Ultrastructural binding sites of endomysium antibodies from sera of patients with dermatitis herpetiformis and coeliac disease

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Abstract

The ultrastructural binding sites of endomysium antibodies, specific serological markers of gluten sensitive enteropathy, were investigated in the rabbit oesophagus using the immunogold technique. Endomysium antibodies from sera of patients with dermatitis herpetiformis and with coeliac disease bound in an identical manner in a non-fibrillar material closely associated with fine collagenous-reticulin fibrils and also with similar fibrils connecting smooth muscle cells and elastic tissue in the endomysial connective tissue. These observations suggest that IgA antibodies in sera from patients with dermatitis herpetiformis and coeliac disease recognise a common antigen in an amorphous component associated with the reticular connective tissue of oesophageal lamina muscularis mucosae and thus confirm the probable identity of IgA class endomysium and jejunal antibodies.

Sera from patients with dermatitis herpetiformis and with coeliac disease contain three types of IgA class antibodies detected by an immunofluorescence technique on human and animal tissues: antireticulin,^{1,2} antijejunal,^{3,4} and anti-endomysium antibodies.^{5,6} The last two types are accepted as specific markers of gluten sensitive enteropathy.^{3,6} IgA, in contrast to IgG, antireticulin antibodies are also characteristic of coeliac disease when detected on the proper substrate.^{1,2} IgA antibodies from the sera of both types of patients which bind to normal jejunum show a similar staining pattern to that detected in the diseased jejunum of patients with gluten sensitive enteropathy.^{7,8} Therefore these antibodies were interpreted as the target organ related IgA class autoantibodies in coeliac disease.⁴

All three antibody types can be detected on both human and animal tissues.^{4,9} Recently, endomysium and antireticulin antibodies of human subtype,⁹ as well as endomysium and antijejunal antibodies⁴ were found to be identical. Previously the ultrastructural binding sites of serum IgA from dermatitis herpetiformis patients was determined on rabbit small bowel.¹⁰ To further examine possible differences between IgA class antibodies detected on different substrates, as well as between IgA class antibodies in sera from patients with dermatitis herpetiformis and coeliac disease, we carried out the first investigation to identify the target tissue constituents recognised by IgA antibodies from patients with these diseases in oesophagus at electron microscopic level using immunogold labelling.

Methods

IMMUNOFLUORESCENCE STUDIES

Serum samples taken from one coeliac and one dermatitis patient positive for endomysium antibodies in a titre 1:320, as well as serum from a patient with malabsorption syndrome without coeliac disease, were studied on rabbit and fetal human jejunum and on monkey and rabbit oesophagus as described previously.³

INDIRECT IMMUNOELECTRON MICROSCOPY (IEM)

Since the most characteristic feature of endomysium antibodies is a honeycomb like fluorescence along the lamina muscularis mucosae, we studied this part of the oesophagus, as described previously.¹⁰ Briefly, small pieces of tissue were fixed for two hours at room temperature in 4% paraformaldehyde and 0.1% glutaraldehyde in phosphate buffer (0.1 mol/l, pH 7.2) and were cut into 0.5-1 mm strips, rinsed in graded series of 10-20-30% sucrose-NaCl (0.9%) solution, and quick frozen in liquid nitrogen. The samples were then incubated for 48 hours at 4°C with endomysium antibody positive and negative sera diluted 1:20 in phos-

Figure 1: Indirect immunofluorescence: binding of IgA from sera of dermatitis herpetiformis (A) and coeliac (B) patients along the endomysium of oesophageal (A) and intestinal (B) smooth muscle layers. Note the similar honeycomb like fluorescence. (Original magnification: A $\times 120$, B $\times 180$.)

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Figure 2: Electron micrographs showing rabbit oesophagus stained by endomysium antibodies from serum of a patient with coeliac disease. Immunogold labelling: note gold deposition in a non-fibrillar component next to (A) fine collagenous-reticulin fibrils in the endomysial connective tissue of lamina muscularis; (B) fine collagenous-reticulin fibrils connecting the basement membranes of neighbouring smooth muscle cells. (Original magnification: A $\times 29\,000$, inset $\times 120\,000$, B $\times 82\,900$, inset $\times 140\,000$.)

phate buffered saline (0.01 mol/l, PH: 7.4), with mouse monoclonal antibody to human IgA (Zymed Laboratories, USA) (diluted 1:10 in phosphate buffered saline) and with 5 nm gold-conjugated goat antimouse serum (Janssen Life Sciences, Belgium), diluted 1:15 in Tris-HCl buffer (Tris 20 mmol/l, NaN_3 20 mmol/l, pH 7.6) at room temperature for two hours. The tissue was postfixed in Karnovsky and 1% OsO_4 solutions and embedded in epoxy resin (Epon 812) (Serva, Germany).

