Alpha-1-antitrypsin immunoreactivity in the small bowel in coeliac disease

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Abstract

The role of alpha-1-antitrypsin (A,AT) in the small intestinal mucosa in health and disease is poorly understood. We studied the prevalence and distribution of A,AT positive cells in small bowel biopsies from 35 coeliac disease patients and 25 normal controls retrieved from the records of the Department of Pathology, University of Oxford. Serial 6 micron thick paraffin sections were stained with H&E, PAS, PAS-diastase and for A,AT employing an indirect immunoperoxidase technique. None of these cases had biochemical evidence of A,AT deficiency. In the present study, 24 out of 35 small bowel biopsies among coeliacs (68.5%) compared to 13 out of normal controls (52%) showed A,AT immunoreactivity. Thus our findings point to the preservation of A,AT in coeliac disease.

Key words: Alpha-1-antitrypsin, small bowel, coeliac disease, immunoperoxidase.

INTRODUCTION

Alpha-1-antitrypsin is a glycoprotein, an alpha-1-globulin, that inhibits several proteolytic enzymes including trypsin/chymotrypsin, pancreatic elastase, skin collagenase, plasmin, thrombin, renin, urokinase and neutral proteases of polymorphs. Raised levels in the serum are found in infections and inflammatory processes.

The functional role of A,AT under normal conditions and in the pathogenesis of disease processes is poorly understood. The reported absence of A,AT positive epithelial cells in many adult coeliacs prompted us to examine small bowel biopsies received in the Pathology Department, University of Oxford. Unlike a previous study, our aim was to carry out A,AT demonstration by an immunoperoxidase staining procedure.

MATERIALS AND METHODS

Thirty-five small bowel biopsies from patients with untreated coeliac disease earlier obtained by standard Meditech catheter technique were collected. 25 small bowel biopsies obtained from ileocolonectomy specimens (operated for malignancy or intestinal obstruction or diverticular disease, etc) and irritable bowel syndrome served as normal controls. None of the above patients had biochemical evidence of A,AT deficiency.

For routine light microscopy, 6 micron thick paraffin sections were cut on a rotary microtome and stained with haematoxylin-eosin, periodic acid Schiff and periodic acid Schiff-diastase. For immunohistochemical localization of A,AT, an indirect immunoperoxidase technique was applied using corresponding paraffin sections of each biopsy. Rabbit antiserum to human A,AT (DAKOPATTS) in a dilution of 1:100 was used. The specificity of the immunoreaction was assessed as negative or positive.

RESULTS

In the normal controls, A,AT was present as brownish material predominantly located in the crypt epithelium (Fig.2). These positive cells, though distributed unevenly, were chiefly seen in the basal region of the glands resting on the muscularis mucosae. The staining was conspicuous by its absence in the epithelium covering the villi. Among coeliac biopsies showing moderate to severe villous atrophy, A,AT was distinctly detected in the surface/villus epithelium as tiny globules (Fig.3) and in the crypt epithelium appearing as largely granular form.
Paneth cells did not reveal any positivity. A,AT was observed in neuroendocrine cells which were identified by their small size, triangular shape and antiluminal granular cytoplasm (Fig.5). It was focally present in lymphomononuclear cells and occasional polymorphs in the lamina propria.

Table 1 summarizes the results of immunostaining. Among 35 biopsies of coeliac disease studied, specific A,AT staining was positive in 24 and negative in 11 biopsies. In comparison, among 25 normal controls, A,AT was positive in 13 and negative in 12 biopsies. PAS-positive diastase resistant globules were also seen in all biopsies with positive A,AT immunoreaction.

**DISCUSSION**

Alpha-1-antitrypsin is one of the major protease inhibitors present in human serum. It is synthesized in the yolk sac and foetal liver. During adult life the liver is the major source of its synthesis. A,AT immunoreactivity has been well demonstrated in livers of deficient subjects, in islet cells of normal adult pancreas: small bowel, gastric mucosa, in pulmonary macrophages, mast cells, neutrophils, various body fluids such as tears, lymph, saliva, duodenal fluid, cervical mucus, amniotic fluid, semen and synovial fluid.

The present study has localized A,AT in small bowel mucosa by employing a standard immunoperoxidase technique instead of immunofluorescence. Furthermore, A,AT has been found to be present in a large number of biopsies from patients with coeliac disease (68.5%) compared to an earlier study by Geboes et al., who could not detect A,AT in 10 out of 14 biopsies by immunofluorescence.

Although the exact significance of A,AT in the small intestine is not known, reports have indicated an association between A,AT deficiency in the serum and emphysema, cirrhosis and intestinal mucosal atrophy. Possibly, A,AT may be essential for the physiological and morphological integrity of the intestinal mucosa. Two important points emerge out of our study: (i) as A,AT seems to be preserved in a large proportion of patients with coeliac disease, it does not explain the cause of villous atrophy; (ii) perhaps A,AT positive biopsies represent a separate group among some coeliacs. The validity of this finding, however, should be confirmed by studying more cases in different centres.

On the basis of staining intensity and distribution pattern, A,AT appears to be produced in the crypts and diffuses intracellularly into villous epithelium. As in the lung, in the small bowel, A,AT possibly has a role in protecting the physiologically active mucosa from the potentially destructive proteases released by leucocytes secondary to invading bacteria.

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FIG.2: Distribution of alpha-l-antitrypsin in normal mucosa. (Immunoperoxidase stain x 140).

FIG.3: Micrograph of mucosa (coeliac disease) showing $\Lambda_A$AT immunoreaction as tiny globules in the surface epithelium. (Immunoperoxidase stain x 650).
REFERENCES


