Immunogold-silver technique applied to showing malignant B cell infiltration of gastrointestinal tract in patients with chronic lymphocytic leukaemia and non-Hodgkin's lymphoma

G SMITH,† J CROCKER,* P NAR,* I CHESNER,‡ M J LEYLAND†

*From the Departments of *Histopathology, †Haematology, and the ‡Metabolic Unit, East Birmingham Hospital, Birmingham*

SUMMARY Gastric, duodenal, and rectal biopsy specimens from 20 patients with chronic lymphocytic leukaemia (CLL) and non-Hodgkin's lymphoma (NHL), not primarily of gastrointestinal origin, were examined using the immunogold-silver staining (IGSS) technique. In paraffin sections the presence of κ and λ surface immunoglobulin in lymphoid infiltrates was shown. Using this technique, nine patients were shown to have infiltration of the gastrointestinal mucosa by monoclonal B cells at one or more sites. In a further case a lymphoid aggregate was shown to express both κ and λ surface light chains, suggesting that it had a benign nature.

A review of data collected at this hospital suggested that iron deficiency was a common finding in patients with chronic lymphocytic leukaemia (CLL) and non-Hodgkin's lymphoma (NHL). To assess the possible contribution of lymphoid infiltration of the gastrointestinal mucosa to occult blood loss or malabsorption of iron, we applied the immunogold-silver staining (IGSS) technique to gastric, duodenal, and rectal biopsy specimens. To distinguish benign from malignant B cell lymphoid infiltrates it is necessary to show that the constituent cells are monotypic. Up to 20% of normal mucosal biopsy specimens from the small intestine may contain lymphoid follicles in the lamina propria, whereas at the epithelial surface lymphocytes account for 30% of the nucleated cells.¹ Lymphoid aggregates are also common in all stages of inflammatory bowel disease, and should express both κ and λ surface immunoglobulin whereas malignant aggregates should express only one or other light chain. For the examination of surface immunoglobulin (sIg), peroxidase-antiperoxidase sequences cannot readily be applied to paraffin sections and therefore frozen sections have been used.² Any study using biopsy material obtained via an endoscope or sigmoidoscope is hampered by the inevita-

ble small size of the specimens, which makes the use of frozen sections, and the consequent loss of morphological detail, unsatisfactory. Accordingly, an IGSS method, known to show sIg in CLL, was applied.

Material and methods

Twenty patients (12 men, eight women) aged between 39 and 77 years were studied. There were 18 cases of CLL and two of non-Hodgkin's lymphoma. Applying the prognostic staging system of the International Workshop on CLL,³ there were seven cases of stage A CLL, seven stage B, and four stage C cases. Of the patients with non-Hodgkin's lymphoma, one had generalised low grade lymphoma of the centrocytic type, while the other had a high grade tumour apparently localised to the left tibia. None of the patients had any evidence of gastrointestinal disease before being investigated. In all cases subsequently shown to have gastrointestinal infiltration surface marker studies on peripheral lymphocytes confirmed that the patients had B cell neoplasms.

SPECIMENS

Endoscopy was performed and multiple gastric and duodenal biopsy specimens were taken using standard endoscopic biopsy forceps. Sigmoidoscopy was performed without prior bowel preparation and two

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rectal biopsy specimens taken from the posterior wall. Fully informed consent was obtained from all patients before these procedures.

FIXATION AND PROCESSING

All specimens were placed immediately in 5% formal acetic acid (5% glacial acetic acid in 10% aqueous formalin) which has been shown to enhance the response to immunohistological procedures.⁴ The specimens were fixed for 48 hours, dehydrated, and taken to paraffin wax. Sections were cut at 2 μ m thickness, and after rehydration the following immunogold-silver staining technique was applied.

IMMUNOGOLD-SILVER STAINING TECHNIQUE

The staining sequence adopted was a standard one using the Janssen system (Janssen Pharmaceutica Life Sciences Products, B-2340 Beerse, Belgium), which has been previously reported from this department.⁵ Lugol's iodine was applied for five minutes and then cleared with 2.5% sodium thiosulphate solution. The sections were treated with 0.1% trypsin in Tris buffered saline (TBS) for five minutes and after washing in TBS overlaid with normal (non-immune) swine serum diluted 1/5 in TBS for five minutes. The primary antiserum (rabbit antihuman κ or λ light chain) was applied at a dilution of 1/1000 in TBS for 30 minutes at room temperature. The sections were again washed in TBS before the secondary antiserum (colloidal gold adsorbed to goat antirabbit immunoglobulin diluted 1/500 in TBS) was applied. After overnight incubation at 4°C the sections were washed in TBS for 30 minutes and then in distilled water for 15 minutes before being immersed in freshly prepared citrate buffer (2.35 g trisodium citrate; 2.55 g citric acid, in 100 ml distilled water) for five minutes. The silver enhancement process was carried out in a dark-room with a freshly prepared solution of 77 mM hydroquinone and 5.5 mM silver lactate in 200 mM citrate buffer, pH 3.85. The reaction was stopped in 10% fixing solution (Janssen Pharmaceutica) and the sections then thoroughly washed in water before counterstaining with haematoxylin. After dehydration the sections were mounted in synthetic medium.

