

10th International Symposium on Coeliac Disease

Pathogenesis and Outcomes

**Faculté de Médecine Necker
Institut Fédératif de Recherche Necker-Enfants malades (IRNEM)
Inserm
Assistance Publique des Hôpitaux de Paris
Groupe d'Études et de Recherche sur la Maladie Coeliaque
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10th International Symposium on Coeliac Disease
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C1 COMPLEMENTARITY OF GENETIC AND ENVIRONMENTAL FACTORS IN THE ETIOLOGY OF IMMUNE MEDIATED DISEASES. APPLICATION TO CELIAC DISEASE

Jean-François Bach

Coeliac disease is the result of the unfavourable interaction between predisposition genes and environmental factors. These environmental factors include in the first place a major triggering factor gliadin but also most probably protective factors. The nature of these protective factors which represent the plausible explanation for absence of clinical disease in discordant monozygotic twins and in some HLA concordant siblings are still ill-defined. Their role is suggested by the increased incidence of the disease in developed countries in which there is also a consistent increase of the frequency of the autoimmune and allergic diseases. Much information is available on the mechanism of protection in the latter conditions. An important question is to evaluate the modalities of the interaction between these various environmental factors and the products of the predisposition genes. Another practical issue is to search for safe therapeutic methods which could substitute for the insufficient immunostimulation secondary to decrease infectious load which is I assumed to explain the increased frequency of autoimmune and allergic diseases.

C2 CONTRIBUTION OF GENETICS TO THE UNDERSTANDING OF COELIAC DISEASE

Maria Cristina Mazzilli – Department of Experimental Medicine and Pathology

University La Sapienza of Rome - Italy

Coeliac disease (CD) is a complex genetic trait with multiple genetic and environmental components contributing to susceptibility. A number of chromosomal regions containing genes influencing CD have been suggested by different groups, but to date the only established susceptibility factor maps in the HLA complex. The human leukocyte antigen (HLA) system comprises class I and class II genes controlling highly polymorphic proteins involved in the presentation of peptides to the T-cell receptor. Specific alleles at these HLA loci predispose their carriers to the development of diseases, usually those suspected to be of autoimmune aetiology.

The first reports of an association between an HLA antigen and coeliac disease date back to 1972. Due to the high level of linkage disequilibrium (LD) in the HLA region, it took almost 20 years to establish that the primary disease susceptibility was due to particular HLA-DQ heterodimers encoded by the DQA1 and DQB1 genes. However, several data suggest that DQ2 is not the only genetic risk factor for coeliac disease in the HLA region but the LD has hindered identification of other predisposing loci close by.

The ongoing CD Component of the 13th IHWC aims at characterising the influence of non-class II HLA genes on CD susceptibility. The approach is to study microsatellite markers within or near the HLA region in families and in DR-DQ matched patients and controls. The proceedings of this international effort will be discussed.

C3 EVALUATION OF THE GENETIC COMPONENTS OF COELIAC DISEASE IN THE EUROPEAN POPULATION

Françoise Clerget-Darpoux

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On behalf of the European Genetic Cluster

We report the results obtained on the genetic basis of coeliac disease by a European cluster (QLRT-1999-00037) in five European populations : Italy (Greco), Finland (Partanen), Sweden-Norway (Ascher-Sollid), United Kingdom (Ciclitira) and France (Clerget-Darpoux).

The total collection includes 525 families with at least 2 affected sibs, and 644 families with one affected child. All parents are available for the simplex families and most of them for the multiplex.

Four partners performed a full genome scan. Subsequently, replication using independent samples, focussing on implicated genome areas was performed. Apart from HLA, none of the individual results gave a very high level of significance, but some of them seem to pinpoint the same area of the genome. To globally interpret the linkage results, both a meta-analysis and a mega-analysis were performed. The two approaches do not leave any doubt about the presence of a genetic risk factor in the 5q31-33 region. Although less significant, the CTLA4/CD28 region on 2q appears promising. Consequently, we focused on the following regions: HLA, CTLA4 and 5q. In fact, the three regions of interest require different approaches.

- In the HLA region, the involvement of the DQ2 and DQ8 heterodimers was already known. The questions were: a) How are these heterodimers distributed among patients? ; b) Can the DQ genes explain the whole HLA component?

- The CTLA4 region was studied as candidate by TDT of 9 polymorphisms, spanning a 3.3cM region at 2q33 by all partners. Discordant results were obtained. This will be discussed, in light of the linkage disequilibrium observed in the different populations.

- In contrast, the position of the risk factor in the 5q31-33 region is unclear. Two approaches have been adopted to identify the gene(s) involved: a) the study of candidate genes identified by function; b) a systematic search across the region, for association with SNPs using DNA pools.

The synergistic efforts of the European genetics cluster, reported in detail elsewhere at the meeting, have already been very fruitful. Collaboration should be pursued, to fully unravel the genetic component of coeliac disease.

C4 IS THERE A DIRECT SPECIFIC CITOTOXIC EFFECT OF GLIADIN ON THE INTESTINAL MUCOSA ?

S. Auricchio (Naples, Italy)

Gliadin peptides are known to have a “toxic” effect in vitro on cell lines and developing intestine. We found a strict correlation for a number of cereal proteins and gliadin peptides between in vitro/in vivo damaging activity for the celiac intestine on the side, and for the developing fetal rat intestine and K 562 S and CaCo 2 cells, on the other side. Similar results were obtained in other laboratories in various in vitro systems (for a review see 1).

An apparent disagreement exists in the literature between the “immunodominance and “toxicity” of different gliadin fragments.

The A gliadin peptide 31-43 damages the enterocytes of cultured atrophic celiac mucosa and, in treated celiac mucosa, induces expression of HLA-DR on crypt enterocytes as well as appearance of CD 25+cells in lamina propria (2). The same peptide, infused in celiac jejunum, causes, in serum,

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transient increase of IL-1 α and IL-2R and, in the small intestinal mucosa, increase of CD3+ and $\gamma\delta$ + IEL density, in lamina propria swelling, lymphoid infiltration and increase of PGE₂/LTC₄, and, finally decrease of brush border disaccharidases: architectural changes resulted in infiltrative hyperplastic lesions with enlarged crypts (3). Similar results were obtained *in vivo* on small intestinal (4) and oral (5) mucosa with the peptide 31-49. On the contrary, the peptide 31-43 does not activate celiac intestinal CD4+T clones (6).

On the other part the A gliadin peptide 56-68, which is a dominant epitope for celiac intestinal CD4+T cells (6), is not able to damage *in vitro* (7) and *in vivo* (3) the celiac intestine. Furthermore, *in vitro* gliadin challenge of treated celiac mucosa induces "early" immunological modifications, which are not T cell dependent (8).

The hypothesis is that in the celiac intestine, through still unknown specific mechanisms, there is, together with an adaptive T cell response, a direct (toxic?) effect of some gliadin peptides on cells of the innate immune system, such as enterocytes, macrophages and dendritic cells. The results of new studies on the effect of gliadin peptides on these cells will be presented.

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C5 IS THERE A PRIMARY INCREASE IN EPITHELIAL CELL PERMEABILITY IN COELIAC DISEASE?

Alessio Fasano, M.D.

Despite the progress made in understanding the immunological aspects of coeliac disease (CD) pathogenesis, the early steps that allow gliadin to cross the intestinal barrier are still largely unknown. We have recently reported that zonulin, the eukaryotic analogue of the *Vibrio cholerae*-produced zonula occludens toxin (Zot), modulates the permeability of intestinal tight junctions (tj) and is up-regulated in CD. Both zonulin and Zot engage to an intestinal receptor with subsequent activation of an intracellular signaling leading to the disassembly of intercellular tj. To establish the direct effect of gliadin on the activation of the zonulin system, both mammalian intestines (rat, rabbit, and human tissues) and a non-tumoral murine cell line (IEC6 cells) were used. Incubation of IEC6 cells with gliadin led to a reversible, PKC-mediated actin polymerization temporarily coincident with luminal (but not serosal) zonulin release. A significant reduction in tissue resistance (TEER) was observed after mucosal addition of gliadin on either rabbit or rat small intestines. This reduction was paralleled by decreased mRNA expression (evaluated using PCR Real Time with the TaqMan probes technique) of occludin, the main tj protein. Pre-treatment with the zonulin inhibitor FZI/0 abolished the gliadin-

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induced actin polymerization and t_j disassembly but not zonulin release. No significant changes were observed when tissue was treated with similar concentrations of bovine serum albumin used as control. Similar results were obtained when duodenal biopsies from CD treated patients were mounted in the polarized microsnapwell system and exposed to gliadin added to the luminal side of the tissue. Gliadin induced a time-dependent decrement in both TEER and occludin mRNA expression that was blocked by pre-treatment with FZI/0. No significant changes were observed in untreated tissues. Interestingly, intestinal tissues obtained from healthy subjects and exposed to gliadin showed a decrease of the mRNA occluding and TEER similar to that observed in CD-derived tissues. In conclusion, gliadin induces zonulin-dependent actin polymerization, followed by decrement of occludin expression and t_j disassembly both in healthy and CD intestinal tissues. These changes lead to an increased intestinal permeability, suggesting that gliadin may play a pivotal role in facilitating its own passage to the submucosal compartment and, therefore, initiating the immune activation typical of CD in genetically-susceptible individuals.

C6 ARE THERE PRIMARY CHANGES IN THE TRANSPORT AND PROCESSING OF GLIADIN PEPTIDES IN THE MUCOSA OF COELIAC PATIENTS ?

M. Heyman, Matysiak-Budnik T, Candalh C, Dugave C, Cellier C.4 and Cerf-Bensussan N1.

1INSERM E9925, Faculté Necker, Paris, 2Laboratoire DIEP, CEA/Saclay, Gif sur Yvette, and 4Hôpital Européen Georges Pompidou, Paris, France.

Coeliac disease (CD) is a gluten-induced enteropathy whose pathomechanism is not fully understood. The present study analyse the transport and processing of two A-gliadin peptides, peptide 31-49 (toxic) and peptide 57-68 (immunodominant), across duodenal biopsies of 27 CD (active or treated) and 8 control patients.

Methods : Peptides 31-49 or 57-68 were radiolabelled (^3H) and placed in the mucosal compartment (200 $\mu\text{g}/\text{ml}$) of duodenal biopsies mounted in Ussing chambers during 3 hours: Electrical resistance (R), an index of epithelial integrity, was monitored, mucosal to serosal peptide fluxes were quantified and the peptide processing during transport was assessed using HPLC radio-chromatography.

