IS CELIAC DISEASE DUE TO MOLECULAR MIMICRY BETWEEN GLIADIN PEPTIDE-HLA CLASS II MOLECULE-T CELL INTERACTIONS AND THOSE OF SOME UNIDENTIFIED SUPERANTIGEN?

WILLIAM E. BARBEAU,* MARY ANN NOVASCONE† and KLAUS D. ELGERT‡

†Department of Human Nutrition, Foods and Exercise, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061-0430, U.S.A.; ‡Department of Biology, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061-0406, U.S.A.

(First received 11 March 1997: accepted in revised form 22 April 1997)

Abstract—This paper presents a new hypothesis for the etiology and pathogenesis of celiac disease (CD). It is our contention that CD is triggered by the binding of one or more gliadin peptides to CD-associated HLA class II molecules. Furthermore, we propose that these putative CD peptides bind to oligosaccharide residues on HLA class II molecules distal to the peptide-binding groove invoking recognition and binding by specialized subsets of γδ T cell receptor-bearing lymphocytes. The binding of these γδ T cells serves as a signal for abrogation of oral tolerance to ingested proteins setting in motion a series of immune responses directed against the small intestinal epithelium of CD patients. CD patients are victimized by this self-destructed immune response because of inheritance of certain combinations of HLA-DQ and DR haplotypes. Dimers encoded by HLA-DR haplotypes may be the primary restriction elements for lectin-like, gliadin peptides while the degree of immune suppression (or lack thereof) to ingested gliadins is governed by inherited HLA-DQ haplotypes. Finally, we speculate that molecular mimicry between one or more gliadin peptides and some, as yet unidentified, bacterial or viral superantigen plays a role in disease pathogenesis.

Key words: celiac disease, gliadin peptides, HLA-DQ. – DR molecules.

INTRODUCTION

Two earlier theories for the etiology of celiac disease (CD) focused on the possibility that certain gliadin peptides were cytotoxic and accumulated in the small intestine of celiac patients because of either a missing or deficient enzyme (Krainick et al., 1959; Phelan et al., 1977), or alternatively that some gliadin peptides had unrecognized lectin activity (Weiser and Douglas, 1976). A third theory, called the immune hypothesis, supercedes them. Briefly, this hypothesis states that habitual consumption of wheat and possibly other cereals causes an aberrant immune response in celiac-susceptible individuals which is directed against the epithelial lining of the small intestine (Strober, 1986). Disease susceptibility is believed to be based, in part, on the inheritance of chromosomes 6 HLA class II genes (Kagnoff, 1988; Marsh, 1992; Solliid and Thorsby, 1993), which encode proteins involved in antigen presentation.

Evidence continues to mount in support of the immune hypothesis (Marsh, 1992; O‘Farrell and Gallagher, 1992; Solliid and Thorsby, 1993). This hypothesis is, however, incomplete because it does not account for an event or situation that might serve as a trigger for disease pathogenesis. This triggering event or situation is likely to occur only when genetically predisposed individuals consume wheat, rye, oats or barley. Kagnoff et al. (1984) suggested that CD is triggered only after exposure to adenovirus Ad12. They contend that the disease occurs because of a sequence homology between peptide fragments found in an adenovirus protein, Elb, and A-gliadin. Some experimental evidence supports their hypothesis (Kagnoff et al., 1987; Mantzaris et al., 1990) while other data does not (Howdle et al., 1989; Ellis et al., 1992; Lawler et al., 1994). A weakness in Kagnoff’s hypothesis is that it fails to provide a satisfactory explanation for why individuals inheriting certain HLA haplotypes are more predisposed to CD than others who may also come in contact with the Ad12 adenovirus. In addition, it is unlikely that gliadin toxicity is confined to A-gliadin because the protein is found only in the alpha fraction of gliadin, while toxicity is dispersed among four different gliadin fractions (Howdle et al., 1984).

γδ T CELLS, SUPERANTIGENS AND CELIAC DISEASE

One of the keys to unraveling the complexities of CD is to understand the reason(s) for disproportionate
increases in the number of CD4+CD8+ IELs bearing the γδ TCR in the jejunum of untreated celiac patients as well as those on a gluten-free diet (Halstensen et al., 1989; Trejosiezeicz et al., 1991). This IEL subset utilizes a non-disulfide linked form of the γδ TCR encoded by Vδ1/Jο1 gene segments (Halstensen et al., 1989; Spencer et al., 1989). Two particularly pressing questions are: what restriction elements are recognized by these IELs and what antigen or antigens are they responding to? The present literature is ambiguous as to whether γδ T cells are restricted by class I or class II HLA molecules (Matis et al., 1987; Kozbor et al., 1989). Some studies suggest that γδ T cells may be able to recognize and respond to antigens even in the absence of class I or class II molecules (Holoshitz et al., 1989). Many investigators believe that γδ T cells preferentially respond to heat shock proteins (Haregewoin et al., 1989; Born et al., 1990) and/or to superantigens, a group of protease resistant ligands derived from bacteria and viruses (Pleffer et al., 1990, 1992).

