

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

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Abstract

The role of alpha-1-antitrypsin (A₁AT) in the small intestinal mucosa in health and disease is poorly understood. We studied the prevalence and distribution of A₁AT 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-diacetate and for A₁AT employing an indirect immunoperoxidase technique. None of these cases had biochemical evidence of A₁AT 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 A₁AT immunoreactivity. Thus our findings point to the preservation of A₁AT 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 A₁AT under normal conditions and in the pathogenesis of disease processes is poorly understood. The reported absence of A₁AT 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,¹ our aim was to carry out A₁AT 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 ileocelectomy 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 A₁AT 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-diacetate. For immunohistochemical localization of A₁AT, an indirect immunoperoxidase technique² was applied using corresponding paraffin sections of each biopsy. Rabbit antiserum to human A₁AT (DAKOPATTS) in a dilution of 1:100 was used. The specificity of the immunoreaction was demonstrated by positive staining of known positive control tissue sections of liver (Fig.1) from a patient with A₁AT deficiency. The negative immunoreaction was put up by replacing the primary antiserum with normal swine serum. Immunostaining of A₁AT in each section was assessed as negative or positive.

RESULTS

In the normal controls, A₁AT 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, A₁AT was distinctly detected in the surface/villus epithelium as tiny globules (Fig.3) and in the crypt epithelium appearing as largely granular form

(Fig.4). Paneth cells did not reveal any **positivity**. A_1AT 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_1AT staining was positive in 24 and negative in 11 biopsies. In comparison, among 25 normal controls, A_1AT was positive in 13 and negative in 12 biopsies. PAS-positive diastase resistant globules were also seen in all biopsies with positive A_1AT 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**.³ During adult life the liver is the major source of its **synthesis**.⁴ A_1AT immunoreactivity has been well demonstrated in livers of deficient subjects in islet cells of normal adult pancreas: small bowel,⁵ gastric **mucosa**,⁷ in pulmonary **macrophages**,⁸ mast cells? **neutrophils**,¹⁰ various body fluids such as tears, lymph, saliva, duodenal fluid, cervical mucus, amniotic fluid, semen and synovial fluid."¹¹

The present study has localized A_1AT in small bowel mucosa by employing a standard immunoperoxidase technique instead of immunofluorescence.¹ Furthermore, A_1AT 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*² who could not detect A_1AT in 10 out of 14 biopsies by immunofluorescence.

Although the exact significance of A_1AT in the small intestine is not known, reports have

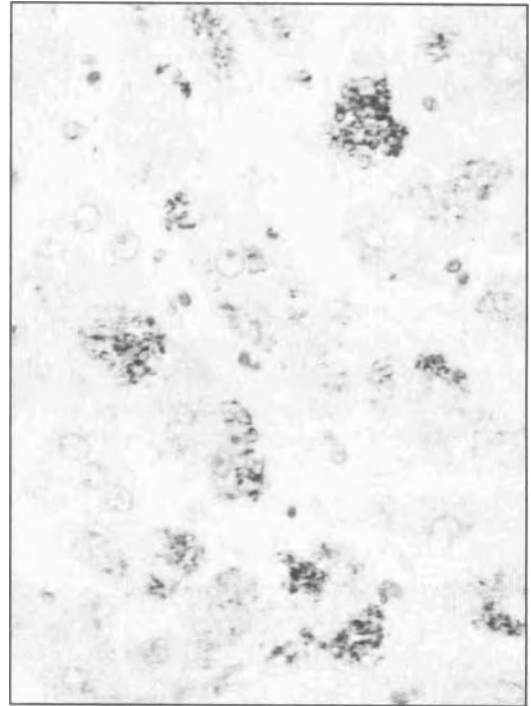


FIG.1: Micrograph of section of liver from a patient with alpha-1-antitrypsin (A_1AT) deficiency showing positive staining. (Immunoperoxidase stain x 650).

