Nature of the inflammatory cell infiltrate in duodenitis

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SUMMARY Counts of lamina propria and intraepithelial cells, lymphoid and polymorphonuclear, have been performed on semithin sections of endoscopic biopsies from the duodenum of patients with ulcer-associated duodenitis, with non-specific duodenitis, and from controls. In both types of duodenitis there were significant increases in lamina propria counts of plasma cells, lymphocytes and eosinophils, and in intraepithelial lymphocyte counts, when compared with controls. In control specimens, neutrophil polymorphs were very infrequent but a substantial neutrophil polymorph infiltration of the epithelium and lamina propria was present in both types of duodenitis. In biopsies from areas of duodenitis scanning electron microscopy showed the presence of cells, which are probably neutrophil polymorphs, on the luminal surface of the mucosa. Abnormalities in cell counts were present only in biopsies taken from visually inflamed areas of the duodenal bulb. These values returned to normal after healing of duodenitis with cimetidine. This study highlights the complex nature of the mucosal cellular infiltrate in duodenitis, particularly the striking increase in polymorphonuclear leucocytes. Histopathological features of ulcer-associated and non-specific duodenitis are identical.

Inflammation of the proximal duodenum, "duodenitis", can be recognised and defined in various ways, for example radiologically (coarse mucosal folds), endoscopically (redness, swelling, bleeding), or pathologically (altered mucosal architecture, increase in "inflammatory" cells). We have defined duodenitis on the basis of visual features at upper gastrointestinal endoscopy and in an earlier study we have reported that in both ulcer-associated and non-specific duodenitis, there are abnormalities of the duodenal mucosa with reduction in villus length, increase in crypt length and increased crypt mitotic counts.^{1,2} We have now compared ulcer-associated and non-specific duodenitis in respect of the non-epithelial cells of the duodenal mucosa—the so-called "inflammatory cellular infiltrate" which is reported to be increased in non-specific duodenitis.^{3,4} Various methods for the grading of duodenitis, based on subjective assessment of inflammatory cellular infiltrate, have been attempted.⁵⁻⁷ However the density of the cell population in the lamina propria of the duodenum varies considerably even in the normal state.^{8,9} We have therefore used a quantitative technique to study the nature of the inflammatory cellular infiltrate in the duodenal

mucosa, to compare this in non-specific duodenitis with that in ulcer-associated duodenitis, and to examine the effects on the infiltrate of successful clinical treatment with cimetidine.

Patients and methods

PATIENTS

The criteria for selection of patients was as described in an earlier study on intestinal mucosal architecture in duodenitis.¹ Upper gastrointestinal endoscopy was performed as part of the clinical investigation or follow-up of patients with abdominal pain or heartburn. The diagnoses of duodenal ulcer and of duodenitis were based on endoscopic findings and the following groups of patients were studied.

Controls (C) were subjects in whom the only endoscopic abnormality was hiatus hernia.

Duodenal ulcer (DU) were patients in whom one or more ulcer craters, with discrete edge and depth, were present in the duodenal bulb.

Non-specific duodenitis (NSD) was divided into two categories. Mild NSD comprised hyperaemia and swelling of the mucosa with slight contact bleeding

severe NSD comprised intense hyperaemia and mucosal swelling, associated with spontaneous bleeding and multiple erosions.

Post cimetidine (DUPC and NSDPC) These were patients with duodenal ulcer or with severe non-specific duodenitis previously diagnosed at endoscopy, in whom after six to 12 weeks treatment with cimetidine (1 g/day) there remained no visual evidence of inflammation in the duodenal bulb at endoscopy.

For the light microscopic studies, between six and 10 patients per group were examined; for scanning electron microscopy biopsies were taken from three patients each in the control, duodenal ulcer and severe non-specific duodenitis groups.

METHODS

Biopsy procedures

Upper gastrointestinal endoscopy was performed with the Olympus GIF K instrument. Perendoscopic biopsies were taken from specified areas of the first and second parts of the duodenum under direct vision. In controls, biopsies were taken at random from the duodenal bulb and from the second part of the duodenum (D2). In the DU and duodenitis patients, various biopsies were taken from visually inflamed areas and from visually normal areas of the duodenal bulb and of D2. In the patients studied after treatment, biopsies were taken from previously inflamed sites. The sites of biopsies in the various groups of patients are summarised in Table 1.