Results

IMMUNOFLUORESCENCE STUDIES (FIG 1)
Sera from both dermatitis herpetiformis and

coeliac patients produced an identical honey-comb like fluroescence along the endomysium of oesophageal (Fig 1A) and jejunal (Fig 1B) smooth muscle layers in titre of 1:160–1:320. No fluorescence was detected when control serum was studied.

ULTRASTRUCTURAL DETERMINATION OF ENDOMYSIUM ANTIBODY BINDING SITES USING IMMUNOGOLD LABELLING (FIGS 2–4)
Both studies with coeliac serum (Fig 2) and dermatitis herpetiformis serum (Fig 3) resulted in similar staining patterns on rabbit oesophagus. Endomysium antibody binding was detected in the endomysial connective tissue between the smooth muscle bands (Fig 2A) as well as between the smooth muscle cells (Fig 2B). Gold particles

Figure 3: Ultrastructural binding of IgA from dermatitis herpetiformis serum to rabbit oesophageal smooth muscle layer. Note gold particles in amorphous material along fine collagenous-reticulin fibrils (B) and (A) along fibrils adjacent endomysial elastic tissue. (Original magnification A $\times 40\,700$, inset $\times 156\,000$, B $\times 173\,000$.)

Figure 4: Control experiment on rabbit oesophagus with serum without endomysium antibodies. (Original magnification $\times 95\,000$.)

were deposited in amorphous material adjacent to fine collagenous-reticulin fibres of different diameters (Fig 2, 3B), connecting the basement membranes of neighbouring smooth muscle cells (Fig 2B), or adjacent marginal smooth muscle cells (Fig 3B). Between the smooth muscle cells and in the marginal perimysial connective tissue, elements of elastic tissue were also recognised. In these regions similar fine fibrils were detected between smooth muscle cells and elastic tissue. Here, too, gold particles were seen in a non-fibrillar material adjacent to the fine fibrils (Fig 3A). Control experiments with endomysium antibody negative serum did not show specific gold depositions (Fig 4).

Discussion

Indirect immunoelectron microscopy of rabbit oesophagus using endomysium antibody positive sera from patients with dermatitis herpetiformis and coeliac disease showed identical IgA antibody binding in an amorphous component adjacent to fine collagenous-reticulin fibrils of the endomysial connective tissue. These fibrils connected neighbouring smooth muscle cells, smooth muscle bands, and elastic tissue and smooth muscle cells.

In previous ultrastructural studies in rabbit jejunum with anti-jejunal antibody positive dermatitis herpetiformis serum, IgA antibodies also recognised amorphous material adjacent to collagenous-reticulin fibres underlying the basement membrane, around capillaries, between smooth muscle cells, and between smooth muscle cells and elastic tissue in jejunal vessel walls.¹⁰ Our recent experiences with anti-jejunal

and endomysium antibodies suggest that these antibodies could be identical.⁴

On the basis of ultrastructural staining characteristics, both endomysium and anti-jejunal antibodies are supposed to be an IgA anti-reticulin antibody. In recent absorption studies Hallström could not distinguish IgA class anti-reticulin antibodies. In recent absorption studies from endomysium antibodies reacting with monkey oesophagus.⁹

The biochemical composition of endomysium, similar to that of reticulin, is not precisely defined, and may correspond to a group of different fibrillar and amorphous components: collagen I and III fibres, elastic tissue, fibronectin, non-collagenous proteins, and proteoglycans.¹¹⁻¹³ Both endomysium and reticulin are argyrophilic and strongly periodic acid-Schiff reactive, probably because of the high proteoglycan content of the interfibrillar matrix. According to our observations, some of these interfibrillar components may represent the antigen recognised in patients with dermatitis herpetiformis and coeliac disease.

The results of the present immunoelectron microscopic study further corroborate the probable identity of endomysium and jejunal antibodies and show that sera of patients with dermatitis herpetiformis and coeliac disease react with an identical tissue component present in different animal and human tissues.

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