35 CHARACTERIZATION OF SERUM ANTI-GLIADIN IgA IN CHILDHOOD COELIAC DISEASE VERSUS OTHER GASTROINTESTINAL DISEASES AND CONTROLS

F.Mascart-Lemone, D.Delacroix, S.Cadranel, J.Duchateau
Dept.of Immunology,St-Pierre Hospital,Brussels,Belgium.

Titers and molecular sizesof anti-gliadin (GL) IgA were compared in the serum of 3 groups of children : (1) 100 "normal" controls, (2) 100 controls with several gastrointestinal diseases (acute gastroenteritis in 82, inflammatory bowel disease in 4, cow's milk allergy in 10 and cirrhosis in 5), (3) 19 coeliac diseases (CD) (13 untreated and 6 on gluten challenge). Anti-GL IgA were detected by solid-phase radioimmunoassay, and their molecular size was analyzed in all sera with > 40 arb.u. antibodies by sucrose density ultracentrifugation gradients. Anti-GL IgA titers were higher in untreated CD than in all other conditions ($p<0.001$), but some overlap of the antibody titers were noted. Though total serum IgA was 80% monomeric (m-IgA), 47% of anti-GL IgA was polymeric (p-IgA) in untreated CD. These antibodies were mainly dimeric and not complexed with secretory piece. After one month on gluten-free diet, anti-GL IgA titers were dramatically lowered, due mainly to the disappearance of p-IgA antibodies. In contrast, 83% of anti-GL IgA were m-IgA in 5 positive controls and respectively 85% and 79% were m-IgA in 2/6 positive CD during gluten challenge.
In conclusion, the serum anti-GL IgA response in untreated CD differs quantitatively (high titers) and qualitatively (high proportion of p-IgA) from that in other diseases and in CD children on gluten challenge (low titers - mainly m-IgA)

36 IMMUNOLOGICAL PROPERTIES OF GLIADIN PRESENTED VIA THE GUT

R Troncone, A Ferguson
Gastro-Intestinal Unit, University of Edinburgh and Western General Hospital, Edinburgh, Scotland, UK

When an antigen is first presented via the gut, either priming or suppression of the systemic immune response (oral tolerance) can result. Aim of this study was to establish if wheat gliadin behaves as an oral immunogen or tolerogen.
BALB/c mice were reared on normal diet (with 0.3% gliadin) or gluten free diet (second or later generation). Antigen was fed either as normal diet or as pure gliadin weighed and presented in an agar pellet. The immune status (tolerant or sensitized) was then defined by the antibody and CMI response to parenteral immunization (ELISA and footpad swelling test).
Mice on a normal diet or fed this diet for a week before immunization showed a significantly ($p<0.05$) reduced humoral response to gliadin compared to mice reared on gluten free diet. CMI was reduced to a lesser extent ($p<0.1$). Mice fed pure gliadin (5, 25 or 125 mg) one week before immunization had suppression of both antibody ($p<0.02$) and CMI ($p<0.05$) at the higher dose. 25 mg feed suppressed only the humoral response.
These results indicate that wheat gliadin can act as an effective oral tolerogen; however the type of immune response is influenced both by the amount and the form in which the antigen is presented to the gut.

37 INTESTINAL MUCOSA OF COELIACS IN REMISSION IS UNABLE TO REMOVE TOXICITY OF GLIADIN PEPTIDES ON IN VITRO DEVELOPING FETAL RAT INTESTINE AND CULTURED ATROPHIC COELIAC MUCOSA.

H.J.Cornell°,S.Auricchio*, G.de Ritis*,M. De Vincenzi°°,L. Maiuri*,V. Raia*,V. Silano°°.
°Dept of Applied Chemistry, Royal Melbourne Institute of Technology, Melbourne, Australia;*Clinical Pediatrics, II Medical School, University of Naples, Naples, Italy;°°Dept of Comparative Toxicology and Ecotoxicology, Istituto Superiore di Sanità, Rome, Italy.

Subfraction 2R of fraction 9 of a wheat gliadin's peptic-tryptic-pancreatic digest is known to be toxic in vivo for coeliacs (H.J.Cornell et al., Clin.Chim. Acta 31,123,1982). We have found that fractions 9 and 2R agglutinate K 562(S) cells and inhibit the in vitro development of fetal rat intestine and the increase of enterocyte height occurring in organ culture of atrophic coeliac mucosa (0.1-0.5 mg/ml medium). Other peptide fractions of the gliadin digest are devoid of such in vitro effects. Fraction 2R, after incubation with morphologically normal small intestinal mucosa of coeliacs in remission and ultrafiltration, was still able to agglutinate K cells and was very active in both culture systems, at low concentration (0.1 mg/ml); on the contrary, fraction 2R was inactivated after incubation with normal mucosa. In intestinal mucosa of coeliacs in remission either a primary (or secondary) enzyme deficiency or some other mechanisms may explain these results which are compatible with the hypothesis that there is a mucosal defect in handling gliadin peptides in coeliac disease.

38 A-GLIADIN RELATED SYNTHETIC PEPTIDES AGGLUTINATE UNDIFFERENTIATED K 562 S CELLS AND AFFECT IN VITRO DEVELOPING FETAL RAT INTESTINE AND CULTURED ATROPHIC COELIAC MUCOSA.