Preparation of specimens for light microscopy

Biopsies were fixed in 5% glutaraldehyde in sodium cacodylate buffer for 4–24 h and post-fixed in 1% osmium tetroxide. They were embedded in Araldite and sections (1 μ m) were cut on an LKB III ultramicrotome using glass knives. After removal of Araldite, sections were stained in either 1% toluidine blue or in haematoxylin and eosin.

As the study progressed it became obvious that intestinal mucosal mast cells could not be identified in these preparations. A special staining technique was applied, in order to demonstrate mast cells in glutaraldehyde fixed mucosal biopsies.¹⁰ Groups of specimens were recut, and the sections stained with Azure II, methylene blue and basic fuchsin.

Cell counts

Counts of lamina propria and intraepithelial cells were performed on coded slides, by light microscopy, with an eyepiece graticule fitted into the microscope dividing the visual field into 100 equal

Table 1 Groups of subjects and sites of duodenal biopsies

Diagnosis	No of patients			
	Total	Biopsy of bulb		Biopsy of second part
		Visually inflamed	Visually normal	
Control	10		10	6
DU	8	8	8	6
NSD—severe	8	8	8	6
NSD—mild	6	6		
DUPC	6		6	
NSDPC	6		6	

squares. Lamina propria cell counts were performed in three parts of the section, to give a total lamina propria area studied of 100 squares. Mast cells in the Azure II methylene blue basic fuchsin stained sections were counted in a total of 500 squares of lamina propria for each section. Results were then converted to number of cells per square millimetre of lamina propria with a correction factor which had been calculated using the eyepiece graticule and a calibration slide. Infiltration of lymphocytes and of neutrophils within the surface epithelium was measured by counting the lymphocytes and neutrophils associated with 200 epithelial cells of the villus surface, and the values expressed as counts per 100 epithelial cells. The distribution of cells within the epithelial layer was also examined. For this, the epithelium was divided into three levels: apical, mid (including the top half of a nucleus) and lower zones. In a total of three specimens for the lymphocyte count, the distribution of 200 of each of these cells within the epithelium was documented, each cell being classified according to its location within the epithelial layer.

Statistics

Comparison of values between the various groups was done both by the Kruskal and Wallis test and by Wilcoxon's sum of ranks test.

Specimens for scanning electron microscopy

For scanning electron microscopy, specimens were immediately fixed in 2.5% glutaraldehyde and later dehydrated with 100% acetone, critical point dried from liquid CO₂, sputter coated with gold and examined under an International Scientific Instrument (Model ISI 60) scanning electron microscope.

Results

LAMINA PROPRIA CELL COUNTS

It was possible to perform lamina propria cell counts in all but four of the specimens taken. No attempt was made to assess changes in the sizes of villi and

crypts since this had been fully documented in the previous study. Epithelial cell damage and pseudostratification were seen frequently in specimens from all of the groups studied. Superficial gastric metaplasia was also seen in specimens from controls as well as from patients with duodenal ulcer and non-specific duodenitis.

Results of the counts of total lamina propria cells, plasma cells, lymphocytes and eosinophils (per square millimetre of lamina propria) are summarised in Figs. 1-4. A similar pattern of results emerges for all of these categories. In biopsies from the duodenal bulb of control patients the mean lamina propria total cell count was $4216/\text{mm}^2$ with 2118 plasma cells, 1647 lymphocytes and 83 eosinophils. The results of the counts in biopsies of mucosa from the second part of the duodenum in controls were similar. In contrast, in biopsies from areas of ulcer-associated and severe non-specific duodenitis significantly higher counts of all of these cell types were found. However, in mild duodenitis and in all of the biopsies taken from visually normal duodenum in the duodenal bulb and second part of the duodenum, no significant differences from con-

trols emerged. In the post-treatment biopsies of the duodenal bulb, lamina propria cell counts were also normal. Mucosal mast cell counts were performed in specimens from the duodenal bulb in six controls, six patients with ulcer-associated duodenitis and six with severe non-specific duodenitis. As shown in Fig. 5, no differences were found between these three groups. Neutrophil polymorphs were very rarely present in the lamina propria of control biopsies (mean $7/\text{mm}^2$ lamina propria). However in ulcer-associated and in severe non-specific duodenitis (Fig. 6), values were considerably higher with 904 ± 123 ($\pm$ SE) in DU and 421 ± 72 in NSD respectively ($p < 0.005$ in each case). There was no difference from controls in the neutrophil polymorph count in the lamina propria in the other groups of specimens from the duodenal bulb and second part of duodenum.