Recently, Schild et al. (1994) used site-directed mutagenesis to make single amino acid substitutions both in and outside the peptide-binding site of antigen presenting cells (APCs) recognized by a particular subset of γδ T cells. They found that substituting one amino acid for another along the peptide-binding groove did not influence γδ T cell recognition; however, APCs were no longer recognized when they substituted glutamic acid for lysine at position 79 of the C-terminal end of the alpha polypeptide chain (Brown et al., 1991) which conceivably might be important in the purported interactions with the human immune system.

DeRitis and others have demonstrated that there are a number of peptides derived from the NH2-terminal region of A-gliadin that are toxic to celiac patients (DeRitis et al., 1988; Sturgess et al., 1994; Maiuri et al., 1996). Active peptides contain the amino sequences QQQP and PSQQ (DeRitis et al., 1988). QQQP may be a more important disease-conferring epitope than PSQQ (Sturgess et al., 1994; Maiuri et al., 1996). Both of these amino acid sequences have a propensity for forming beta reverse turns (Koca et al., 1991) which might be important in their purported interactions with the human immune system.

It occurred to us, after reviewing the models for superantigen-HLA class II-T cell interactions proposed by Herman and Fraser, that infiltration of elevated numbers of γδ T cells into CD lesions, might be a result of molecular mimicry between one or more gliadin peptides and an unidentified bacterial or viral superantigen. CD pathogenesis is, in our view, triggered by the mutual engagement of a gliadin peptide(s), HLA class II molecules and γδ TCR bearing T lymphocytes. A key interaction in formation of this complex is likely to be hydrogen bonding between oxygen atoms of oligosaccharides attached to HLA class II molecules and the amide hydrogens of glutamine side chains in gliadin peptides.

A NEW HYPOTHESIS FOR CD ETIOLOGY AND PATHOGENESIS

We now have a framework on which to construct a new hypothesis for the etiology and pathogenesis of CD. We propose that the critical first step in CD occurs when one or more gliadin peptides bind to HLA class II molecules on the epithelial lining of the small intestine of celiac-susceptible individuals. Furthermore, we hypothesize that these putative peptides bind to oligosaccharide residues on HLA class II molecules distal to the peptide-binding groove, invoking recognition and binding by a specialized subset of γδ TCR-bearing lymphocytes (see Fig. 1). Engagement by these γδ T lymphocytes serves as a signal for abrogation of oral tolerance to ingested proteins and sets in motion a series of self-destructed immune responses directed against the small intestinal epithelium (see Fig. 2). Celiac-susceptible individuals are victimized by this self-destructed immune response because they inherit certain HLA haplotypes that code for γδ class II dimers with affinities for binding putative gliadin peptides at sites distal to the peptide binding groove.

IS THIS HYPOTHESIS SUPPORTED BY OR AT LEAST CONSISTENT WITH EXISTING DATA?

The next question was whether it can be shown that our hypothesis is supported by, or at least consistent with, existing experimental data? First, is there any evidence for the binding of gliadin peptides to HLA class II molecules in situ, either to "empty" class II dimers that might be...
A new hypothesis for the etiology of CD

A new hypothesis for the etiology of CD 537

478 + glycosylated residue 78 on α1 domain of a HLA-DR dimer

lectin-like gliadin peptide

\[ \text{HLA-DR dimer} \]

APC in the jejunum of CD patients

Cytoplasm

Membrane

(Please note that according to Brown et al. (1993) HLA class II molecules may exist in situ as dimers)

Fig. 1. A proposed model for recognition and binding of gliadin peptides and CD-associated HLA-DR complexes by γδT cells.

rarely expressed on cell surfaces (Reid and Watts, 1992), or within endosomal compartments where newly synthesized class II molecules presumably associate with exogenous peptides generated by proteolytic breakdown of proteins (Germain, 1986; Teyton et al., 1990). A recent report suggests that gliadin peptides are translocated into HLA-DR containing lysosomes within enterocytes of patients with untreated CD (Zimmer et al., 1995). This study provides no direct evidence, however, for the binding of gliadin peptides to HLA-DR molecules. Gallagher et al. (1988) failed to detect alpha-gliadin binding to HLA-DP, DQ or DR molecules of B cells isolated from peripheral blood specimens of seven celiac patients. However, a different set of HLA class II molecules might be

Fig. 2. Possible scenarios following ingestion of wheat and other cereals leading to oral tolerance in the general population and to gut-localized delayed hypersensitivity in CD-susceptible individuals.
expressed on cells normally confined to the jejunal mucosa than on circulating B cells; HLA molecules would also be expected to bind to partially digested, proteolytic fragments of alpha-gliadin rather than the intact protein.

