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ARE SOLUBLE AND/OR MEMBRANE-BOUND TRANSGLUTAMINASE ACTIVITIES INVOLVED IN INTESTINAL METABOLISM OF GLIADINS?

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Transglutaminase (TGases) are Ca^{2+}-dependent enzymes, which link peptide-bound glutamine to primary amines. We found that wheat gliadins, purified A-gliadin and their derived peptic-tryptic peptides are effective acyl donor substrate for: 1) TGase purified from guinea pig liver 2) TGase activity of rat small intestine, measured both in jejunum slices and in influx chambers, in which only the jejunal mucosa was exposed to the substrates. The enzyme activity in jejunal mucosa homogenate, expressed as pmol of spermidine incorporated into N,N-dimethylated casein /mg/hour, is low at birth (0.10±0.02,mean±SD), increases up to 0.31±0.065 at 7-10 days and then decreases to the adult level (0.09±0.03) at 15 days of age. On the contrary, the enzyme activity of submucosa+serosa is from the 7th day at values similar to that of the adult intestine (0.5 and 0.8 respectively). 50% of TGase activity was detected in the particulate fraction (not containing brush border membranes) of both young and adult rat mucosa. These results are consistent with the hypothesis that TGase activities may be involved in the metabolism of gliadins, or of their peptides, in the lumen or in the mucosa. The specific cellular localization of the enzyme should be clarified.

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EXPRESSION OF CLASS II MHC ANTIGENS IN THE INTESTINAL EPITHELIUM OF PAEDIATRIC COELIAC DISEASE (CD).

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Since CD of the infancy has been found to be associated with products of HLA-B, -DR, and -DQ loci of MHC, the expression of class II antigens in the intestinal mucosa of 16 children with CD, diagnosed according to ESPGAN criteria (4 m. and 12 f., aged 11 mos.- 14 yrs.) was evaluated immunohistochemically. Biopsy specimens were obtained from patients on gluten-containing diet, showing subtotal mucosal atrophy (8 patients), and on gluten-free diet with normal mucosa (10 patients). Control normal specimens were obtained from biopsies performed in 5 subjects with food allergy or other non inflammatory diseases. 4 microns acetone fixed cryostat sections were stained by indirect immunofluorescence and peroxidase with monoclonal antibodies to non polymorphic determinants of HLA-DR, -DP, and -DQ antigens. The results of the study showed that DR antigens are expressed in 100% of the cases, whereas no detectable levels of DQ antigens were found. DP antigens were demonstrable in 44% of the biopsies of coeliac subjects, without changes related to the diet; on the contrary, no DP antigen expression was found in the control group. Present findings suggest that the pattern of expression of class II MHC antigens in the intestinal epithelium of coeliac children does not show any relationship to the diet. No differences could be drawn between cases and controls, with the only exception of DP expression. This is evident in about the half of coeliac patients.

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HLA-DR EXPRESSION, NK CELLS AND IgE CONTAINING CELLS IN THE JEJUNAL MUCOSA OF COELIAC CHILDREN

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The expression of HLA-DR by surface and crypt epithelium and the numbers of cells of NK phenotype and of IgE containing cells were studied with monoclonal antisera using the peroxidase technique in 48 jejunal biopsy specimens taken from 35 coeliac children and 13 control specimens. The surface epithelial cells stained with HLA-DR antiserum in all specimens. In 21/28 specimens taken from coeliacs when on a gluten containing diet the crypt epithelium showed strong HLA-DR expression, while only 4/20 /$p<0,01$/ specimens of coeliacs on a gluten free diet and 1/13 specimens of controls had similar staining. Among the intraepithelial lymphocytes no NK cells were found. No difference was found in the numbers of IgE containing cells between the patients and controls. The strong expression of HLA-DR by the crypt epithelial cells in coeliac children on a normal diet suggest that these cells are involved in the presentation of the antigen

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JEJUNAL LYMPHOCYTE SUBSETS IN HEALTHY AND COELIAC CHILDREN

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A panel of monoclonal antibodies was used to determine the densities of T lymphocyte subsets in 48 jejunal biopsies of 35 children with coeliac disease (CD), 20 on gluten-free diet (GFD), 11 on normal diet and 17 on gluten challenge (GCh); and of 13 healthy controls. In lamina propria the age of the subject had a great influence on the T cell densities, with the numbers declining with age at a pace similar to that in peripheral blood in the control children. The correlation with the age was less obvious in CD patients. Among surface and crypt intraepithelial lymphocytes (IEL) the T suppressor cells predominated, and their numbers were significantly elevated on GCh as compared with CD patients on GFD, or the controls. The influence of the age on IEL T cell counts was negligible. A cell population bearing the "pan T" cell surface antigens but neither "T suppressor" nor "T helper" markers was identified within CD patients' IEL population, both on GFD and GCh. This cell population may be activated, and thus unable to express their cell surface antigens; or it may represent a unique cell population in the patients, relevant to the pathogenesis of coeliac disease.

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GLUTEN CHALLENGE IN POSTPUBERTAL CHILDREN WITH COELIAC DISEASE (CD): DETECTION OF MUCOSAL RELAPSE WITH IGA-CLASS RETICULIN ANTIBODY (ARA).

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Altogether 31 postpubertal children 15 to 22 years old with established CD were rebiopsied. 10 had mucosal abnormality indicating poor adherence to the diet. Normal gluten containing diet was reintroduced in 21 patients who had normal mucosa and were negative for ARA. The patients were retested for ARA every 3 months. IgA-ARA turned positive (≥1:50) within 3 to 24 months (mean 8 months) in 17 patients before (12) or at the same time with abdominal symptoms (5). Histologically a clear relapse of CD was seen in all cases. One patient developed major abdominal symptoms at 4 months. Her ARA were negative and small bowel mucosa showed slight changes. Three patients had no symptoms and a negative ARA test at 24 months. The mucosa was normal in two and flat in one. Thus the possible recovery of CD was 2/31 or 6.5 %. We conclude that IgA-ARA can in most cases be used to determine the time of rebiopsy during gluten challenge before any symptoms occur. To establish definite recovery of CD in cases with normal mucosa at 2 years of challenge, further follow up studies of ARA and later rebiopsy are needed.

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PREDICTABILITY OF SMALL BOWEL MUCOSAL DAMAGE BY RETICULIN (ARA) AND GLIADIN ANTIBODIES (AGA).

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The sera of 340 children undergoing small bowel biopsy because of suspected coeliac disease were tested for IgA- and IgG-class ARA and AGA. ARA were determined using an immunofluorescence method and AGA were measured by a solid-phase enzyme-linked immunosorbent assay. Altogether 44 children had flat mucosa (SVA), 43 partial villous atrophy (PVA) and 253 normal small bowel mucosa. The sensitivity of IgA-ARA to detect SVA was 93 %, specificity 100 % and positive predictive value 100 %. The corresponding figures for IgA-AGA were 86 %, 78 % and 41 %. IgA-ARA gave no wrong positive tests in contrast to that of IgA-AGA (59 %). The sensitivity increased (98 %) when the tests were combined meaning that the individual tests missed different patients. IgA-ARA was not suitable for screening SVA (sensitivity 41 %, positive predictive value 78 %) whereas IgG-AGA was a sensitive test (86 %) but its positive predictive value was only 25 %. In PVA the sensitivity of combined AGA tests was 88 % in contrast to that of ARA (1 %). We conclude that AGA tell when small bowel mucosal abnormality caused by gluten or other factors should be suspected and IgA-ARA emphasizes the need of small bowel biopsy to detect children with small, flat intestinal mucosa and coeliac disease.