INTRAEPITHELIAL CELL COUNTS

Just as in the case of lamina propria cells, intraepithelial lymphocyte counts were increased in inflamed areas of DU and severe NSD, as compared to controls, whereas there were no significant differ-

Fig. 1 Total number of cells per square millimetre (mean $\pm$ SE) of lamina propria in duodenal mucosa.

Fig. 2 Number of plasma cells per square millimetre of lamina propria in duodenal mucosa.

Fig. 3 Number of lymphocytes per square millimetre of lamina propria in duodenal mucosa.

Fig. 4 Number of eosinophils per square millimetre of lamina propria in duodenal mucosa.

Fig. 5 Number of mast cells per square millimetre of lamina propria in duodenal mucosa.

Fig. 6 Number of neutrophil polymorphs per square millimetre of lamina propria in duodenal mucosa.

Fig. 7 Intraepithelial lymphocyte count per 100 epithelial cells in duodenal mucosa.

ences from controls in the biopsies taken from visually normal mucosa in the duodenal bulb or second part of the duodenum (Fig. 7). With regard to neutrophil polymorphs, no neutrophil polymorph was observed within the epithelium in any of the control specimens. In contrast, in specimens from DU and severe NSD, the figures were 4 ± 0.8 (mean $\pm$ SE) and 4.6 ± 0.9 polymorphs per 100 epithelial cells (Fig. 8) ($p < 0.005$ for each comparison with control). Intraepithelial neutrophil polymorphs were rarely found in the other groups of specimens from DU and NSD patients, and for these, values showed no statistically significant differences from controls.

An interesting difference emerged when the location within the epithelium of lymphocytes and neutrophils was studied. As shown in Table 2, neutrophils were evenly distributed within the epithelium, in the apical, mid and basal regions, whereas intraepithelial lymphocytes were basally situated.

Fig. 8 Intraepithelial neutrophil polymorph count per 100 epithelial cells in duodenal mucosa.

Table 2 Distribution of intraepithelial lymphocytes and polymorphs within the epithelium of the duodenal mucosa

	Base	Middle	Top
Lymphocytes	69	23	8
Neutrophil polymorphs	28	38	34

SCANNING ELECTRON MICROSCOPY

Examination of control specimens from the duodenal bulb showed the presence of villi of various shapes and sizes with no surface exudate and no intraluminal cells adherent to the surface (Fig. 9). In contrast in the specimens from both DU and severe NSD there were cells on the surfaces of the villi which had the scanning EM features of neutrophils, and some red blood cells were also present (Figs. 10 and 11).

Discussion

Visual and histological changes of inflammation often occur in the proximal duodenum in association with peptic ulcer and similar changes, when seen in the absence of frank ulceration, are termed non-specific duodenitis. The overall aim of our work in duodenitis has been to elucidate the pathogenesis of non-specific duodenitis and its relation to peptic

Figs 9 (above) and 10 (below) Scanning electron microscopy showing surface of villi from control duodenal mucosa. Original magnification $\times 500$. (Below) Scanning electron microscopy showing surface of villi from inflamed duodenal mucosa. Original magnification $\times 510$.

Fig. 11 Scanning electron microscopy showing cells on the surface of villi from inflamed duodenal mucosa. Original magnification $\times 5100$.

ulcer of the duodenum. For these reasons, we chose to use visual features at upper gastrointestinal endoscopy as initial diagnostic criteria. This then allowed formal examination of various pathological features of mucosal biopsies in patient categories so defined.

We have already reported that it is feasible to perform morphometric studies of the architecture of villi and crypts in endoscopic biopsies of the duodenum. Similar biopsies, Araldite-embedded and cut as semithin sections, were found to be adequate for inflammatory cell counts in the lamina propria and within the epithelial layer. These biopsies were also adequate for study by scanning electron microscopy. Some variation in cell density was noted in several biopsies from one area to another. Counts were done in three areas of each specimen to avoid errors in estimation of cellular infiltrate due to this variation.