Results: ^3H -equivalent-peptide fluxes were similar for both peptides in control patients (3564 ± 1733 and 4217 ± 1179 $\text{ng}/\text{h}\cdot\text{cm}^2$ for peptide 31-49 and peptide 57-68 respectively). In patients with active CD, peptide 31-49 fluxes doubled compared to Controls (8221 ± 3322 $\text{ng}/\text{h}\cdot\text{cm}^2$, $p < 0.001$) whereas peptide 57-68 fluxes were not significantly enhanced (4913 ± 1627 $\text{ng}/\text{h}\cdot\text{cm}^2$, $p = 0.6$). R was decreased in active CD (12.9 ± 4.4 vs 17.4 ± 3.4 $\text{ohms}\cdot\text{cm}^2$) but no major peptide leakage occurred, since in any case, less than 0.3% was transported during the 3 hour-experiment. The intestinal processing was very different for the two peptides: gliadin 57-68 started to be degraded in the mucosal compartment (brush border enzymes) and was almost totally degraded after intestinal transport, both in CD and control patients. Peptide 31-49 was resistant to brush border enzymes degradation in CD and Controls. It was highly degraded during transepithelial transport in control or treated CD patients, but in active CD patients, high amounts of intact peptide 31-49 and/or hydrophobic metabolites were recovered in the serosal compartment. Such features were not found for peptide 57-68.

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Conclusion: The intestinal mucosa (epithelium) seems to process differently gliadin peptides involved in coeliac disease. The abnormal processing of gliadin 31-49 in active CD patients may be related to its toxicity. By contrast, the high rate of mucosal degradation of gliadin 57-68 is difficult to reconcile with its immunodominance, but may explain its apparent lack of

C7 IMMUNOELECTRON MICROSCOPICAL ANALYSIS OF GLIADIN TRANSPORT PATHWAYS WITHIN ENTEROCYTES

K.-P. Zimmer, T. Mothes, E. Méndez, P. Ciclitira

Klinik und Poliklinik für Kinderheilkunde, Universitätsklinikum Münster, Albert-Schweitzer-Str. 33, 48149-Münster, Germany

There is increasing evidence that enterocytes modulate immunological functions of the intestinal mucosa. Enterocytes are able to express HLA class II antigens and to present antigens to T-lymphocytes. Our knowledge how antigens cross the intestinal barrier is limited. Several studies indicated to a transcellular as well as a paracellular pathway of antigen uptake into the lamina propria. We have recently shown that ovalbumin is quickly transported into HLA class II positive late endosomes of naive enterocytes from BALB/c mice which generate oral tolerance to this food antigen. The aim of our study is to characterize the transcellular pathways of specific gliadin peptides within enterocytes.

Duodenal biopsies of celiac disease (CD) patients as well as healthy and diseased controls were incubated with Frazer's Fraction under in-vitro and in-vivo conditions at 0, 10 and 30 min. We prepared thin frozen sections from these biopsies, labeled them by antibodies against gliadin and visualized their binding sites by immunogold particles within the electron microscope. A polyclonal antibody against α -gliadin and two monoclonal antibodies against specific gliadin peptides (WB8 and R5) were applied to examine whether the intracellular transport of toxic and non-toxic gliadin peptide sequences differ. Polyclonal antibodies against tissue transglutaminase (tTG) and lysosome-associated membrane proteins (LAMP) were used to define co-localization sites of gliadin with tTG and HLA class II antigens in late endosomes.

We found gliadin within vacuoles and Golgi complexes of enterocytes in the biopsies of most CD patients. Antibodies recognizing toxic gliadin peptide sequences did not label late endosomes but Golgi complexes, the apical and basolateral membrane of enterocytes. tTG co-localized with gliadin in Golgi complexes, vacuoles, the apical and the basolateral membrane of enterocytes. In addition we found enterocytes which quickly took up gliadin into the cytosol of enterocytes.

C8 EPIDERMAL TRANSGLUTAMINASE (TGASE 3) IS THE AUTOANTIGEN OF DERMATITIS HERPETIFORMIS

Miklós Sárdy, Sarolta Kárpáti*, Barbara Merkl‡, Mats Paulsson‡, Neil Smyth‡*

*Department of Dermato-Venereology, Semmelweis University, Budapest, Hungary

‡Institute for Biochemistry II, Medical Faculty, University of Cologne, Köln, Germany

Gluten sensitivity typically presents as celiac disease, a common chronic small intestinal disorder. However, in certain individuals it is associated with dermatitis herpetiformis, a blistering skin disease

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characterized by granular IgA deposits in the papillary dermis. While tissue transglutaminase has been implicated as the major autoantigen of gluten sensitive disease, there has been no explanation as to why this condition appears in two distinct forms. Here we show that while sera from patients with either form of gluten sensitive disease react both with tissue transglutaminase and the related enzyme epidermal (type 3) transglutaminase, antibodies in patients having dermatitis herpetiformis show a markedly higher avidity for epidermal transglutaminase. Further, these patients have an antibody population specific for this enzyme. We also show that the IgA precipitates in the papillary dermis of patients with dermatitis herpetiformis, the defining signs of the disease, contain epidermal transglutaminase, but not tissue transglutaminase or keratinocyte transglutaminase. These findings demonstrate that epidermal transglutaminase, rather than tissue transglutaminase, is the dominant autoantigen in dermatitis herpetiformis and explain why skin symptoms appear in a proportion of patients having gluten sensitive disease.

C9 GENERATION OF THE INTESTINAL T CELL REPERTOIRE AGAINST GLIADIN IN CHILDREN

Frits Koning,

Department of Immunohematology and Blood Transfusion, Leiden University Medical Center, Leiden
Coeliac disease is strongly associated with HLA-DQ2 and HLA-DQ8. In the small intestine of coeliac disease patients, T cells are present that respond to gluten in the context of these HLA-DQ2/8 molecules. Gluten is a complex mixture of gliadin and glutenin proteins, and we have identified and characterised a series of gliadin and glutenin peptides that are recognised by HLA-DQ2/8 restricted intestinal T cells isolated from adults and children with coeliac disease. While some of these peptides are recognized in their native form, the majority of the peptides are recognised only, or better, when particular glutamine residues in the peptides are modified to glutamic acid. This modification is mediated by the enzyme tissue transglutaminase (tTG), which is expressed in the small intestine. We have demonstrated that tTG modifies only particular glutamines in the peptides, and that the specificity of the enzyme matches the specificity of the gluten specific T-cells. Since this indicated that the selective modification of gluten peptides by tTG is tightly linked to gluten toxicity, we have investigated the specificity of the enzyme. We found that the spacing between glutamine and proline, the two most abundant amino acids in gluten, plays an essential role in the specificity of modification by tissue transglutaminase. On the basis of the resolved specificity of tTG, we were able to design algorithms that could be used to identify T cell stimulatory peptides in gluten molecules. Moreover, these algorithms identified many similar peptides in the gluten like hordeins from barley and secalins from rye, but not in the avenins from oats. Oats is the only cereal that is tolerated by coeliac disease patients and since the avenins contain significantly lower percentages of proline residues this offers a plausible explanation for the lack of toxicity of oats. Thus, the unique amino acid composition of gluten and related proteins in barley and rye favours the generation of toxic T cell stimulatory gluten peptides by tTG, and explains their toxicity in genetically predisposed individuals.

These results offer an explanation for the association of coeliac disease with HLA-DQ2/8, because the modification of gluten by tTG generates peptides that bind with high affinity to these HLA-molecules. This knowledge may now be used to devise novel strategies for intervention in the disease

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development. These range from blocking tTG activity or HLA-DQ peptide binding capacity, to more specific intervention by reintroduction of specific tolerance to the identified toxic gluten peptides. Moreover, the identification of the toxic gluten sequences and the non-toxic counterparts in the oats derived avenins, provides an innovative rationale for the development of non-toxic gluten molecules. Finally, the results offer possibilities for the development of a novel test to detect toxic gluten peptides in food products, a test that can also be used to screen for less toxic wheat varieties.

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Vader W, de Ru A, van der Wal Y, Kooy Y, Benckhuijsen W, Mearin L, Drijfhout JW, van Veelen, P and Koning F. Specificity of tissue transglutaminase explains cereal toxicity in celiac disease. *J. Exp. Med.* 195: 643-649 (2002).

C10 THE T CELL RESPONSE AND CONTROL OF COELIAC DISEASE DEVELOPMENT

Ludvig M. Sollid, Helene Arentz-Hansen, Burkhard Fleckenstein, Knut E.A. Lundin, Stephen McAdam, Øyvind Molberg, Shuo-Wang Qiao and Hanne Quarsten.

Institute of Immunology and Department of Medicine, Rikshospitalet, University of Oslo.

The coeliac enteropathy develops as a result of interplay between genetic and environmental factors. Gluten is clearly a critical environmental component and both HLA and non-HLA genes are predisposing genetic factors. The HLA effect is chiefly mediated by DQ2 (DQA1*05/DQB1*02) in the majority and by DQ8 (DQA1*0301/DQB1*0302) in the minority of the patients. CD4+ T lymphocytes, which recognise gluten peptides presented by DQ2 or DQ8, can be isolated from small intestinal biopsies of coeliac patients but not from controls. Several distinct gluten T cell epitopes exist in adult patients. There is a hierarchy with respect to how frequently the various epitopes are recognised by the patients. Some of the epitopes are immunodominant. In gliadin proteins, the epitopes cluster in regions rich in proline residues. Most of the epitopes are recognised in a deamidated form where specific glutamine residues have been converted to glutamic acid. Accumulating evidence indicates that this deamidation is mediated by the enzyme tissue transglutaminase. Tissue transglutaminase can either transamidate or deamidate glutamine. The ratio of deamidation to transamidation is drastically influenced by local factors. Tissue transglutaminase has different affinities for different gluten peptides. The characterised specificity of the enzyme explains the differences in affinities and indicates that the enzyme is involved in selection of gluten T cell epitopes. The enzyme specificity is also a contributing factor to the clustering of epitopes to proline rich regions. Oats is considered safe to coeliac patients. However, in one coeliac patient who is clinically hypersensitive to oats, we have isolated intestinal DQ2 restricted T cells that recognise tissue transglutaminase modified avenins. This finding may indicate that oats are harmful to at least some coeliac disease patients. Overall, our results point to control of the immune response to gluten and similar proteins by intestinal T cells restricted by the DQ2 or DQ8 molecules. This is likely to be a critical checkpoint for the development of coeliac disease and could explain the dominant genetic role of HLA in the disorder.

