47 MURINE T-CELL RESPONSES TO WHEAT GLIADINS

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There is evidence that not only alpha but beta, gamma and omega gliadins induce the mucosal changes of coeliac disease. There is also evidence that untreated coeliacs have high antibody titres to all these gliadins. We have used mouse T-cell lines to investigate the immunological cross-reactivity between these gliadins. Gluten-free Balb c mice were immunised with either alpha, beta, gamma or omega gliadin. Lymphocytes, isolated from the draining nodes, were cultured through three complete stimulation and rest cycles using the corresponding gliadins for stimulation. The specifically sensitised cell lines were tested in a stimulation assay against the other gliadins. The results show some cross-reactivity in all cases. Alpha-sensitive cell lines were most restricted, responding principally to alpha gliadin; omega cells responded equally to all gliadins. Conversely, alpha gliadin was the most effective stimulus across cell lines; omega was the least, stimulating principally omega cell lines.

We conclude that alpha gliadin is the most antigenic of the gliadins to these murine T-cells, presumably carrying the most determinants. However, the considerable cross-reactivity supports the belief that the immunological reaction in coeliac disease is against determinant(s) present in all the gliadins.

48 ORGAN CULTURE OF FOETAL RAT SMALL INTESTINE FOR TESTING GLUTEN TOXICITY - A REAPPRAISAL

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Recent reports of the use of organ culture of animal foetal small intestine to detect cereal toxicity have proposed its use for screening the toxicity of cereal peptides for coeliac mucosa. Some authors have assessed toxicity using morphological means, others by more objective biochemical means; the results have been variable. We have used foetal rat small intestine organ cultures to test the toxicity of gluten fraction III (GFIII), assessing the effect by morphological and biochemical means.

Small intestinal segments from 18-day old rat foetuses were used and were obtained from several foetuses in one litter for each experiment. They were cultured with and without GFIII for 48 hours. Segments before and after culture were observed histologically and significantly more developed definite tall villi after culture in the absence of GFIII ($p = 0.009$), associated with significantly less stratification ($p = 0.014$) and more columnar epithelial cells ($p = 0.024$). Alkaline phosphatase activity fell during culture, whereas α-glucosidase activity increased but there was no difference whether GFIII was present or not.

Gluten toxicity for foetal rat intestine was detected using morphological, but not biochemical, means. Morphological assessment was difficult however, due to considerable variability within sections. We do not consider this type of culture to be sufficiently reliable for routine investigation of cereal toxicity, and advise caution in the interpretation of the results.

49 CLONING AND EXPRESSION OF A COLONOCYTE-SPECIFIC FIMBRIAL ADHESIN FROM AN ESCHERICHIA COLI STRAIN ISOLATED FROM AN INFANT WITH SEVERE DYSENTERY-LIKE DIARRHOEA.

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E.coli strain 469-3 (021:NM) expresses mannose-resistant haemagglutination (MRHA) of human erythrocytes, adheres to cultured human epithelial (HEp-2) cells and to the brush border of human colonic (but not duodenal) enterocytes. The adhesin, an aggregate of a 14 kilodalton protein subunit, has now been identified by electron microscopy and consists of fine ~2-nm diameter fibrils. The chromosomally located genetic determinants of the adhesin were isolated by cosmid cloning and expressed in *E.coli* K12. Several recombinant cosmids expressing a MRHA phenotype were identified and 1 such clone was used to subclone smaller DNA fragments able to confer the same MRHA and adherence properties as the parent strain. A 16.4 kilobase chromosomal DNA fragment cloned in pBR322 (pGTH1) expressed MRHA of human erythrocytes, adhered to HEp-2 cells and to human colonocyte brush borders. The identity of the cloned adhesin was confirmed by biochemical, genetic, electron microscopical and immunological comparison with the adhesin synthesized by 469-3.