S. Auricchio*,A.Arco°°,G.D'Auria°, G.de Ritis*,M.De Vincenzi**,G.Magazzù°°,L.Maiuri*,V.Pavone°,V.Raia*, V.Silano**.
*Clinical Pediatrics,II Medical School, University of Naples, Naples,Italy;°Dept of Chemistry, University of Naples, Naples, Italy;**Dept of Comparative Toxicology and Ecotoxicology, Istituto Superiore di Sanità, Rome, Italy;°°II Clinical Pediatrics, University of Messina, Messina, Italy.

Peptides from wheat gliadins, A-gliadin and prolamins from cereals toxic for coeliac patients agglutinate K 562(S) cells; they also damage in vitro cultured fetal rat intestine and atrophic coeliac mucosa. The largest common sequences among the in vitro active A-gliadin peptides were -Pro-Ser-Gln-Gln and $-(Gln)_3$-Pro-. The following peptides all containing the aminoacid sequence $-(Gln)_3$-Pro have been synthesized: the pentapeptide Tyr-$(Gln)_3$-Pro, its dimer and tetramer and the eptapeptide Gln-Pro-Tyr-$(Gln)_3$-Pro in their free and N-acetylated forms and the Pyroglutamic derivate of the heptapeptide (Pyr 7). Pyr 7 agglutinated cells and inhibited the in vitro development of fetal rat intestine (medium's concentration 0.5-2 mg/ml); it was non toxic on the in vitro cultured coeliac atrophic mucosa. The N-acetylated form of the pentapeptide's tetramer (1 mg/ml) also damaged the atrophic coeliac mucosa in 3 cultured biopsies. These results suggest that the sequence $-(Gln)_3$-Pro when part of a larger peptide may be toxic in vitro for the atrophic coeliac mucosa.

39 AN IN VITRO MODEL SYSTEM FOR STUDYING THE BIOSYNTHESIS OF BRUSH BORDER MEMBRANE (BBM) HYDROLASES IN COELIAC DISEASE (CD).

H.Y. Naim, P. Ambühl, E.E. Sterchi, H. Gaze, M.J. Lentze. Dept. of Pediatric Gastroenterology, University of Berne, Inselspital, Switzerland.

In an effort to mimic the in vitro situation in CD, the effect of gliadin peptides III and IV on the biosynthesis of a number of BBM hydrolases was studied. Thus, biopsies from patients with CD in remission were labelled with ^{35}S-methionine for 24 hours in the presence or absence of gliadin and α-casein. Sucrase (SI), lactase (LPH), maltase (MGA), aminopeptidase N (APN), dipeptidylpeptidase IV (DPPIV) and angiotensin-converting enzyme (ACE) were immunoprecipitated with monoclonal antibodies and analyzed by SDS-PAGE, fluorography and densitometric scannings. Gliadin inhibitory effect on the synthesis of the hydrolases is expressed as % decrease in labelling intensity versus control. Thus SI synthesis was inhibited by 72%, that of MGA and APN by 85-88%, whereas DPPIV, ACE and LPH were completely absent in gliadin-treated biopsies. α-Casein affected the synthesis of SI, LPH and ACE by about 35%, APN was not affected whereas DPPIV was to about 55 %. These values are significantly lower than their counterparts in gliadin-treated biopsies. Conclusion: This in vitro system provides therefore a promising approach for studying the mechanisms involved in the induction of mucosal damage at the molecular level.

40 SELECTIVE BINDING TO MANNAN OF GLIADIN PEPTIDES WHICH AGGLUTINATE UNDIFFERENTIATED K 562 S CELLS AND INHIBIT IN VITRO DEVELOPMENT OF FETAL RAT INTESTINE.

L. Maiuri°,S. Auricchio°,M. Cardelli*,G. de Ritis°,M. De Vincenzi*,F. Latte°,E.Mancini*,V. Raia°,V. Silano*.
°Clinical Pediatrics,II Medical School, University of Naples,Naples, Italy;*Dept of Comparative Toxicology and Ecotoxicology, Istituto Superiore di Sanità, Rome, Italy.

Mannan and N,N'-diacetyl-chitobiose and N,N',N''-triacetyl-chitotriose prevent the agglutinating activity on K 562(S) cells and the damaging in vitro effect on fetal rat intestine and atrophic coeliac mucosa of mixtures of gliadin peptides and pure A-gliadin peptides (S.Auricchio et al.,J. Ped. Gastroenterol. and Nutr. 4,923, 1985). We separated by cromatography on mannan-Sepharose 4-B of peptic-tryptic(PT) digest of gliadins: 1) A small fraction (C) which was very active in agglutinating cells and inhibiting the in vitro development of fetal rat intestine (20γ/ml medium). Both effects were prevented by the sugars. 2) A much larger fraction (B) which is devoid of both activities. These results show that only a few peptides of a gliadin digest, which are specifically bound by mannan, are active in the two in vitro systems.

Peptide fraction	Minimal concentration agglutinating all cells (mg/l)	Percentage of the total digest
A) PT digest	73	100
B) Fraction not bound by mannan	No agglutination up to 4666	92-97
C) Fraction bound by mannan	0.8	0.15-0.2