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C11 RELATIONSHIP BETWEEN CLINICALLY TOXIC AND EXPERIMENTALLY IMMUNOSTIMULATORY PEPTIDES

Paul J Ciclitira MD PhD FRCP

Gastroenterology Unit (KCL) Rayne Institute, St Thomas' Hospital London SE 1 7EH

Coeliac disease is a result of a breakdown in oral tolerance to dietary gluten leading to inappropriate stimulation of small intestinal CD4+ lamina propria T cells which secrete IFN-gamma. Enteropathy characteristic of coeliac disease is induced in vitro in normal small intestine by supernatants from activated gluten sensitive T cells clones.

There are discrepancies in the literature between in vitro T cell antigenicity and in vivo toxicity of gluten peptides. A-gliadin 31-49 is celiac toxic by in vitro organ culture and when infused in vivo. However no small intestinal T cell clone reactive to this peptide has ever been isolated. On the other hand, T cells specific to the peptide 55-75 of α -gliadins have been isolated and cloned. This peptide has also been shown to be toxic in vivo. It has been suggested that peptide(s) in this region may be immunodominant.

A-gliadins 31-49 may be a minor epitope with low precursor cell numbers. Additionally some in vitro T cell growth protocols may favour selection of cells specific to the immunodominant peptide(s).

C12 IMMUNOREGULATORY ROLE OF NKT CELLS.

Agnès Lehuen, Véronique Laloux, Jan Novak and Lucie Beaudoin.

INSERM U561, Hôpital St Vincent de Paul,

In coeliac disease, the role of genetic (particular HLA genes) and environmental (gluten toxicity) factors is well established, however little is known about defects in immuno-regulation in these patients. All individuals harbor auto-reactive T cells that are normally kept in check by several tolerance mechanisms among them suppression by regulatory T cells. Recently, many advances have been done in the characterization of such regulatory T cells. Both Tr1 cells and CD25+ CD4+ T cells have been shown to inhibit the development of various pathogenic T cells responses, among them intestinal inflammation. These both regulatory T cells are conventional $\alpha\beta$ T cells expressing a diverse repertoire. Another subset of T cells, namely NKT cells, are also able to prevent the development of auto-immune diseases.

NKT cells, which are non-conventional $\alpha\beta$ T cells, have been conserved through mammalian evolution. NKT cells are restricted by CD1d, a non-polymorphic MHC class I-like molecule, that presents glycolipids. Most NKT cells express an invariant TCR α chain composed of V α 14-J α 281 segments in mice and V α 24-J α Q segments in human. NKT cells express molecules common to the NK cell lineage and have an activated phenotype. After activation, NKT cells rapidly release massive amounts of cytokines, such as IFN- γ and IL-4, and trans-activate various cells of the immune system. This can explain why they have been involved in many types of immune responses. Importantly, NKT cells also can prevent the development of pathological autoimmune responses leading to disorders such as type 1 diabetes, experimental autoimmune encephalomyelitis and graft-versus-host disease.

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Recent insights on the role of NKT cells in the prevention of auto-immune attack will be shown. In particular, we have analyzed the effect of NKT cells on the activation, proliferation, differentiation and migration of pathogenic CD4 T cells.

C13 REGULATORY CHANGES INDUCED BY MICROENVIRONMENTAL FACTORS:ROLE OF INTERFERON ALPHA

Thomas T MacDonald and Giovanni Monteleone

Introduction

The demonstration that lamina propria T cells of celiac patients recognise deamidated gluten peptides in the context of HLA-DQ2 and secrete Th1 type cytokines has given structural insights into the immunology of celiac disease. Nonetheless, the numbers of healthy DQ2+ individuals eating gluten is far greater than the number of individuals with celiac disease (including silent and latent types). It is therefore likely that some microenvironmental factors, which may be stochastic, play a role in triggering the tissue damaging T cell response.

Human mucosal T cell responses are strongly Th1 biased

An additional important consideration is that the normal human mucosal immune system is strongly Th1 biased due to the presence of IL-12 in Peyer's patches (PP, Nagata et al 2000). Thus when PP T cells see food antigens, they are driven along the Th1 pathway and express pSTAT4 and T-bet. These cells then migrate to the lamina propria, where they still secrete large amounts of interferon- γ . In healthy individuals it is probable that these cells die, probably due to multiple mechanisms (Fas/FasL, lack of antigen, poor antigen-presenting cells, local TGF β 1, PGE2). Although not formally shown yet in celiac disease, it is also likely that gluten reactive T cells persist in the lamina propria, secrete excess amounts of IFN- γ and drive remodelling and the generation of the flat mucosa.

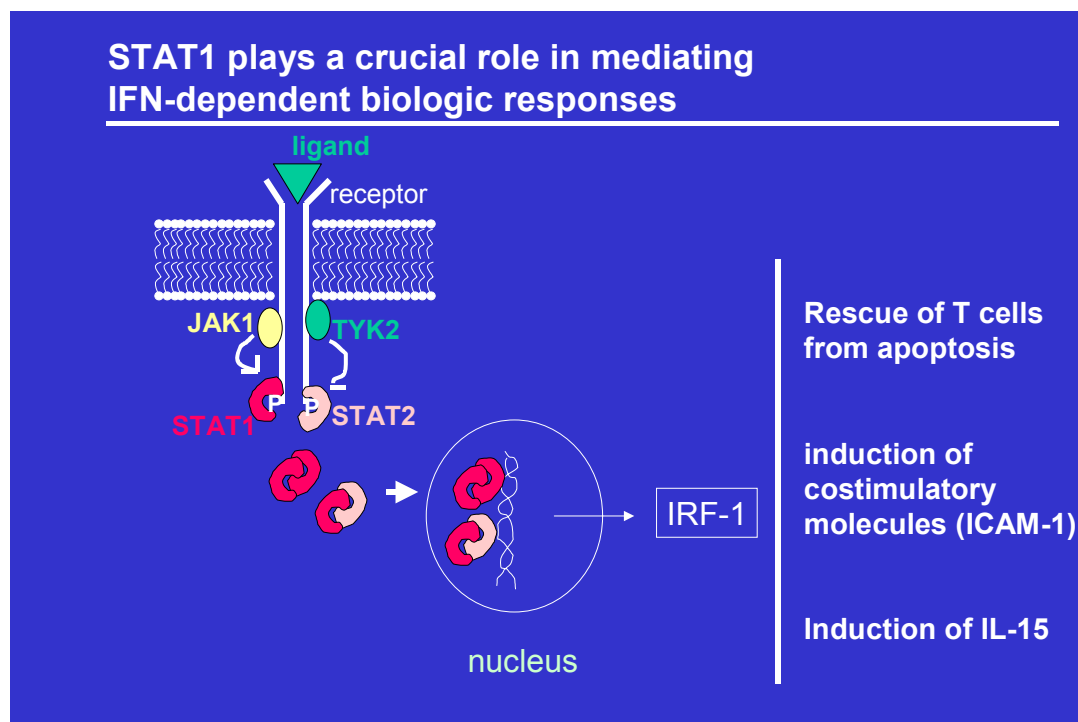
Type 1 interferons and celiac disease

To try to link these 2 notions together, we have been interested in other signalling pathways which may allow gluten-reactive lamina propria Th1 cells to persist. In man, but not mice, type 1 interferons signalling through the JAK/STAT pathway, can interact both with TCR signalling and drive Th1 responses. Initial suggestions that type 1 interferons might play a role in celiac disease came from case reports of the appearance of celiac disease in individuals treated with type 1 interferons for other conditions, in our case, a patient with CML (Bourliere et al 2000, Cammarotta et al 2001, Monteleone et al 2001a). Duodenal biopsy revealed a flat mucosa which healed on a gluten-free diet Unfortunately sera were not available to determine if the patient had anti-tTG antibodies, which would allow us to determine if interferon unmasked silent celiac disease, or precipitated the condition de novo. Direct functional evidence that interferon- α could drive Th1 responses came from studies using a fetal gut model of T cell driven crypt hyperplasia (the hallmark of untreated celiac disease). Anti-CD3 stimulation of resident T cells produced only a weak Th1 response with little crypt hyperplasia, associated with low production of the epithelial mitogen, KGF (Monteleone et al 2001b). However titration of interferon- α into the cultures enhanced Th1 responses, activated the STAT1 and STAT3 signal transduction pathways, increased KGF and produced greater epithelial division.

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STAT1 activation in celiac disease

To link these studies back into celiac disease we have recently investigated STAT1 activity in this condition. Duodenal biopsies, taken from CD patients and normal controls, were analysed for STAT1 by Western blotting, EMSA, immunohistochemistry, and SOCS-1 (the endogenous inhibitor of STAT signalling) was analysed by Southern and Western blotting (Mazzarella et al 2002). In ex vivo organ cultures of treated CD biopsies, the effect of a JAK/STAT1 inhibitor on the gliadin-mediated induction of costimulatory molecules was examined. High local concentration of IFN- α and a more pronounced phosphorylation and DNA-binding activity of STAT1 were seen in CD in comparison to controls. By immunohistochemistry, STAT1 was localised within the nucleus of epithelial and lamina propria cells. Staining was intense in CD patients compared to controls. Despite CD samples containing high SOCS-1 RNA, SOCS-1 protein was undetectable. In explant cultures of treated CD biopsies, gliadin induced activation of STAT1 but not SOCS-1. Furthermore, inhibition of STAT1 prevented the gliadin-mediated induction of ICAM-1 and B7-2. These data suggest that exaggerated IFN- α and defective SOCS-1 protein expression results in persistent STAT1 activation in CD, thereby maintaining and expand the local inflammatory response. STAT1 can induce the production of IRF-1 whose biological activities are consistent with a pathway to promote T cell survival in the lamina propria.



Summary

Taken together these data are suggestive that type 1 interferons might play a role on promoting Th1 responses in celiac disease. Given that type 1 interferons are produced in the gut during viral infections, this suggests a link between infection and gluten sensitisation. Nonetheless it is clearly only

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part of the story because even normal individuals demonstrate endogenous STAT-1 activation, presumably due to local interferon- γ production.

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C14 CHANGES IN THE MUCOSAL MICROENVIRONMENT IN COELIAC DISEASE

Marco Londei

Imperial College of Science Technology and Medicine

Celiac disease is caused by the antigenic recognition of gluten by T cells. Many studies have now unequivocally defined the nature of these 'dominant' T cell epitopes. T cell recognition and the potential pathogenic outcome are, however, not only controlled by the trimolecular complex MHC-peptide and TCR. It is now apparent that the context in which such recognition occurs will dictate how T cell will react and ultimately cause pathology. For this reason we have explored how the mucosal, small intestine, microenvironment of CD patients is modified upon exposure to different fragment of α -gliadin as well as cytokines normally detected in CD patients. Our studies reassert the importance of the α -gliadin immuno-dominant T cell epitopes but also provide new evidence for a more complex pathogenic cascade. The outcome and significance of our studies will be discussed in the presentation.