indicated an association between A_1AT deficiency in the serum and emphysema, cirrhosis and intestinal mucosal **atrophy**.^{12,13} Possibly, A_1AT may be essential for the physiological and morphological integrity of the intestinal mucosa.¹ Two important points emerge out of our study: (i) as A_1AT 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_1AT 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_1AT appears to be produced in the crypts and diffuses **intracellularly** into villous **epithelium**.¹⁴ As in the lung, in the small bowel, A_1AT possibly has a role in protecting the physiologically active mucosa from the potentially destructive proteases released by leucocytes secondary to invading **bacteria**.¹⁵

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TABLE 1: A_1AT immunostaining in small bowel

Staining reaction	Coeliac disease No. (%)	Normal controls No. (%)
Positive	24 (68.5)	13 (52)
Negative	11 (31.5)	12 (48)
Total tested	35	25

Mean positivity = 61.7%; mean negativity = 38.3%

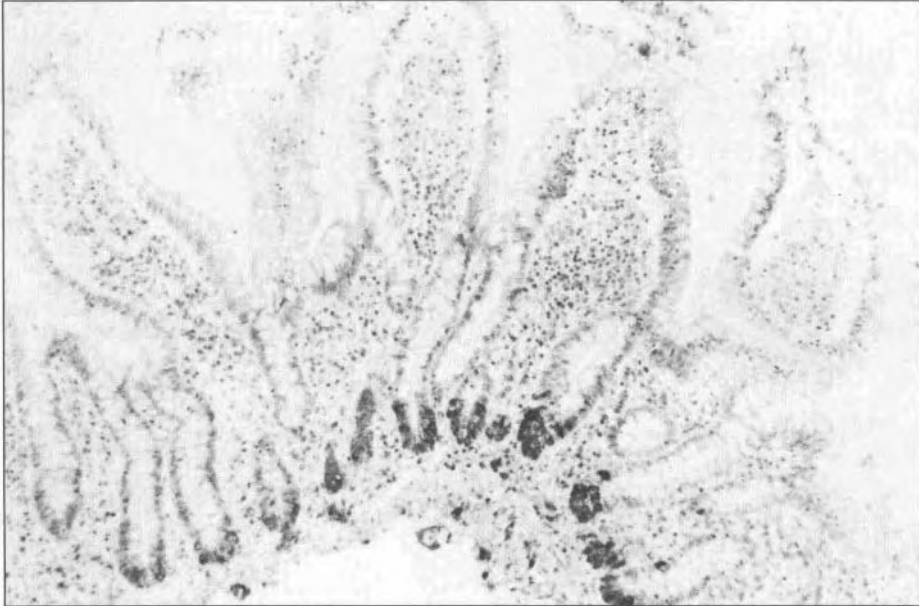


FIG.2: Distribution of alpha-1-antitrypsin in normal mucosa. (Immunoperoxidase stain x 140).

