
Immunoperoxidase staining of intermediate filaments in human small bowel

It has been shown that alcoholic hyaline has a complex antigenic structure. Using monoclonal and polyclonal antibodies to Mallory’s body protein, Mallory’s bodies contain not only unique antigens but also antigens that are common to intermediate filaments of normal hepatocytes and bile duct epithelium. Two of these monoclonal antibodies also react with an epitope of intermediate filaments in normal epithelium of the small bowel. 

A total of 45 small bowel biopsy specimens from 41 patients were obtained with a standard Meditech catheter. Biopsy specimens were available before and after gluten challenge from four patients with coeliac disease. Six micron sections were cut at −24°C in cryostat and dried overnight at 37°C. These were fixed in cold acetone (−20°C) for 15 minutes. After drying at room temperature two monoclonal antibodies produced in our laboratory, anti-JMB1 (51) and anti-JMB2 (31) in the form of undiluted culture fluid, were applied to the sections for 60 minutes. Sections were washed in phosphate buffered saline (PBS) for 10 minutes. Peroxidase conjugated rabbit anti-mouse IgG (diluted 1/20) was then placed on the sections for 30 minutes. After washing in PBS 3.3’-diaminobenzidine tetrahydrochloride (500 µg/ml) dissolved in PBS containing hydrogen peroxide (0.003%) was applied for five minutes. Slides were counterstained by weak haematoxylin, dehydrated in alcohol, cleared in xylene and mounted in DPX.

From the results, antibody from second clone (designated as 31 or anti-JMB2) gave a uniform staining pattern of ++ to ++++ in the epithelial cells of villi and crypts (figure) in biopsy specimens obtained from patients with and without coeliac disease. It also reacted with Brunner’s glands of the duodenum whenever included in the section. No staining was seen in the lamina propria cells and connective tissues implying its epithelial specificity. The notable finding of preservation of intermediate filaments, especially in the four patients before and after gluten challenge possibly reflects prompt recovery and formation of these microstructures. With regard to first monoclonal antibody (51 or anti-JMB1), staining was apparently similar as above but considerably weaker in intensity with an average score of +. No obvious differences were found in biopsies before and after gluten challenge.

Thus it seems that anti-JMB2 or 31 stain intermediate filaments distinctly in normal and diseased biopsy specimens showing the severe villous atrophy of coeliac disease. A consistently uniform staining score of ++ to +++++ was observed in all coeliac biopsy specimens showing severe villous atrophy. There appeared to be no disintegration of these unique filaments in biopsy specimens from patients with untreated coeliac disease. This suggests a structural but poor functional integrity of mucosa in coeliac disease. Further work is necessary to confirm these observations.

AK MALIK*
P RAWCLIFFE
JA MORTON*
DP JEWELL
JO’D MCxEE*

*Nuffield Department of Pathology, University of Oxford, Gastroenterology Unit, John Radcliffe Hospital, Oxford

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A K Malik, P Rawcliffe, J A Morton, D P Jewell and J O McGee

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