C15 ROLE OF IEL IN THE ENTEROPATHY INDUCED BY TOXOPLASMA GONDII

Dominique Buzoni-Gatel(1) , Franck Mennechet(2), Nicolas Rachinel(2), Souphalone Luangsay(2), Lloyd Kasper(2)

(1)Institut Pasteur, Unite de Biologie Moleculaire du gene, 75015 Paris.

(2)Microbiology Department, Dartmouth college, Lebanon, NH, 03756

Most experimental models of IBD utilize genetically or immunologically altered mice. One hypothesis for the development of IBD is a hyperinflammatory immune dysregulatory response to microbial

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antigens. C57BL/6 mice when orally infected with *Toxoplasma gondii* develop an acute lethal ileitis with histologic changes consistent with human inflammatory ileitis.

The development of this inflammatory process is dependent upon the induction of a robust Th1 response including overproduction of INF- γ , TNF- α , IL-1 β and NO. Primed Lamina Propria CD4+ T cells isolated from parasite infected mice produce substantial quantities of both IFN- γ and TNF and synergize with infected immortalized mouse intestinal epithelial mIC_{cl2} cells to enhance the production of several inflammatory chemokines.

Using CD40 KO chimeric mice reconstituted with competent bone marrow, we further demonstrate the requirement for CD40-CD40L interaction in this pathogen driven model. Intestinal immune homeostasis is dependent upon the production of TGF- β and IL-10. Adoptive transfer of parasite primed IEL prevents the development of ileitis via a TGF- β dependant mechanism. TGF- β producing IEL abrogate chemokine production of infected enterocytes and interact with CD4 LP to downregulate their IFN- γ and TNF- α production. IEL trafficking to the intestine is necessary to achieve their homeostatic functions and is dependant upon $\alpha 4\beta 7$, $\alpha E\beta 7$, and CCR5 interaction with their appropriate ligand.

We have engineered a parasite devoid of the major surface antigen, SAG1. Susceptible mice infected with the $\Delta sag1-7$ mutant fail to develop the acute ileitis. BALB/C mice are resistant to develop acute ileitis post-oral infection. BALB/c mice pre-sensitized with SAG-1 and cholera toxin via nasal immunization exhibit an acute inflammatory ileitis upon infection with intact SAG1+ parasites but not the $\Delta sag1-7$ mutant. These data suggest that a single microbial antigen can influence the outcoming of T cells mediated immune response in pathogen induced ileitis in susceptible and resistant mice.

C16 ISOTYPES OF THE NHG2 FAMILY OF NK RECEPTORS TIGHTLY REGULATE THE FUNCTION OF NORMAL AND CELIAC INTESTINAL $\alpha\beta$ T LYMPHOCYTES

Bertrand Meresse, Leanne Lee, Arthur Roberts, Veronique Groh, Thomas Spies, Ellen C. Ebert,, Nadine Cerf-Bensussan, Peter Green and Bana Jabri.

Department of Molecular Biology, Princeton University, Princeton; Department of Medicine, University of Medicine & Dentistry of New Jersey, New Brunswick; Clinical Research Division, Fred Hutchinson Cancer Research Center, Seattle; OF INSERM 97-10, Faculte Necker, Paris; Division of Gastroenterology, College of Physicians & Surgeons, Columbia University, New York.

Human intestinal T lymphocytes (T-IELs) are activated/effector cells with potent cytolytic properties. We report here, that in the intestinal epithelium CTL are critically controlled by a set of activating NKG2 molecules which function as costimulatory receptors for non-classical MHC class I-like ligands, and that IL-15, a cytokine secreted by tissue cells, plays a pivotal role in arming these costimulatory receptors. Because both IL-15 and the MHC-like ligands MICAIMICB and HLA-E are induced on tissue cells upon stress and inflammation, this costimulatory pathway appears to be tightly controlled by local innate immunity. Thus, by transiently lowering the antigenic threshold for TCR activation in tissues undergoing stress and inflammation, NKG2 receptors exert a new level of regulation of adaptive immunity at its effector CTL stage within the tissue environment. Uncontrolled secretion of IL-

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15, as found in celiac disease and arthritis, could lead to inappropriate activation of NKG2 receptors and favor the development of autoimmune disease by lowering the activation threshold of T cells with low affinity for self-antigens and by promoting NK-like lysis.

C17 MECHANISMS OF ENTEROCYTE APOPTOSIS IN COELIAC DISEASE

GR Corazza, R Ciccocioppo, A Di Sabatino, B Cinque, MG Cifone

Gastroenterology Unit, IRCCS Policlinico S. Matteo, University of Pavia;

Department of Experimental Medicine, University of L'Aquila, Italy

Dysregulation of apoptosis has been associated with the pathogenesis of a wide array of diseases. In particular, at small bowel level, an excessive enterocyte apoptosis has been implicated in the remodelling of intestinal mucosa in active coeliac disease (CD). This increase correlates with higher enterocyte proliferation, falls to normal levels after short-term gluten-free diet before histological improvement, and correlates with the degree of villous atrophy. Two mechanisms shown to be involved in mediating the increased enterocyte apoptosis in CD are the Fas/Fas ligand (FasL) system and the perforin/granzyme pathway. FasL, a type II transmembrane protein which belongs to the tumor necrosis factor family, induces apoptosis after the binding with Fas, a type I transmembrane protein member of the tumor necrosis factor receptor family, expressed on the target cell. Fas receptor is overexpressed by coeliac enterocytes, making them an easy target for the FasL-mediated cytotoxicity by intraepithelial (IELs) and lamina propria lymphocytes (LPLs). Perforin is a molecule contained, together with granzymes, in the cytoplasmic granules of cytolytic T cells. Its release causes pore formation on cellular membrane that permits penetration of granzymes into cytoplasm, leading to apoptosis of target cells. The proportion of perforin-expressing IELs is increased in untreated coeliac mucosa and correlates with the proportion of apoptotic enterocytes, suggesting that the perforin pathway could be a further mechanism able to induce increased enterocyte apoptosis in CD. Although it is conceivable that cytotoxic lymphocytes can kill their target by several mechanisms, these findings suggest a pathophysiological model whereby enhanced presentation of tissue transglutaminase-deamidated gliadin peptides by DQ2 or DQ8 molecules to CD4-positive cells in the lamina propria results in secretion of Th1 cytokines that may induce Fas enterocyte overexpression and increased FasL- and perforin-mediated cytotoxicity by IELs and LPLs, leading to increased enterocyte apoptosis and, then, to villous atrophy in CD.

C18 ENTEROCYTE-DERIVED INTERLEUKIN-15, A KEY LINK BETWEEN INTRAEPITHELIAL LYMPHOCYTE HYPERPLASIA AND LYMPHOID MALIGNANCIES IN CELIAC DISEASE

Jean-Jacques Mention, Mélika Ben Ahmed, Virginie Verkarre , Vahid Asnafi, Elisabeth McIntyre,

Nicole Brousse, Christophe Cellier, Nadine Cerf-Bensussan

INSERM EMI-0212. Faculté Necker. Paris V. France

Background

One hallmark of celiac disease (CD) is massive hyperplasia of intraepithelial lymphocytes (IEL) which gives rise in a subset of patients to malignant lymphoid complications: refractory sprue (RS) characterized by the expansion of clonal IEL with an abnormal phenotype and T cell lymphomas. The

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mechanisms underlying the changes in IEL homeostasis in CD are unknown. They do not seem to involve direct IEL stimulation by gliadin-derived peptides, and may rather result from changes in the microenvironment secondary to the inflammatory reaction, and perhaps favoured by unknown genetic factors. Several clues pointed to the possible role of IL15. In mice, IL15 plays a key role in IEL homeostasis, and its overexpression in transgenic mice leads to the emergence of CD8⁺ and NK leukemias (14). Our own work indirectly suggested an increased production of IL15 in CD favouring the increased expression of the CD94 NK marker on IEL in active CD.

Results

1- Immunohistochemical studies revealed a massive increase in IL5 protein in the intestinal epithelium of patients with active CD and in RS, but no significant changes in the ileum of patients with active Crohn's disease (not associated with IEL hyperplasia or lymphoma). IL15 expression decreased in patients on GFD but remained significantly increased over controls, suggesting a possible primary defect. Quantitative PCR showed that the regulatory defect was at the post-transcriptional level, but sequencing did not reveal any mutation in the regions of the IL15 mRNAs that control translation. IL15 was not secreted in detectable amounts by epithelial cells but was expressed at the surface of enterocytes in active CD or RS. Current experiments indicate that IL15 became detectable on enterocyte surface only after induction of apoptosis/necrosis.

2- IL15 selectively induced the expansion of abnormal clonal IEL derived from RS patients which were able to overgrow the remaining normal residual T. IL-15 prevented the apoptosis of clonal IEL, induced their IFN γ secretion and their granzyme/perforin-dependent cytotoxicity, effects which must be compared with the massive increase in ARNm encoding granzyme B and IFN γ two proteins induced by IL15 in the mucosa of patients with active CD and RS. The effects of soluble IL15 on apoptosis and IFN γ secretion could be reproduced by the coculture with epithelial cell line T84, and the effects of coculture were blocked by antibodies against IL15 and CD103.

Conclusion

These data sustain the hypothesis that IL15 plays a key role in the change in IEL homeostasis in CD. Furthermore they suggest a self-sustaining loop perpetuating IEL activation and epithelial damage: thus IL15 released by damaged enterocytes promotes IEL expansion and activation, which then induces further epithelial damage via IFN γ release or cytotoxicity. Persistent changes in IEL homeostasis might ultimately lead to the emergence of clonal malignant proliferations, which can further sustain epithelial lesions as seen in RS and T cell lymphomas associated with CD. Altogether, these results suggest that blockade IL15 signalisation might be a novel therapeutical strategy in MC and moreover in RS, a severe disease resistant to most current treatments..

C19 SCREENING EPIDEMIOLOGY OF COELIAC DISEASE IN EUROPE : PREVALENCE 1:100-200 ; SHOULD WE SCREEN THE GENERAL POPULATION AS A ROUTINE ??