50 BINDING, INTERNALIZATION AND DEGRADATION OF VIP BY PIG JEJUNUM EPITHELIAL CELLS

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As VIP is involved in the intestinal water and electrolyte secretion and a VIP-ergic innervation of the epithelial layer has been demonstrated, we investigated binding, internalization and degredation of VIP by pig jejunum epithelial cells. Enterocytes were isolated by a non-enzymatic dissociation procedure. ^{125}I-VIP binding was time- and temperature dependent and was inhibited dose-dependently by VIP (K_D=1.5±0.2 nM, mean±SD; n=5) and secretin (half maximal binding 10μM) but not by other peptides. Internalized radioactivity was separated from cell surface-bound radioactivity by washing the cells with isotonic saline, pH2.5. At 37°C the amount of internalized radioactivity increased till 30 min and represented 30% of cell bound radioactivity. At 10°C, all radioactivity bound to the cells was acid dissociable. To investigate degredation of VIP, cells were incubated with VIP(5-20 nmol/l) and the reaction mixture was analysed by HPLC and metabolites were identified by amino acid analysis. After only 30 sec incubation, des(His)VIP(fragment 2-28) was identified as a major metabolite, representing 30% of the substrate In conclusion normal enterocytes internalized VIP but, in view of the rapid cell-surface degradation, it is suggested that VIP is not internalized as intact peptide. As VIP(2-28) has only 1% of the biological activity of VIP, its action in the gut may be terminated by aminopeptidases.

51 DETOXICATION ENZYMES IN SMALL INTESTINAL MUCOSA IN CHILDREN

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Liver is the main organ of detoxication. However, detoxication enzymes are present in the intestinal mucosa. We studied the activities of these enzymes in relation to mucosal structure.

Activities of different detoxication enzymes were measured in 194 samples of peroral small intestinal capsule biopsies from children in whom the biopsies were taken as a part of gastrointestinal investigations for various reasons. Alkaline phosphatase was used as reference and its activity was 161±9, 118±17, and 61±10 nmol/min/mg protein in samples with normal mucosa, partial villous atrophy, and total villous atrophy, respectively. Aryl hydrocarbon hydroxylase and ethoxycoumarin O-deethylase activities did not vary in relation to mucosal structure. On the contrary, glutathione peroxidase activity was significantly decreased in villous atrophy: 27.3±2.1, 21.4±4.0, and 4.8±1.6 pmol/min/mg protein in normal, partially atrophied, and severely atrophied samples, respectively. Also epoxide hydrolase was diminished in relation to mucosal structure: 43.6±2.0, 39.2±4.0, and 26.3±4.5 pmol/min/mg protein in the same order as above.

Changes in villous structure alter mucosal capacity to detoxify ingested foreign compounds and potentially toxic products of metabolism.

52 APPLE JUICE: INCOMPLETE ABSORPTION, BUT OF WHICH CARBOHYDRATE?

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Apple juice (AJ) is a popular drink in toddlers. Due to the widespread belief that it has an anti-diarrhoeal effect, it frequently is advocated in case of chronic diarrhoea. Recently, however, it has been suggested that AJ promotes diarrhoea in toddlers either due to fructose (F) or to sorbitol (S). AJ contains about 60g/l of F, 20g/l of glucose, and possibly up to 10g/l of S. Aim of this study was to investigate the incidence of incomplete absorption of AJ carbohydrates and to identify the responsible carbohydrate, using breath hydrogen (BH) tests. Methods. We studied 10 children, 7 without and 3 with chronic nonspecific diarrhoea (CNSD), all AJ consumers, ages 1-4½y. BH tests were done with 150-250ml of AJ. In 7 (2 CNSD), tests were repeated with F (10g); in 4/7 (1 CNSD) also with S (5g). BH increase >10ppm indicated positive (+ve) tests. Results. AJ tests were +ve in 5/7 controls (20-55ppm) and in all 3 CNSD patients (42-69ppm). In addition, 2/5 controls and 2/2 CNSD patients had +ve F tests (16-55ppm). The 3 controls with negative F tests had +ve S tests (16-29ppm); one CNSD patient had a negative S test. Actual S content of AJ (assessed by GLC) was 4.3g/l. In the CNSD patients, stools became normal after AJ elimination. Conclusions. These results indicate that AJ absorption frequently is incomplete in toddlers and may result in CNSD, and that neither F nor S can be held solely responsible for this phenomenon.