Our hypothesis assumes that the binding of gliadin peptides to HLA class II dimers is insufficient to trigger CD and that disease pathogenesis ensues only when these peptide–HLA complexes are recognized and bound by the T cell. Is there any evidence suggesting that T cells play a role in CD pathogenesis and for the idea that CD T-cell binding of gut–presented gliadin peptide–HLA complexes serves as a signal for abrogation of oral tolerance? Fujihashi et al. (1989) reported abrogation of oral tolerance in nude mice by adoptive transfer of DQα TCR-bearing CD3+ CD4+ CD8+ T cells. This phenomenon also occurs in C3H/HeN mice where Fujihashi and coworkers have shown that DQβ T cells abrogate oral tolerance and and T cell provide helper function to T cells (Fujihashi et al., 1992). No human studies have been reported in this area. Thus, no direct evidence exists for T cell involvement in contrasuppression of human immune responses against wheat gliadins or other dietary antigens.

Stokes et al. (1972) were the first to report on a significant association between the incidence of adult CD and inheritance of certain HLA phenotypes. Investigators now believe that disease susceptibility is primarily conferred by DQA1*0501 and DQB1*0201 genes inherited either in the cis or the trans position that encode for a particular HLA-DQ heterodimer (Mazzioli et al., 1992; Ploski et al., 1993; Congia et al., 1994): the expressed form of which is presumably involved in antigen presentation of gliadin derived peptides. In fact, gliadin-specific, HLA DQ (z1*0501, b1*0201) restricted T cells have been isolated from jejunal biopsies of CD patients (Lundin et al., 1993). Disease susceptibility cannot be confined to a combination of DQA1*0501 and DQB1*0201 alleles; however, because some people carrying these alleles never develop CD and the two alleles are absent in others with the disease (Tighe et al., 1993; Sollid and Thorsby, 1993). We envision a hierarchy of T cell responses to the parasite.

According to our hypothesis, one or more of the putative gliadin peptides must bind to an, yet unidentified, HLA class II dimer at a site distal to the peptide-binding groove formed by the N-terminal domains of α and β polypeptides (Brown et al., 1988). We think that a DQ (z1*0501, b1*0201) heterodimer is unlikely to be a restricting element for these putative peptides. We are supported in this belief by the fact that while although HLA-DQ (z1*0501, b1*0201) restricted T cells exhibited strong proliferative responses to gluten, they failed to respond to overlapping peptides from the celiac active, NH2-terminal end of A-gliadin (Lundin et al., 1993).

We believe that CD is triggered by the binding of gliadin peptides to an oligosaccharide moiety on a HLA-DR dimer. This supposition is congruent with the finding that HLA-DQ-restricted T cells suppress rather than activate antigen-specific immune responses (Salgame et al., 1991). Hirayama et al. (1987) discovered that human T cell responses to the parasite, Schistosoma japonicum, are controlled by genes that encode both HLA-DQ and HLA-DR molecules. Non-responder haplotypes carry a gene expressing a DR2 molecule capable of presenting schistosomal antigens to CD4+ T cells, but helper T cell responses are suppressed in non-responder haplotypes by T cells controlled by a DQw1 molecule. High-responder haplotypes express a different DQw1 molecule that does not suppress helper T cell responses to the same degree as in non-responder haplotypes.

A similar scenario may be operative in CD with phenotypic expression of the disease occurring only when one has inherited genes encoding for a particular set of HLA-DQ and HLA-DR molecules. Approximately 10% of CD patients are DQ (z1*0501, b1*0201) negative and appear to share a second CD associated DQ (z1*0301, b1*0302) heterodimer (Lundin et al., 1994). These DQ heterodimers may be strongly associated with CD because they somehow fail to suppress T cell-mediated responses to ingested wheat gliadins. The DQ (z1*0501, b1*0201) and DQ (z1*0301, b1*0302) heterodimers occur at a much higher frequency in the human population than the overall frequency at which symptomatic cases of CD occur (worldwide average of less than 1/1000 individuals; Troncone and Auricchio, 1991) making it unlikely that these heterodimers are the only inheritable disease determinants. Linkage disequilibrium is found between genes coding for these DQ heterodimers and some HLA-DR haplotypes (Roep et al., 1988; Lundin et al., 1994) suggesting that there may be a second critical set of CD determinants encoded by these DR haplotypes.