Chris Mulder

10th International Symposium on Coeliac Disease Pathogenesis and Outcomes

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In recent years, large-scale screening studies on Coeliac Disease have been published and suggest a prevalence of CD in America, Europe, Australia, The Middle East etc. of about 1:200. Coeliac Disease can be classified due to all those studies to be an important health problem. It concerns a disease with a high prevalence, associated with low to high-grade morbidity and severe long-term complications such as osteoporosis in the majority of patients diagnosed in adulthood and T-cell lymphoma's. Epidemiological studies should assess the costs of CD screening compared with the savings in relation with prevention of complications and the extra costs of delay of the diagnosis due to unawareness of the medical societies.

The current approach is screening of high risk populations such as auto-immune diseases, infertility-disorders, first degree relatives and publish about it and convince scientific societies to look for CD. The question is if the natural course of these high risk groups will be changed by GFD? Medical societies have to make protocols, which risk conditions must be screened, once a life or with certain intervals.

The second approach is to ask endoscopists to take biopsies during routine upper endoscopy, not only from the stomach, but also from the duodenum, as has been advised by the UEGW working group (2001). Data about this second approach are limited.

The third approach is screening of the general population, data about children in different age groups are available. However, no data are available about the most optimal age to screen in childhood. Assuming that screening once a lifetime is not enough, what about other age-groups to screen? No data are available of cohorts of certain age groups (f.i. 40 or 60 yrs).

Data in relation to cost-benefit are mandatory.

Mass screening for CD in populations with a high gluten-intake, so to say pasta/pizza/bread countries might be justified and/or accepted before 2010 (?).

Epidemiology has to focus which test is the best for disease detection. Recent work in adults has suggested, that antibodies fail to identify a subgroup of patients with villous atrophy, especially in smokers. The endomysial test is the golden standard. However t-TG testing seems promising, however data for sensitivity in partial, subtotal or total villous atrophy have to be awaited. A combination of DQ typing and serology might be more attractive, however DQ2 typing is still expensive. Genetic screening as part of national new-born screening programmes for metabolic defects, using the same blood sample might be a possibility.

The fourth and maybe best approach is to train the medical community and patients to consider CD even with minor symptoms. CD is not born f.i. in the mind in doctors diagnosing auto-immune diseases. However, more and more patient associations for auto-immune disease are giving information to their members about being aware of so-called associated diseases, such as CD.

C20 COELIAC DISEASE IN A SCANDINAVIAN POPULATION LIVING IN THE USA, 1950-2001

JA Murray, C Van Dyke, M plevak, R Dierkhising R AR Zinsmeister, LJ Melton III. Mayo Clinic, Rochester, MN.

Celiac disease has been considered a rare disorder in North America and a relatively common one in Western Europe. This is despite the ethnic similarity between the large Caucasian majorities of both

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continents. Substantial temporal changes in the incidence and age specific trends have been reported from several European countries over the last several decades. A dramatic rise and fall in early childhood celiac disease occurred in Ireland and the UK in 60's and 70's and a decade later in Sweden. Similarly an increased mortality has been reported in cohorts of with celiac disease from Europe predominantly due to malignant disease. Olmsted County, Minnesota has maintained complete medical records for all its inhabitants for over 100 years. This resource allows for a precise enumeration of celiac disease over the last 50 years. We report the incidence, clinical and genetic features and outcome of celiac disease in this well enumerated community.

Methods

Population: Olmsted County has a predominantly Caucasian even Scandinavian population. All individuals residing in Olmsted County, Minnesota with a diagnosis of celiac disease or dermatitis herpetiformis (DH) first made between the 1950 and 2001 were identified using the Rochester Epidemiological Project. Ninety-five percent confidence intervals for incidence rates (95% CI) were calculated assuming a Poisson distribution. A Poisson regression model was used to assess incidence rate changes with respect to calendar year, age, and gender. Survival was compared to the expected for the Caucasian US population.

Results

A total of 82 new cases of celiac disease were identified (58 females and 24 males) in this 51-year period. There was a marked female predominance ($p=0.003$) and the incidence increased with age ($p<0.001$) mean age 45 years. The annual age- and gender-adjusted incidence of celiac disease and DH was 2.1 per 100,000 (95% CI, 1.7 to 2.6) and 0.8 per 100,000 (95% CI, 0.5 to 1.1), respectively, for the entire period. The annual incidence of celiac disease rose from 0.8 per 100,000 in the 1960's, 70's and 80's was 3.2 per 100,000 (95% CI, 2.2 to 4.3) in the 1990's and even greater in the last 2 years, 9.4 per 100,000 (95% CI,). Clinical features also changed over time with less frequent diarrhea and a higher body mass index at presentation. Interestingly, the occurrence of DH was constant over the same period. Serology aided in diagnosis of 53% of the recently identified cases of celiac disease. The HLA type for the subjects living in Olmsted County was uniformly DQ2. Overall survival was not different from that expected for the general population, however there was a predominance of cancer related mortality and dementia was a common cause of death.

Preliminary results from screening projects has identified a prevalence of CD of 10% of first degree family members, 5 % of those individuals with osteoporosis (<75 years) and 5% of type one diabetes. We estimate that the screening of high-risk groups will double the measured prevalence of CD. General population estimates by general population screening are not available for this population but if other US studies are correct this strategy of screening high risk groups will still leave many undiagnosed.

Conclusion

The apparent incidence rate of celiac disease in this geographically and medically well characterized population has increased significantly in the last 10 years, and dramatically so in the last 2 years. There has been no change in the predominantly adult onset over the last 50 years. Pediatric cases

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have always been rare in this community. While milder clinical features and increased use of serology suggests an increased detection rate, a true increase in disease occurrence cannot be excluded. The recent incidence rate matches most areas in Europe where accurate enumeration is available. The life expectancy of celiacs is not altered despite the late age of diagnosis /onset. This is despite racial and genetic similarity with Western Europe. Differences may be due to infant feeding practices. Celiac disease in this US community is largely a covert disease, with minimal symptoms and no increase in mortality. The iceberg may be as large as the European Iceberg but may not be as dangerous.

C21 ENDOSCOPIC MARKERS IN THE DIAGNOSIS OF CELIAC DISEASE; WHAT IS SPECIFIC?

PETER H R GREEN MD

COLUMBIA UNIVERSITY COLLEGE OF PHYSICIANS AND SURGEONS NEW YORK

The endoscopic markers of celiac disease include: absent or reduced duodenal folds 1, mosaic pattern or nodular appearance 2 3, scalloping of folds 4, mucosal fissures or grooves, a visible vascular pattern

These markers are valuable in identifying patients who are undergoing endoscopy for reasons other than for the assessment of diarrhea or malabsorption for their recognition will result in the diagnosis of celiac disease when it had not been suspected'. These markers had been considered to be highly predictive of celiac disease 5,8 . However they are not specific. They are instead a marker of a pathologic process involving the mucosa that may include villous atrophy. These changes have been recognized the markers in patients with opportunistic infections, amyloid, systemic mastocytosis, Crohn's disease, HIV enteropathy, *Giardiasis* and tropical sprue 9. The markers are very sensitive predictor of a pathologic process involving the mucosa 9. In a study, by Murray, of patients evaluated for iron deficiency anemia the sensitivity for any marker was 59% and specificity 92%. Similar sensitivities were described by Bardella 10 and Dickey". In the latter study the prevalence of markers was lower in patients with partial villous atrophy (58%) versus subtotal or total villous atrophy (82%)

Factors affecting visualization of the endoscopic markers include the resolution of the endoscope, whether the examiner includes thorough visualization the duodenal bulb, the use of chromoendoscopy and underwater techniques.

Endoscopic markers of celiac disease are an insensitive markers for the disease, though when present are highly predictive of mucosal disease which in the right clinical situation may be celiac disease.

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C22 ARE CLINICAL PRESENTATION AND EPIDEMIOLOGY IN THE USA AND EUROPE DIFFERENT?

Carlo Catassi

University Department of Pediatrics, Ancona, Italy

Center For Celiac Research, Baltimore MD, USA

Available data suggest that the incidence of celiac disease (CD) (only cases diagnosed on clinical ground) is much lower in the US than in Europe, with a reported figure of 1:6000 and 1:1000-2000, respectively. However recent surveys found a similar CD prevalence (including all cases found through serological screening) in either continents, both in the general population (0.5-1 %) and in at-risk groups, e.g. CD relatives (5-10 %) and patients with type I diabetes mellitus (5 %). Despite this similar disease frequency, CD is diagnosed less commonly in the US.

Typical CD cases, with early onset and classical gastrointestinal complaints, are less common in the US than in Europe, a situation that generated poor awareness of disease in the US. Due to the similar disease prevalence, it follows that the proportion of atypical and silent cases is higher in the US. It has been suggested that a lower degree of the celiac enteropathy parallels (and possibly explains) this clinical situation. Such a difference could theoretically be related to variability in the: (a) genetic background. The frequency of the major CD predisposing genes (the HLA-DR3 and more recently the

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HLA-DQ2 and DQ8) is similar across the US and European countries. Any difference in the frequency of not-HLA related predisposing genes is a matter of speculation. It should however be noted that the segment of the population showing an ethnical background at low-risk for CD, e.g. sub-Saharan African and Asian origin, is wider in the US than in Europe; (b) environmental factors. Wheat, the main gluten-containing cereal, is a staple food both in Europe and the US. Subtle differences in infant dietary patterns could influence the epidemiology of CD, as recently suggested by the Swedish “epidemics” of CD. A protective pattern of infant feeding (prolonged breast feeding and lower gluten intake) seems to be more common in the US than in Europe.

The diffuse misconception that CD is a rare disorder in the US is self-perpetuating, for several reasons: serological CD markers are rarely checked by primary care physicians, few laboratories perform a complete battery of CD screening tests, biopsies are rarely adequate for a proper investigation of the small intestinal architecture, health insurances not always refund patients for the cost of a diagnostic endoscopy. An active case-finding policy seems to be the best buy for increasing the awareness of CD both in the US and Europe.

C23 THE CELIAC OSTEOPATHY

Prof. Julio C. Bai, Md

Head of the department of medicine, “Carlos Bonorino Udaondo” Gastroenterology Hospital., Gastroenterology Chair, School Of Medicine, Universidad Del Salvador; Buenos Aires; Argentina.

