Endomysium antibodies in coeliac disease: an improved method

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Abstract

The ultra structural binding sites of endomysium antibodies have been studied on human umbilical cord tissue. The sensitivity and specificity of IgA endomysium antibodies were compared with recently described methods using basement membrane of smooth muscle of monkey oesophagus. Thirty adults affected by coeliac disease (10 in remission) and 75 healthy adult controls with normal intestinal mucosa (35 false antigliadin positive) were investigated. Sensitivity and correlation of endomysium antibodies with total villous atrophy in untreated coeliac disease patients were 100% on the human umbilical cord smooth muscles, and only 90% on the muscular layer of primate oesophagus. Indirect immunofluorescence was superior to peroxidase staining in detecting these IgA antibodies. The easy availability and enhanced testing sensitivity of the umbilical cord is an advance towards a better diagnostic tool for coeliac disease.

Patients and methods

COELIAC DISEASE

Serum samples from 30 patients (20 women and 10 men) with coeliac disease proved by biopsy according to the revised ESPGAN criteria,12 mean age 35 years (range 5–68), were examined. Twenty patients had flat intestinal mucosa; 10 were in remission while receiving a gluten free diet and had a histologically normal jejunal mucosa.

IMMUNOHISTOCHEMICAL EXAMINATION

Immunohistochemical examination to determine the presence of EmA were carried out on 5 μm thin cryostat sections of human umbilical cord and on commercially available sections of monkey oesophagus (EmA Kit Biosystems, Genova, Italy).

The umbilical cord was cut and embedded immediately after excision in tissue tek OCT (Miles-Bayer, Milano, Italy) or was frozen at −70°C for several days and then embedded in OCT.

The umbilical cord sections were fixed for 10 minutes in acetone at −20°C and for 30 minutes in chloroform at room temperature. After washing in phosphate buffered saline (PBS) 0·05 M pH 7·4 (Sigma, Milano, Italy) sections were incubated with 1% bovine serum albumin (Sigma), and after that, with undiluted or 1:5, 1:10, 1:50, and 1:100 diluted patient serum samples. After washing in PBS, sections were incubated in a dark room with fluoresceinisothiocyanate (FITC) conjugated antiserum samples against human IgA (Behring, Aquila, Italy) at a dilution of 1:10 in PBS. The slides were washed again, mounted in aqueous mounting medium
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(Merk, Roma, Italy), and examined by fluorescence microscopy.

Tissue sections, after incubation with five serum samples per group, were incubated with 1:15 diluted biotinylated antihuman IgA (Sigma) and, after washing in PBS, with avidin-biotin peroxidase (ABC Dako, Milano, Italy). After staining with aminoethylcarbazole and counter staining with haematoxylin, sections were mounted, and viewed by light microscope. The same procedure was used for staining formalin fixed umbilical sections. These sections were treated with 1% trypsin and endogenous peroxidase was inhibited by incubation with 1% H2O2.

Fifteen sections of umbilical cord were fixed in acetone and then stored at −20°C for several days. The fluorescence staining procedure was continued as described before using five serum samples per group.

The determination of EmA on monkey oesophagus was performed according to the instructions of the kit used.

Positive and negative controls for EmA (Biosystems) were used for each batch and reproducibility of the two different tests was checked on separate days.

Results

A comparison was made between the binding specificities of EmA on commercial available monkey oesophagus or human umbilical cord specimens.

Patients with Coeliac Disease

All 20 patients with active coeliac disease, flat jejunal mucosa, and positive for AGA were also positive for EmA. Their serum samples showed a honeycomb like fluorescence along the peritubular muscle layers of vessels on umbilical cord, a typical feature, staining the extracellular connective tissue (Figure). On monkey oesophagus the same reticular fluorescence pattern was seen around smooth muscle fibres in the muscularis mucosae. Only two patients with active disease were negative for EmA on monkey oesophagus but positive on human umbilical cord.

The 10 coeliac disease patients in remission with an histologically normal mucosa were all negative for EmA, both on umbilical cord and monkey oesophagus. The honeycomb pattern was absent, whereas a weak cytoplasmic fluorescence was present in the muscle cells.

Control Group

All patients with positive AGA but normal jejunal mucosa and without gastrointestinal symptoms were negative for EmA, as well as patients with normal mucosa and no AGA. No difference was seen between the two different methods. A weak, un specific cytoplasmic fluorescence could be seen in the muscle cells of both tissues.

The appropriate dilution tested on frozen umbilical cord sections was between 1:5 and 1:10. Differences in staining pattern on the endomysium were seen between sections embedded in OCT and those previously frozen, the second being less intensive and poorly defined. Only weak reaction was seen on acetone fixed frozen specimens incubated with coeliac serum samples. Peroxidase stained frozen or formalin fixed sections showed not a typical honeycomb pattern but only a weak uniform staining in the muscle layer around the vessel, without any difference between coeliac disease patients and control group.

Discussion

Indirect immunofluorescence was used to determine EmA in serum samples of coeliac disease patients and controls on monkey oesophagus and human umbilical cord. EmA present in serum samples of subjects with active coeliac disease are primarily of the IgA class binding in an amorphous component adjacent to fine collagenous reticular fibrils of the endomysial connective tissue. These fibrils connect neighbouring smooth muscle cells, smooth muscle bands, and elastic tissue.

We found the specific honeycomb binding of EmA to the connective tissue surrounding muscle cells of vessels within the umbilical cord in all tested coeliac disease patients with flat mucosa. Binding was absent in remission patients and normal subjects with false positive or negative AGA. In two untreated coeliac disease cases EmA bound only to human tissue but not to monkey oesophagus, thus confirming that human tissue is a more sensitive material than that of primates. The higher specificity and sensitivity of EmA in adult coeliac disease patients and healthy controls on human umbilical cord are an important step towards detecting a simple and highly precise diagnostic tool for coeliac disease. Furthermore, this simple immunohistochemical method permits unlimited testing and characterisation of EmA in any immunology department. Umbilical cord is an easily and commonly available and inexpensive human tissue, which is a perfectly adequate substitute for the distal oesophagus of endangered species. In our experience, the method based on FITC conjugated antihuman IgA antibodies is superior to that of peroxidase staining because of its improved reading. The only disadvantage is the short stability of the fluorescence.
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