the median staining scores for secretory component and IgA were significantly reduced ($P < 0.01$) in both dysplastic and

hyperplastic lesions compared with the controls, although the ranges of individual scores were large (Figs. 1-4). For both marker proteins the fluorescence intensity tended to decrease in the order of controls, hyperplasia, hyperplastic lesions associated with

dysplasia, and dysplastic lesions (Fig. 4). Decreased staining intensity, moreover, seemed to be related to the severity of dysplasia (Fig. 5) and the degree of inflammation when present (Fig. 6). The latter relationship was indicated in both dysplastic and hyperplastic lesions. Titration experiments confirmed that the fluorescence intensity scores truly reflected the relative expression of secretory component in the two types of lesion. (IgA was not treated in this way.)

Discussion

It is important to search for objective criteria when it comes to definition and grading of dysplasia in ulcerative colitis patients who are evaluated for colectomy. The fact that some authors recommend surgery when 'moderate dysplasia' is detected,^{15,27} whereas others require 'severe dysplasia' for such a decision,²⁹⁻³¹ reflects partly lack of standardisation in conventional histopathology and partly differences in clinical approach. Functional histopathology, based on immunohistochemical studies of carcinoembryonic antigen was, therefore, performed by Isaacson¹⁶ in 1976 as an attempt to solve the diagnostic problem. He reported that this antigen was present in carcinomas and dysplastic premalignant lesions but consistently absent from hyperplastic epithelium (by him called inflammatory dysplasia); this observation has been quoted as promising by several authors.^{14,29,30} However, we were unable to confirm the result of Isaacson. Moreover, we did not succeed in obtaining a useful individual distinction between dysplasia and reactive hyperplasia by studying two other epithelial proteins—namely, secretory IgA and secretory component—although these markers revealed significant group differences between the two types of ulcerative colitis lesion.

We cannot offer any explanation of the discrepancy between our result with regard to carcinoembryonic antigen and that of Isaacson.¹⁶ He emphasised that discrimination between dysplasia and reactive hyperplasia depended on selection of an appropriate working dilution of the immunological reagent. In a control series we, therefore, applied our anti-carcinoembryonic antigen conjugate in twofold dilutions but the end-point of detectable staining did not depend on the type of lesion. Moreover, the hyperplastic lesions tended to show brighter antigen staining than the dysplastic ones (Fig. 4).

The fact that Isaacson¹⁶ used immunoperoxidase while we used immunofluorescence staining for carcinoembryonic antigen is not likely to explain the discrepancy; both methods are routinely applied on various substrates in our laboratory with comparable results (unpublished data). Also, in the retrospective part of our study, the tissue specimens had been

processed by a routine method similar to that used by Isaacson.^{16,32} In this formalin-fixed material the antigen was undetectable in several cases of both dysplasia and reactive hyperplasia. Moreover, in a methodological study,²¹ we found that five out of eight samples from histologically normal colon mucosa were negative for carcinoembryonic antigen after formalin-fixation, whereas the adjacent cold ethanol-fixed duplicate samples showed positive staining. Similar results were obtained by Goldenberg *et al.*³³ Thus, the apparent absence of this antigen from colon mucosa seems to be a quantitative rather than a qualitative phenomenon. Isaacson¹⁶ was, in fact, able to demonstrate this antigen in formalin-fixed normal colon mucosa when he increased the concentration of anti-carcinoembryonic antigen serum in his peroxidase method.

We are aware of the antigenic heterogeneity of this antigen and the resulting disparities among corresponding antisera. Nevertheless, by extensive performance testing we have excluded the possibility that our reagent contains unwanted antibodies reactive in the dilution used for this study.¹⁹ We, therefore, await with great interest the results of the carcinoembryonic antigen field trial that, according to Isaacson,¹⁶ is in progress in his laboratory on ulcerative colitis specimens.

The lack of relationship between the staining intensity for this antigen and the grade of dysplastic change (Fig. 5) was, in our opinion, not surprising, as the expression of carcinoembryonic antigen in large bowel carcinomas shows no clear relationship to the degree of tumour differentiation.¹⁹ Also, the degree of inflammation did not generally seem to influence the expression of the antigen, except in patients with only reactive hyperplasia. Increased production of carcinoembryonic antigen in the latter type of lesion might reflect accelerated turnover of the epithelial cells³⁴ caused by the inflammatory process. The expression of the antigen seen in the control specimens showed a fairly uniform pattern in accordance with that previously found in histologically normal colonic mucosa.^{21,35}

Also the fluorescence staining for secretory component and epithelial IgA varied over a wide range in both groups of patients, whereas rather uniform and normal staining patterns were observed in the control specimens. The expression of secretory component and epithelial IgA tended to be decreased when the severity of dysplasia was increased (Fig. 5). This finding accords with previous studies of polyps and adenocarcinomas in the large bowel.^{19,21,36-39} However, it is unknown whether loss of secretory component production, and thereby lacking IgA transport capacity,⁴⁰ are primary events in the neoplastic development or merely reflect secondary

alterations of the epithelial cells. The latter possibility was supported by the observation that dysplastic lesions expressed significantly less secretory component (and epithelial IgA) when they were associated with inflammation (Fig. 6).

Das *et al.*⁴¹ applied a similar immunohistochemical scoring system in a study of idiopathic proctitis: for one-third of the patients secretory component and epithelial IgA were reported to be markedly diminished even in histologically uninvolved areas. The authors, therefore, suggested that a primary disturbance of the secretory IgA system might be involved in the pathogenesis of idiopathic proctitis. However, they did not consider the problem of dysplasia and reactive hyperplasia. Moreover, the nutritional status of the patients can influence the function of the secretory IgA system^{42,43} and perhaps the expression of secretory component and IgA in histologically normal mucosal areas.

In conclusion, because of large individual variations, it is not possible from a practical point of view to distinguish between dysplasia and reactive hyperplasia by the carcinoembryonic antigen, secretory component, or epithelial IgA as immunohistochemical markers. However, the expression of secretory component and IgA showed interesting associations with the epithelial changes in ulcerative colitis. Additional studies of these and other marker proteins may thus add to the understanding of both carcinogenesis and the immunophysiology of the gut.

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