Summary

Osteopenia, osteoporosis and osteoporotic fractures are well-recognized complications of celiac disease (CD) and several recent studies have confirmed that they are very relevant clinical problems for patients. Recently, an increased prevalence of fractures in the peripheral skeleton but not the axial skeleton of CD patients was shown. Moreover, fractures are directly associated with the severity of the clinical compromise of CD. Thus, while patients with classical symptoms of CD have a significant increased number of patients with fractures, those with subclinical or silent forms of the disease have similar figures to the sex- and age-matched healthy population. The difference between both subgroups of patients was highly significant. New information was recently incorporated to the knowledge on the pathogenesis of the increased risk of fractures. While most former studies pay attention to the low bone mineral mass of patients, very recent information from novel tools evidenced defects in the architectural design and strength of bones. Furthermore, ancillary results detected that the nutritional and metabolic affectation of muscles may participate in the weakening of protective structures (regional muscles) and the redesign of the cortical bone. Our knowledge on the pathogenesis of the bone disease of CD patients was recently increased by the advent of new findings. In association with the well-established increased bone turnover as consequence of a secondary hyperparathyroidism, induced by low serum levels of calcium (malabsorption and increased intestinal excretion) and vitamin D, new potential factors are gaining relevance. More recently, the attention has been focused on immunologic factors such as cytokines (IL-1 β , IL-1 α and IL-6) and their effects leading to an increased osteoclast activity (bone resorption). The recently identification of the enzyme tTG as the antiendomysial autoantigen and the well-established importance of bone-specific

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tTG in the mineralization and remineralization of bones have allowed us to hypothesize that endomysial antibodies (EmA) could play a pathogenic role in the celiac osteopathy. This observation is concordant with the recently discovered presence of anti-bone antibodies in serum of patients with active CD. Unpublished investigations have demonstrated that both are the same antibodies. Although a cause effect was not yet shown, we suggest that EmA could neutralize the physiologic role of tTG in the bone metabolism. Finally, we recently detected a genetic predisposition for osteoporosis in CD patients. Thus, patients carrying the allele 2 of the IL1 β -511 gene, who are hypersecretors of IL-1 β , have significantly lower bone mineral density than non-carriers. In conclusion we suggest that the celiac osteopathy may be the consequence of a multifactorial pathogenesis which includes metabolic, nutritional, immunologic and genetic factors.

C24 OSTEOPATHY IN CHILDREN WITH SILENT COELIAC DISEASE

Stefano Mora, Graziano Barera

Laboratory of Paediatric Endocrinology, IRCCS H San Raffaele, Milan, Italy

Osteomalacia, rickets, bone pain, and osteoporosis have been frequently reported in coeliac disease, even as signs of presentation of the disease. Following the widespread use of non-invasive techniques for the study of bone mineral content (BMC) and bone mineral density (BMD), it has become increasingly clear that low bone mass is a common feature of untreated disease. The number of studies involving adult patients largely outstrips those regarding children and adolescents, probably because the evaluation of growing individuals poses critical problems in the interpretation of the bone measurements. Nevertheless, all published studies agree on the presence of extremely low BMC or BMD values at diagnosis in coeliac youth, regardless of clinical presentation. Cross-sectional studies demonstrated that at diagnosis of coeliac disease a marked reduction of BMC was present in the peripheral skeleton (radius), in the lumbar spine, and in the whole skeleton. The effect of gluten-free diet on bone mass has been evaluated both in cross-sectional and longitudinal studies. Children and adolescents with different duration of GFD showed BMD or BMC measurements that were similar to those of healthy controls. The changes occurring during GFD have been documented in a series of longitudinal studies. BMD annual increments were significantly higher in coeliac children and adolescents than in their healthy counterparts during the first year of treatment. This remarkable increment of bone mass contributed to fill the gap, so that after 12 months of diet the BMD measurements of coeliac young patients were no longer different from those of healthy children. The annual increment of bone mass decreased after the first year of diet, delineating a substantial normalisation of bone accretion rate. Studies on coeliac patients on long-term GFD showed that good compliance to the diet is able to assure normal BMD. The biochemical mechanisms associated to osteopathy in this disease are still poorly understood. One study showed that in a small number of young coeliac patients, bone formation markers were lower than those of healthy peers. Interestingly, it has been shown that after long-term GFD both bone formation and bone resorption markers were significantly elevated, especially in patients with a suboptimal compliance to the diet.

The data on the recovery of bone mass due to the dietary intervention are encouraging. Because childhood and adolescence are critical periods for the achievement of optimal peak bone mass (a

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major determinant of osteoporosis and fracture risk in adulthood and senescence), early diagnosis of coeliac disease should be implemented, especially in asymptomatic patients.

C25 GLUTEN SENSITIVITY AS A NEUROLOGICAL ILLNESS.

Marios Hadjivassiliou - Sheffield

It has taken nearly 2000 years to appreciate that a common dietary protein introduced to the human diet relatively late in evolutionary terms, can produce human disease not only of the gut and the skin but also the nervous system. The protean neurological manifestations of gluten sensitivity can occur without gut involvement and neurologists must therefore become familiar with the common neurological presentations and means of diagnosis of this disease.

Over the last 8 years we have used antigliadin antibodies to screen patients with neurological dysfunction of unknown aetiology. Our original study concluded that gluten sensitivity played an important part in neurological illness. The evidence was statistical: Patients with neurological disease of unknown aetiology were found to have a much higher prevalence of circulating antigliadin antibodies (57%) in their blood than either healthy control subjects (12%) or those with neurological disorders of known aetiology (5%). Since then we have identified 158 patients with gluten sensitivity and neurological disorders of unknown aetiology. Perhaps not surprisingly the commonest manifestations are ataxia (also known as gluten ataxia) in 64 patients and peripheral neuropathy (sensorimotor axonal in 35 patients, mononeuropathy multiplex in 16 patients, motor neuropathy in 10 and small fiber neuropathy in 3). Other manifestations include myopathy (10 patients), encephalopathy characterised by episodic severe headaches and abnormal brain white matter on MRI scan (11 patients). Less common manifestations include stiff person syndrome (4), myelopathy (2), and neuromyotonia. We have studied the effect of gluten free diet on gluten ataxia and gluten neuropathy. Preliminary data (to be presented) suggest that gluten-free diet is beneficial for patients with gluten ataxia and gluten neuropathy.

Given that only one third of these patients had evidence of an enteropathy, the response to gluten free diet strengthens our contention that gluten triggered neurological dysfunction can exist without associated enteropathy (coeliac disease). The absence of an enteropathy should therefore not preclude patients from treatment with gluten-free diet. Early diagnosis and removal of the trigger factor by the introduction of gluten-free diet is a promising therapeutic intervention. The pathophysiology of gluten related neurological dysfunction is beginning to be unravelled. We have demonstrated Purkinje cell antibodies in patients with gluten ataxia. We have also found cross reactivity between antigliadin antibodies and Purkinje cells. Post mortem data however suggest additional cell mediated mechanisms contribute to the neurological insult.

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*Alessandro Ventura e Roberto Marzari **

Clinica Pediatrica e *Dipartimento di Biologia, Università di Trieste, Italy

Celiac disease (CD) and other autoimmune disorders are frequently associated. In particular, silent celiac disease may be found in around 2-10% of patients with IDDM, autoimmune Thyroiditis,

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autoimmune hepatitis, biliary cirrhosis, juvenile chronic arthritis, and even in a larger rate of patients with autoimmune ataxia related to anti Purkinje cells antibodies. Patients affected by autoimmune disorders seem to have a particularly increased risk to be celiac when affected by multiple autoimmune diseases (11%) or when they are relatives of a celiac patient (24%). Recently we have shown that silent celiac disease is present in 2.5% of patients affected by idiopathic dilated cardiomyopathy (IDCM) and in 7% of their first degree relatives with pre-clinic ECG abnormalities. Moreover, anti heart autoantibodies were present in the cases in which IDCM were associated to CD. So we have possibly identified a subpopulation of autoimmune IDCM related to gluten intolerance.

The relationship between CD and autoimmune disorders may be explained on the basis of the shared HLA antigens. However, celiac patients produce a large series of gluten dependent autoantibodies (both organ and no organ specific) and seem to develop autoimmune disorders in relationship to the duration of gluten exposure.

The mechanisms involved in gluten dependent autoimmunity as well as its specificity and peculiarity are still largely unknown. By using phage display antibody libraries, that can mimic the specificity of original lymphocytes, we are able to show that anti transglutaminase autoantibodies, the ones considered specific for CD, are produced exclusively at intestinal level. All celiac patients make these autoantibodies using the same VH-5heavy chain family genes and all these antibodies recognize the same limited number of conformational epitopes on tTG molecule.

Studying celiac disease derived libraries we have also showed that even other autoantibodies founded in some celiac patients, such as anti brain autoantibodies related to gluten ataxia, are produced by lymphocytes harboured in the intestinal mucosa.

Some preliminary evidence regarding an IDDM patient, DQ8 positive and anti tTG negative with normal intestinal mucosa, suggest that an autoimmune, anti TTG-gluten dependent response may be present at intestinal level without detectable autoantibodies in the blood and independently by the mucosal damage. On the basis of this preliminary evidence and considering that, in celiac patient IDDM related autoantibodies may disappear during gluten free diet, we are now testing the hypothesis that these kind of autoantibodies may be induced by gluten even in the absence of serological markers of CD. This fact could be of some interest in defining an effective strategy for preventing IDDM in at risk people, as in the case of GAD-ICA positive, first degree relative of the IDDM patient.

C27 CELIAC DISEASE AND AUTOIMMUNITY

Cosnes C, Cosnes A, Contou JF, Gendre JP, Cosnes J. Hôpital Rothschild, Paris 12

Patients with celiac disease (CD) have an increased risk of auto-immune disease (AID). In children, prevalence of AID is related to the age of diagnosis of CD, suggesting that protracted gluten ingestion promotes the development of AID. Prevalence of AID has not been studied in adult CD in relation to symptoms and diagnosis in infancy.

Occurrence of a clinical AID according to a positive list was specified in 188 adult patients with biopsy-proven CD, 188 patients with Crohn's disease, and 188 controls (proctology and dermatology outpatients) matched for age and sex.

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Results: 46 CD patients (24%) had developed an AID, vs. 14 Crohn's disease and 5 controls (OR: 11.9; IC 95%: 4.4-35). Most frequent AID were thyroiditis, inflammatory bowel disease and dermatitis herpetiformis. The prevalence of AID was similar in CD patients diagnosed in infancy (22%), in those symptomatic during infancy but diagnosed in adulthood (27%), and in those asymptomatic before adulthood (24%).

Conclusion: the risk of AID associated with CD appears unrelated to the precocity of diagnosis nor symptoms.