Results

A total of eight of 18 patients with CLL and one of the two patients with non-Hodgkin's lymphoma were shown to have malignant infiltration of the gastrointestinal tract. Table 1 summarises the site of disease. In the patient with gastric disease the mucosa and submucosa were extensively infiltrated by small rounded cells which possessed the features of lymphocytes (fig 1). The biopsy specimens taken from the body and

Table 1 Site of infiltration by monoclonal B cells

Biopsy site	No of cases
Stomach	1
Duodenum	2
Rectum	8

pyloric antrum were also heavily infiltrated. The two cases of duodenal infiltration took the form of an extensive aggregation of lymphocytes in the lamina propria extending into the submucosal layers in one patient, and a smaller focal submucosal aggregate in the second. Rectal disease varied from fairly discrete mucosal aggregates to extensive infiltration of all layers through the bowel wall. In none of the specimens was any evidence of secondary follicle centres observed. The immunogold silver staining showed that most cells in these areas bore either κ or λ sIg light chain but not both (fig 2). In one patient a lymphoid aggregate noted in a rectal biopsy specimen was subsequently shown to express both κ or λ surface light chains, indicating that it was benign.

Discussion

Disease in the gastrointestinal mucosa in CLL has

Fig 1 Gastric mucosa containing large aggregation of lymphoid cells of uncertain nature, from patient with established B-CLL. (Haematoxylin and eosin.)

Fig 2 Left (a) Gastric mucosa from the same patient as that in Fig 1, stained for κ Ig light chain. All cells stain intensely black; right (b) section from same biopsy specimen stained for λ Ig light chain. Scattered cells stain positively but most are negative. (IGSS method.)

been reported at necropsy in several studies. It varies from diffuse infiltration of the gastric mucosa⁶ to ulcerating lesions of the ileum and colon.⁷ Most studies, however, have reported only gross macroscopic evidence of gastrointestinal disease,⁸ and in studies where microscopic analysis was carried out this was not performed in every case.⁷ Furthermore, no attempt was made to differentiate between benign and malignant infiltrates. The highest reported incidence of gastrointestinal involvement was 15%.⁶ The necropsy evidence for gastrointestinal disease in non-Hodgkin's lymphoma, not primarily of gastrointestinal origin, suggests that the incidence may be as high as 46%.⁹ In a study of plasma cell populations in rectal biopsy specimens from patients with myeloma and other B cell neoplasms Leonard *et al* found that up to a quarter of patients with non-Hodgkin's lymphoma had evidence of tumour spread to the rectum.¹⁰ Infiltration of the gastrointestinal mucosa by B cell neoplasms has been associated with haemorrhage^{6,8} and may be associated with malabsorption.^{7,11}

This study has shown that occult spread to the gastrointestinal tract is common in patients with CLL with an incidence in this study of 44%. The immunogold technique can be applied to paraffin wax sections of gut biopsy specimens to confirm the malig-

nant nature of lymphocytic infiltrates by virtue of surface Ig activity. The need for frozen sections to show this activity, which would be impractical because of the small size of the endoscopic biopsy material, is thus avoided.

There was no apparent correlation between the clinical staging of the CLL and the incidence of gastrointestinal disease (table 2). In at least one case, however, treatment was affected by the presence or absence of positive gastrointestinal tract biopsy specimens. A patient with apparent stage A(O) disease who would normally not have received treatment was given chemotherapy because of extensive infiltration of the stomach.

We are presently investigating any functional ab-

Table 2 Staging of patients with CLL and gastrointestinal disease

Case No	Site of disease	Stage
1	Stomach	A(O)
2	Rectum	A(O)
3	Duodenum/rectum	A(O)
4	Rectum	A(I)
5	Rectum	A(II)
6	Rectum	B
7	Rectum	B
8	Duodenum/rectum	C

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Table 3 Relation between gastrointestinal disease and occult blood loss in patients with iron deficiency

Case No	Site of disease	Faecal occult blood samples
1	Stomach	Negative
2	Duodenum/rectum	Positive
3	Duodenum/rectum	Negative
4	Rectum	Positive
5	Rectum	Not done
6	Nil	Positive
7	Nil	Positive
8	Nil	Positive
9	Nil	Negative
10	Nil	Negative

normality that may result from the gastrointestinal infiltration noted in this study. Iron deficiency, measured by absent or reduced marrow iron stores or a low serum ferritin, was present in 10 patients: five of these had gastrointestinal disease. Faecal occult blood samples, however, were only positive in two cases, although a further three patients without apparent gastrointestinal lesions had evidence of blood loss (table 3). This lack of correlation may suggest that the infiltration is patchy and not picked up in every case. We are, however, investigating the possibility that malabsorption, as well as occult bleeding, may contribute to the iron deficiency.

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Requests for reprints to: Dr J Crocker, Department of Histopathology, East Birmingham Hospital, Bordesley Green East, Birmingham B9 5ST, England.