Most studies of inflammatory cellular infiltrate in duodenitis have been done on the basis of subjective assessment. Only three previous studies have attempted to quantify the changes objectively. Thompson and Holme⁴ performed cell counts with the help of a graticule in specimens from duodenitis. Only an abstract has been published and results are not provided. Whitehead *et al*⁵ used a point counting

technique to assess the cellularity of the lamina propria. Values have been expressed as ratio of number of cells to volume of lamina propria and therefore cannot be compared with our figures. Cheli and Giacosa³ used a graticule to perform cell counts in biopsies from controls and patients with non-specific duodenitis, but they provide no details of their endoscopic criteria and findings. Their control values for inflammatory cell counts in lamina propria are higher than ours, and cell counts in lamina propria of normal jejunal biopsies¹¹⁻¹⁴ are also higher than those for our control subjects. However, in all of these studies, sections used were of 5 μm thickness. Intraepithelial lymphocyte counts have been expressed as per millimetre length of epithelium in three of these studies¹¹⁻¹³ and results are therefore not comparable with ours. Montgomery and Shearer¹⁴ expressed intraepithelial lymphocyte counts as per 100 epithelial cells and reported a range of 7 to 22 in 20 controls. Values in our 10 controls ranged from 7 to 14 per 100 epithelial cells.

In our study significant increases in the number of plasma cells, lymphocytes and eosinophils in the lamina propria have been demonstrated in both ulcer-associated and severe non-specific duodenitis. A striking finding was the appearance of significant numbers of neutrophil polymorphs in the lamina propria and in the epithelial layers of the villi. Neutrophils are rarely present in normal mucosa^{7,9} and this is confirmed by our study. Visually defined mild duodenitis did not show any significant difference from controls as regards the inflammatory cells in lamina propria and surface epithelium. The visually recognised changes in mild duodenitis probably represent local changes in blood flow only and do not denote a state of true inflammation. Our previous study had shown no mucosal architectural change in mild duodenitis.¹

Abnormalities in cell infiltration were localised to visually abnormal areas of the duodenal bulb and disappeared after healing of inflammation with cimetidine treatment. Our earlier study demonstrated that mucosal architectural changes were found in areas of ulcer-associated and severe non-specific duodenitis, and that these returned to normal after successful treatment with cimetidine.^{1,2} The present study has shown that abnormal cell infiltration is restricted to those areas where alteration of mucosal architecture was found.

Although there are several reports of increased numbers of lamina propria and intraepithelial lymphoid cells and polymorphs in non-specific duodenitis,^{3,4,5,15,16} the value of an increased inflammatory cell infiltrate as a criterion for the diagnosis of non-specific duodenitis has remained controversial^{9,19} and the relations between ulcer-

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associated and non-specific duodenitis have not been clarified.^{4,19} On subjective assessment, using histological sections from surgical specimens, similarities in the histological appearances of duodenitis and duodenal ulcer have been reported¹⁷ and Joffe *et al*¹⁸ considered that the histological changes in these two conditions were indistinguishable. This present study is the first quantitative comparison of the components of the inflammatory cellular infiltrate in ulcer-associated and non-specific duodenitis, and firmly demonstrates that the changes are identical in these two conditions. There was no overlap between results for controls and for the two types of duodenitis, in the various inflammatory cells studied. However, one specimen from an unaffected area of ulcer-associated duodenitis and one specimen from an unaffected area in a patient with severe non-specific duodenitis showed increased inflammatory cell counts.

A generalised expansion of the mucosal immune cells in both ulcer-associated and non-specific duodenitis could be due to stimulation of gut-associated lymphoid tissues in these conditions. This appears an unlikely explanation, as we have shown that the changes are very localised. Local factors at sites of inflammation seem a more likely mechanism—these may either attract cells into the tissues, increase the rate of proliferation locally, or immobilise these cells and prevent them from leaving the mucosa in blood or lymph.

Neutrophils are not a normal constituent of the lamina propria or the epithelium of the duodenal bulb in normal subjects. The results of this study now confirm that the presence of neutrophils within the mucosa is a striking and consistent feature, both in ulcer-associated and in non-specific duodenitis. A substantial neutrophil infiltrate was present in every biopsy taken from a visually abnormal area of the duodenal bulb in severe duodenitis and in duodenal ulcer patients; and in none of the control subjects was there a substantial neutrophil infiltrate in the mucosa. The study confirms the presence of neutrophils as a crucial factor for the histological diagnosis of duodenitis. These cells appear to be migrating through the epithelial lining and are present on the mucosal surface, as indicated by scanning electron microscopy. Mediators of neutrophil chemotaxis in this situation remain unknown. Possible substances include complement components, immunoglobulin fragments, fragments of fibrinogen and collagen, kallikrein and prostaglandin E₁. The substances causing chemotaxis may play an important role in the pathogenesis of duodenal inflammation. The substance or substances involved may be the same as those responsible for generalised expansion of mucosal immune cells and this aspect of

duodenal inflammation requires further study. The localised nature of inflammation and return to normality after cimetidine treatment have led us to believe that the responsible substance or substances are most likely to be derived from luminal contents.

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