C28 MANAGING COELIAC DISEASE: AN INDUSTRIAL PERSPECTIVE

B.P. Jarry

Tate & Lyle - Amylum, Aalst, Belgium

Our understanding of Coeliac Disease (CD), its prevalence in the population and its genetic cause are growing rapidly, thanks to the scientific achievements obtained recently in the clinical, immunological and genetic fields. However, viewed from the patients side, the management of the disease remains very much of the same, with gluten-free diet the only safe treatment available to the gluten intolerant person.

Still, this very same scientific data now brings some hope that solutions could be found which could over time help to make life easier for the affected patients.

Several research approaches are now in preparation for exploring new initiatives in this respect:

(i) Determination of the toxicity level of gluten for coeliac patients; (ii) development of innovative therapeutic treatment; (iii) searching of wheat varieties with low toxicity for the patients and (iv) improved gluten-free food with better nutritional value and texture.

These focused research areas which bring together some of the best researchers in the related scientific fields are strongly encouraged by the European Starch and Gluten Industry and by the European Commission Research Directorate. It is expected that this concerted effort will lead to much improved solutions in the coming decade, helping there to overcome this unfortunate situation where a sizeable portion of the population is hampered to benefit from one of the most anciently available food raw material.

C29 STANDARDIZATION OF SCREENING TESTS: SENSITIVITY, SPECIFICITY AND REPRODUCIBILITY OF SERUM ANTIBODY DETERMINATIONS

M. Stern, University Children's Clinic, Tübingen (for the EMRC/ESPGHAN Working Group "Serologic Screening for Coeliac Disease")

From 1993 to 2000 the Working Group has produced new evidence on serologic tests for coeliac disease under standardized conditions. Tools of standardization applied were: robust basic test protocols, calibration of test results, use of positive and negative reference sera, use of receiver operating characteristic curves (ROC) wherever possible, determination of intralaboratory and intralaboratory variation as a measure of test reproducibility.

For the final collaborative study seven European laboratories were provided with 252 randomized sera classified by clear-cut clinical and histologic definitions of diagnosis (52 children, 51 adults, and

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controls). Non-commercial test protocols for IgG and IgA gliadin antibodies (ELISA) and for IgA autoantibodies against endomysium (immunofluorescence) and tissue transglutaminase (ELISA) were used with defined joint cutoff values. All antibody determinations were done blindly in duplicate. Statistical evaluation was done by an independent institute.

IgA autoantibodies against endomysium and tissue transglutaminase rendered superior sensitivity (90-93%) and specificity (92-99%) compared to gliadin antibodies. Tissue transglutaminase antibody testing exhibited superior ROC curves (AUC 99.8) compared to gliadin antibodies (AUC 87.4-92.9). Interlaboratory reproducibility indicated by Cohen's kappa values produced the following ranking: 1. IgA EMA (0.93), 2. IgA tTG (0.83), 3. IgG AGA (0.82), 4. IgA AGA (0.62).

Basic criteria of standardization and quality assessment must be fulfilled by any given test protocol in coeliac serology proposed for use in screening. The Working Group has produced robust test protocols and reference materials for future work.

C30 THE PROBLEM OF ANTI-EMA NEGATIVE PATIENTS.

C. Feighery. Dublin, Ireland.

In the diagnosis of coeliac disease (CD), the specificity of a positive IgA endomysial antibody (EMA) test is calculated to be greater than 99%. A lower sensitivity is regularly reported, although in some studies sensitivities of 95% or greater were originally described. However, this may reflect the patient groups selected for study and a more accurate sensitivity level may approximate to 85%. Thus, the EMA test may under-diagnose coeliac disease (CD) by 15%. Various factors may explain a negative test result: some 2% of CD patients are IgA deficient and inevitably will be EMA negative. In addition there are reports that patients with a less severe histological lesion are often (as many as 50%) EMA negative: however, in our experience many patients with minimal lesions are nonetheless EMA positive.

Nowadays, screening for CD frequently involves testing for anti-tissue transglutaminase (tTG) antibodies by ELISA as an initial step, followed by EMA measurement as a confirmatory test. An excellent correlation exists between these two antibody tests. However, negative results are sometimes observed, sometimes for a single test and occasionally for both tests. A negative anti-tTG test may result in no EMA investigation being performed and the diagnosis of CD may be missed. In other patients with active CD, elevated tTG antibodies are found but EMA antibodies are either absent or considered to be negative because an atypical pattern is present. A group of patients with this atypical EMA pattern are reported at this meeting.

There is currently no evidence to suggest that the clinical presentation or course of EMA negative CD patients differs from those with positive antibodies, although this is a subject that should be studied more closely. The main issue is that, over-reliance on these screening tests could result in under-diagnosis of patients with CD. Some benefit could be obtained by additional measurement of anti-gliadin antibodies or testing for IgG EMA: however, these latter tests suffer from low specificity and

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sensitivity. Hence, in clinical practice when CD is suspected, a small intestinal biopsy is warranted even when serological tests are negative.

C31 REFRACTORY SPRUE (RS), COELIAC DISEASE (CD) AND LYMPHOMA.

Prof Christophe Cellier. European Georges Pompidou Hospital, Paris, France.

RS is rare disorder, mainly described in adults and defined as a severe villous atrophy refractory to a gluten-free diet (GFD). RS may be classified as refractory coeliac sprue (RCD) or unclassified sprue.

1- The most frequent form is RCD (85% of 60 cases observed in our database), a CD related disorder characterised by either the presence of circulating anti-gliadin or anti-endomysial antibodies and/or HLA-DQ2/8 status, and/or an increased number of intra-epithelial lymphocytes (IEL) and/or an initial response to a GFD. RCD may be classified according to the IEL phenotype, which may exhibit a clonal configuration associated with an aberrant phenotype (intra-cellular CD3 expression and absence of surface CD8 expression) in almost 80% of RCD. This aberrant clonal IEL population may disseminate to the blood and to the whole intestinal tract and is frequently complicated by ulcerative jejuno-ileitis and/or invasive overt T-cell lymphoma. RCD with a clonal IEL configuration may be considered as a cryptic enteropathy-associated T-cell lymphoma and may be one of the missing link between CD and overt lymphoma complicating CD. The best treatment of RCD remains elusive.

2- The second form of RS (or unclassified sprue) represents 15% of cases in our experience, is not related to CD and comprises several very rare disorders, such as auto-immune enteropathy, common variable immunoglobulin deficiency and T-cell small intestinal proliferation of the lamina propria.

References : Cellier et al. Abnormal intestinal intraepithelial lymphocytes in refractory sprue. *Gastroenterology* 1998;114:471-81. Cellier et al. Refractory sprue, coeliac disease and enteropathy-associated T-cell lymphoma. *Lancet* 2000;356:203-8.

C32 COELIAC DISEASE AND NON-HODGKIN'S LYMPHOMA

M. L. Mearin on behalf of the Biomed European Working Group on Coeliac Disease and Malignancy

1Dept of Paediatrics, Leiden University Medical Centre, the Netherlands

Objective

To evaluate the risk of non-Hodgkin's lymphoma (NHL) in coeliac disease (CD).

Methods

This prospective, multi-center, case-control study was done under the auspices of the Biomed 2 Program- Concerted Action of the European Community (PL-96 3091). 12 working groups in 10 European countries conducted the investigation: the Netherlands, Italy, United Kingdom, Sweden, Finland, Ireland, Spain, France, Poland and Yugoslavia. Newly diagnosed cases of NHL were collected from May 1998 -April 2001. All the countries provided a group of controls. Previously diagnosed CD was noted. CD was screened by determination of IgA antiendomysial antibodies (EMA) in serum. The positive subjects were offered duodenal biopsy. CD was diagnosed in case of flat mucosa characteristic of CD.

The Medical Ethics Committees of all the participant working groups approved the study.

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Results

3213 cases of NHL were diagnosed and CD was screened in 1324 of them. Nine patients, 1 with already diagnosed CD, had high titers of EMA. The other 8 underwent small intestinal biopsy, which showed flat mucosa in 4. Thirteen patients were already known to have CD before the screening. In total there were 17 cases with NHL and CD.

9674 individuals formed the control group. 63 (0.7%) were EMA positive, 42 underwent a small bowel biopsy and in 38 (0.4%) of them a flat mucosa was found. Eleven controls were known to have CD before the screening. Compared with matched controls, CD was significantly associated with NHL (odds ratio = 2,6 (95% CI 1,4-4,9; p<0,01). Enteropathy associated T-cell lymphoma (EATL) was the most common form of NHL in CD.

Conclusions

In CD there is an increased risk for NHL (1), but, with the exception of the EALT's, this risk is much lower than thought before (2). The findings suggest that screening for CD is not indicated in NHL.

(1) JAMA 2002;287:1413; (2) Lancet 1983;1:111.

C33 SMALL BOWEL NON-HODGKIN LYMPHOMA IN COELIAC DISEASE

G K T Holmes, Derbyshire Royal Infirmary, London Rd, Derby. UK

The most serious complication of coeliac disease (CD) is malignancy but the frequency has been difficult to determine for several reasons. Thus the prevalence of CD is unknown, patients with malignancy may have CD that remains undiagnosed and data reported from referral centres may not be representative of the whole coeliac population. Even in series that did attempt to determine prevalence, figures vary widely from 3% to 11% for all cancers and 0% to 7% for lymphoma.

Recently studies of the malignant risk have produced more reliable data. It is evident that malignant complications, especially lymphoma and particularly small bowel lymphoma are much rarer than previously supposed. Some studies have shown a low relative risk for lymphoma and others, report only a small number of tumours arising.

Under the auspices of the British Society of Gastroenterology we carried out a national survey of primary small bowel malignancy in the United Kingdom over a two year period and found 107 lymphomas of which 42 (39%) were associated with CD. Most were EATL.

Malignant complications have been explored in long term studies in my coeliac clinic established in 1978. By the end of 2001, 870 patients were listed on the coeliac register. 12 lymphomas have occurred of which 5 primarily affected the small bowel and of these 3 were EATL. From these data and the prevalence of CD in Derby city it can be calculated that 23 small bowel lymphomas would occur in the coeliac population of the UK in 1 year. By making certain assumptions the observed numbers of small bowel lymphomas in my clinic may represent a 10-fold excess over that encountered in the general population. The occurrence of lymphoma in my clinic at least 2 years after the diagnosis of CD was compared to expected numbers derived using age and sex specific general population data. The risk of small bowel lymphoma in treated CD is tiny. In this large clinical practice no excess was detectable.