We theorize that CD is triggered by the engagement of γδ 1 cells to a specific set of HLA-DR-restricted gliadin peptides. Reactive subsets of γδ T cells may be programmed to home to the small intestinal epithelium and to function in immune surveillance to bacterial or viral superantigens (Bonneville et al., 1988; Janeway et al., 1989). However, the primary effector cells in CD pathogenesis may not be these γδ T cells but αβ T cells recruited to jejunal lesions from the underlying lamina propria (Griffiths et al., 1988; Kutlin et al., 1993). This implies that these CD4+ αβ TCR-bearing T cells also bind to putative gliadin peptide–HLA-DR complexes or, alternatively, that activation of these helper T cells is somehow modulated by gliadin-reactive γδ T cells. There is evidence for “crossstalk” between αβ and γδ T cells, possibly via γδ T cell production of interleukin-2 and/or cell membrane display of heat shock proteins (Kaufmann et al., 1993). Interleukin-2 positive T cells were recently detected in eight out of eight jejunal biopsies of individuals with silent CD and in 24/54 of their first degree relatives but in none of 19 control specimens (Holm et al., 1994), which suggests a role for cytokine secretion and T cell communication in CD pathogenesis.
CONCLUSION

We propose, here, a new hypothesis for the etiology and pathogenesis of CD. Our hypothesis is similar to that of Kagnoff's in that there may be sufficient molecular mimicry between HLA class II dimer bound forms of gliadin peptides and those of particular bacterial or viral superantigens, to be recognized by identical \( \gamma \delta \) T cell subsets. However, in our model it would be unnecessary for CD patients to have a prior infection with an enteric pathogen to illicit strong immune responses to dietary gliadin because according to our hypothesis such responses are primarily predicated on the inheritance of genes encoding HLA-DQ and -DR molecules.

We believe that the preceding discussion will help to stimulate renewed efforts at deciphering human immune system responses to dietary antigens in CD and in other food-related diseases.

Acknowledgements—The authors would like to acknowledge Dr Asim Esen of the Department of Biology, Virginia Polytechnic Institute and State University, for getting us to reconsider a role for molecular mimicry in the etiology of celiac disease.

REFERENCES


Fujihashi K., Kiyono H., Aicher W. K., Green D. R., Singh B., Eldridge J. H. et al. (1989) Immunoregulatory function of CD3\(^+\), CD4\(^+\) and CD8\(^+\) T cells. \( \gamma \delta \) T cell receptor-positive T cells from nude mice abrogate oral tolerance. Journal of Immunology 143, 3415-3427.

Fujihashi K., Taguchi T., Aicher W. K., McGhee J. R., Bluestone J. A., Eldridge J. H. et al. (1992) Immunoregulatory functions for murine intraepithelial lymphocytes: \( \gamma \delta \) T cell receptor-positive (TCR\(^+\)) T cells abrogate oral tolerance, while \( \gamma \beta \) TCR+ T cells provide B cell help. Journal of Experimental Medicine 175, 695-707.


Halsensten T. S., Scott H. and Brandtzæg P. (1989) Intraepithelial T cells of the TcR \( \gamma \delta \) CD8\(^-\) and V\(\alpha\)I\(\delta\) + phenotypes are increased in coeliac disease. Scandinavian Journal of Immunology 30, 683-692.


Janeway C. A., Jones B. and Hayday A. (1989) Specificity and function of T cells bearing \( \gamma \delta \) receptors. Immunology Today 10, 73-75.


Krug O., Lambrichts P., Kehrer P. et al. (1990) A study of Italian pediatric celiac disease patients confirms that the primary HLA association is to the DQ (x1 x0501, β1 x0201) heterodimer. *Human Immunology* **33**, 133–139.


Mazilli M. C., Ferrante P., Mariani P., Martone E., Petronzelli F., Triglione P. et al. (1992) A study of Italian pediatric celiac disease patients confirms that the primary HLA association is to the DQαβ (x1 x0501, β1 x0201) heterodimer. *Human Immunology* **33**, 133–139.


A new hypothesis for the etiology of CD 541