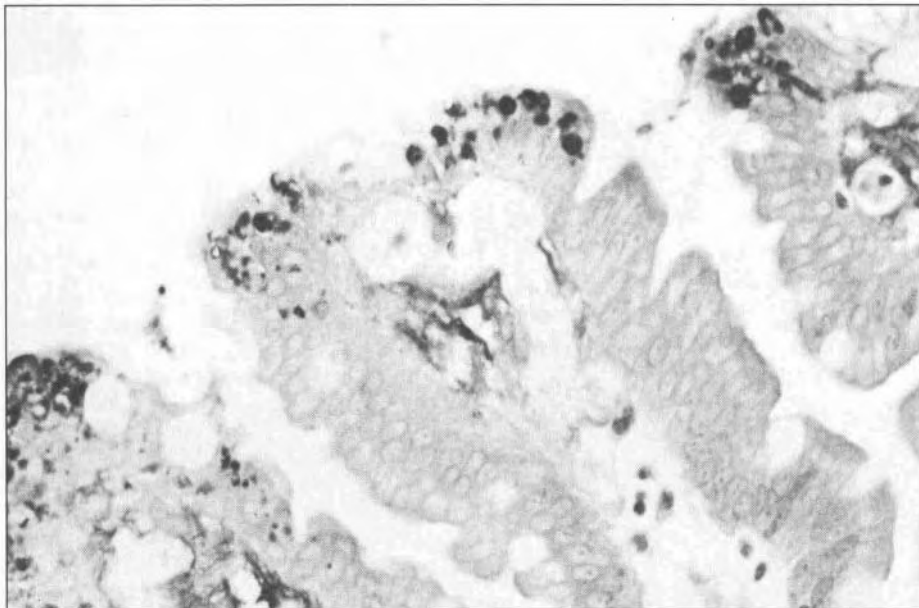


FIG.3: Micrograph of mucosa (coeliac disease) showing A₁AT immunoreaction as tiny globules in the surface epithelium. (Immunoperoxidase stain x 650).

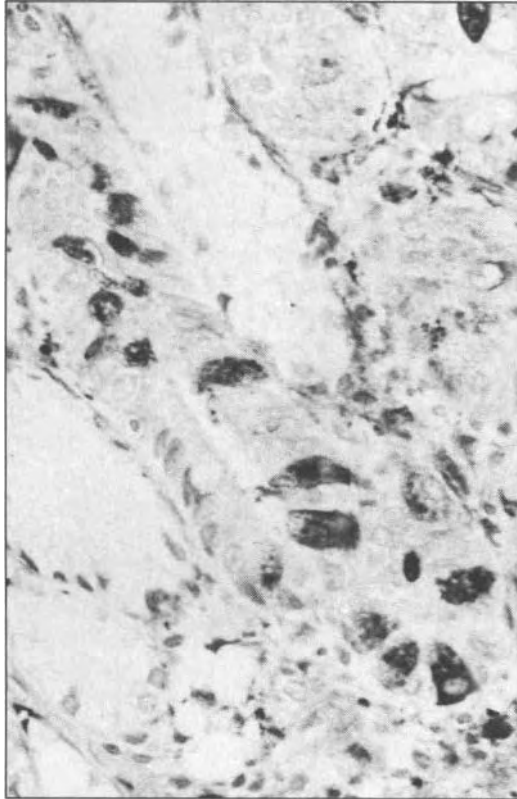


FIG.4: Micrograph of mucosa (coeliac disease) showing positive A₁AT within the crypt epithelial cells. Note the absence of staining in Brunner's glands (Immunoperoxidase stain x 650).

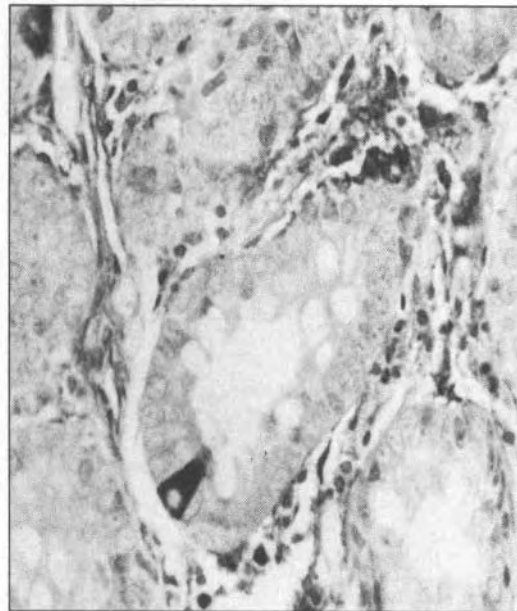


FIG.5: Micrograph of mucosa (coeliac disease) showing distinctly positive A₁AT staining of neuroendocrine cells and lymphomononuclear cells in the lamina propria (Immunoperoxidase stain x 650).

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