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C34 IMMUNOTHERAPY WITH GLIADIN PEPTIDES IN MICE

Riccardo Troncone

Department of Pediatrics & European Laboratory for the Investigation of Food Induced Diseases,
University Federico II, Naples

The mucosal lesion in coeliac disease (CD) represents an immunologically mediated injury triggered by gliadin and restricted by a particular assortment of major histocompatibility complex (MHC) class II genes. Immunomodulatory strategies to tolerize gliadin-specific class II restricted T cell responses could represent an alternative to current dietary treatment. A very efficient route to induce tolerance to a specific antigen is the intravenous (i.v.) administration, but the induction of tolerance via mucosae is a strategy more often implemented in the experimental therapy of autoimmune diseases. Despite its peculiar physicochemical properties we have shown gliadin to be a good oral tolerogen. Nevertheless, in coeliac disease, being the small intestine the target of the disease, mucosae other than those of the small intestine are candidate to elicit mucosal tolerance.

We studied the potential for tolerance induction in gluten-free diet Balb/c mice by intranasal (i.n.) administration of whole antigen. Our results showed that, like the i.v. route, i.n. administration of native gliadin before parenteral immunization abrogated the specific T cell proliferative response; moreover, a marked decrease in γ -interferon and IL-2 levels, and normal levels of IL-4 expression were detected; lower IgG2a serum antibody titres were also observed. Gliadin is a very complex antigen mixture; when gliadin fractions were purified and administered intranasally to study their ability to induce tolerance to whole gliadin, alpha gliadin was found to be particularly effective in downregulating both T cell proliferation and interferon gamma production to whole gliadin. The results indicate alpha gliadin to be a good candidate for further identification of short peptides to be used as tolerogens. Our studies were extended to HLA-DQ8 transgenic mice, this being an allelotype associated to CD even if in a minority of patients. Also DQ8 mice could be tolerated by intranasal administration of a recombinant alpha gliadin. Deamidation sites of this recombinant gliadin were identified and such deamidated gliadin was used to map immunogenic and tolerogenic epitopes.

C35 THE PROLAMIN WORKING GROUP (PWG) GLIADIN STANDARD AND CODEX ALIMENTARIUS WORK

M. Stern, University Children's Clinic, Tübingen (for the International Working Group on Prolamin Analysis and Toxicity)

PWG was founded in 1985 by Wim Hekkens to coordinate research on laboratory gluten analysis in food and on clinical evaluation of patients' sensitivity to prolamins. In 1999 the group has obtained official status of observer non-governmental organization at the FAO/WHO Codex Alimentarius Commission.

Summarizing recent work on the analysis and effects of gluten in coeliac disease it is evident that coeliac toxicity and immunogenicity of various prolamins are not identical. Clinical heterogeneity and inconclusive clinical challenge data do not allow a definition of a threshold of gluten sensitivity which is valid for all coeliac patients. The current Codex Alimentarius limit of 200 ppm gluten is therefore questionable.

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Since a reliable standard method of gluten/gliadin analysis is not yet available, the PWG is now providing a new reference gliadin preparation as a basis for further analysis and for improvement of immunochemical methods. 28 wheat varieties commonly grown in Europe were used for the preparation. PWG gliadin exhibited good solubility, homogeneity and stability with regard to biochemical and immunochemical analysis (HPLC, SDS-PAGE, capillary electrophoresis, mass spectrometry, immuno-blotting, ELISA). All gliadin components of the individual wheat varieties are present in the preparation. PWG gliadin is now available in gram batches for standardization.

Currently PWG is carrying out an international collaborative study on the evaluation of two defined test protocols for gluten analysis in foods (ELISA based on a R5 monoclonal antibody). Definitive results are expected this year to contribute to further propagation of Codex Alimentarius regulations.

C36 OATS AND WHEAT STARCH-BASED GLUTEN-FREE PRODUCTS: TWO CONTENTIOUS DIETS IN THE TREATMENT OF COELIAC DISEASE

Kaukinen K and Collin P

Departments of Internal Medicine, Tampere University Hospital, and University of Tampere, Tampere, Finland

Wheat, rye and barley prolamins should be withdrawn from the coeliac diet, but the issue whether oats can be safely consumed by coeliac patients has been debated since the gluten-free diet was advocated over 40 years ago. In earlier studies the effect of oats was assessed for only short periods in a small number of patients, and the results were contradictory. The conclusion of oat toxicity was based on observation of symptoms or fat malabsorption without small bowel biopsies. In recent larger controlled studies no adverse effects on small bowel mucosal integrity has been found after consumption of moderate amounts of oats in both adults and children suffering from coeliac disease or dermatitis herpetiformis. Furthermore, in vitro studies have indicated that oat prolamins do not stimulate any immune reaction in the coeliac intestinal mucosa.

Wheat starch-based gluten-free products, meeting today's Codex Alimentarius standard, may contain residual gluten. Theoretically these trace amounts of gluten can be harmful, but in recent studies wheat starch-based gluten-free products have been well-tolerated and they have had no harmful effect to clinical outcome or to the recovery of small-bowel mucosa in coeliac disease. We also have preliminary data showing that naturally gluten-free diet is not superior to wheat starch-based diet in newly-detected coeliac disease. Moreover, epidemiological studies suggest that treatment with wheat starch-based gluten-free products has not resulted in excess morbidity or mortality in coeliac disease patients.

As to the treatment of coeliac disease, a poor dietary compliance is much more deleterious than trace amounts of gluten in otherwise gluten-free products. Wheat contamination of gluten-free products may be one reason for poor clinical or histological recovery, even in naturally gluten-free products.

C37 LABELLING OF FOOD PRODUCTS IN EUROPE CONCERNING GLUTEN AND GLUTEN-CONTAINING STARCHES

Hertha Deutsch, Vienna, Austria

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The gluten-free diet is the only treatment in coeliac disease, however because of insufficient labelling directives the risk of unknown gluten-intake was high.

Because of the request of coeliacs the Codex Alimentarius Commission started to consider the food intolerance issue and accepted the Association Of European Coeliac Societies AO ECS as observer. Parallel to the work in Codex AO ECS requested the European Commission to abolish the exceptions of gluten-labelling regarding the 25 % rule, class names etc. Additionally, gluten containing wheat protein products were used for technological reasons and not labelled, because gluten is not a food additive in EU-legislation.

Results

The Codex Alimentarius Commission adopted in 1999 that “cereals containing gluten: i.e. wheat, rye, barley, oats, spelt or their hybridized strains and products of these” and further 7 groups of ingredients shall always be declared (Labelling Standard 4.2.1.4., 4.2.2.1, 4.2.3.2.). Based on the Codex decision Switzerland adopted this “allergen-labelling” in national legislation. The European Commission adopted that “starch and modified starch must always be complemented by the indication of its specific vegetable origin, when that ingredient may contain gluten” (Directive 2000/13/EC) and proposed with Document 2001/0199(COD) to abolish all labelling exceptions regarding gluten.

Conclusion

The improvements of food legislation protect the health of coeliacs.

C38 HEALTH RELATED QUALITY OF LIFE (HRQOL) IN CHILDREN WITH CELIAC DISEASE

H.M. Koopman, R.M. Baars, L.M. Mearin, Leiden University Medical Center, The Netherlands

Having a chronic disease is a known stressor for a child and its family. The outcome of various research and population studies vary, but it is estimated that children with a chronic disease have one and a half to three times as much chance of psychosocial adjustment problems compared to their healthy peers. The psychosocial adaptation to a chronic illness is influenced by several factors such as general and specific characteristics of the chronic illness, life events, stress, the child's personality, the family and the social environment. Adaptation to a chronic disease should also be seen in the context of the present developmental stage of the child or adolescent. Children with a chronic disease confront the same developmental steps as healthy children. Overcoming these tasks and successfully handling the stressful events of childhood development is difficult. The continuing presence of the illness influences the physical and mental functioning of the child as well as the interaction with its surroundings. Children with a chronic illness are also confronted with specific tasks, as managing unpleasant and sometimes painful symptoms of a disease, the (invasive) examinations and the (often drastic) treatments. Functional limitations and Health Related Quality of Life (HrQoL) are indicators which can be used to get an impression of the (long-term) consequences of a chronic disease.

In 1947 the World Health Organisation defined health not only as the absence of illness, but as a state of physical, mental and social wellbeing. From that time onwards QoL was seen as part of ones health. Subsequently the distinction was made between health related and not-health related QoL. HrQoL refers to the subjective and objective influence of disfunctioning related to illness, injury and / or medical treatment. Research shows that a chronic illness can hinder a child's development and

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affect their HrQoL. Next to the possible negative physical and social effects the chronically ill often experience psychological effects as feeling unsure of the future, fears, depression and loneliness. In literature children and adolescents with celiac disease have been described as being dependent and held back in their responses. They experience problems with handling emotions as anger and sadness. Children with celiac disease are found to have a decrease in their HrQoL, especially in the social domain. While physical functioning usually shows no large problems (if children keep to their diet), the diet these children and adolescents need to keep to is often drastic and concerns several food groups. Therefore, keeping to this diet can give social problems. At a party or during the holidays the child with celiac disease has an exceptional position when food or sweets are concerned. During mealtimes children need a continuous alertness to see to it that they eat gluten-free. In times when children want to be like their friends as much as possible it is difficult to be different. Furthermore treatment of celiac can influence their emotional state. They can be sad or angry because they always need to keep to the diet.

Results of HRQoL instruments show that children with celiac disease generally do not differ in their experienced HRQoL compared to healthy peers. Adolescents with celiac do differ on aspects as physical wellbeing and functioning at home. In both aspects they are less content than their healthy peers. The parents view of children aged 8-11 years is similar to the children's own views. When the children reach adolescence the opinions start to differ. In that stage the self-reports and the proxy reports by the parents differ in comparison to the self-report of the children. Both opinions should thus be included in treatment plans.

C39 LIVING WITH COELIAC DISEASE

C Hallert

Coeliac centre, linköping university, linköping, sweden.

Coeliac disease is generally regarded as a very treatable condition. However, signs of depression continue to be seen in patients taking a gluten-free diet, resulting in a poorer quality of life than general population. This appears to be particularly true for coeliac women. This may not necessarily be related to their higher rate of bowel complaints. Instead, the dietary restrictions and the dissatisfaction with the outcome of treatment are features that make the disease burden greater in women with longstanding coeliac disease.

Women and men tend to perceive their gluten intolerance differently. This may have social consequences and implications for their subjective well-being. Presumably, it takes more than a strict gluten-free diet to manage a life with coeliac disease.