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

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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 $\gamma\delta$ T cell receptor-bearing lymphocytes. The binding of these $\gamma\delta$ 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. © 1997 Elsevier Science Ltd.

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 chromosome 6 HLA class II genes (Kagnoff, 1988; Marsh, 1992; Sollid and Thorsby, 1993), which encode proteins involved in antigen presentation.

Evidence continues to mount in support of the immune hypothesis (Marsh, 1992; O'Farrelly and Gallagher, 1992; Sollid 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, E1b, 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).

$\gamma\delta$ T CELLS, SUPERANTIGENS AND CELIAC DISEASE

One of the keys to unraveling the complexities of CD is to understand the reason(s) for disproportionate

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increases in the number of $CD4^-CD8^-$ IELs bearing the $\gamma\delta$ TCR in the jejunum of untreated celiac patients as well as those on a gluten-free diet (Halstensen *et al.*, 1989; Trejdosiewicz *et al.*, 1991). This IEL subset utilizes a non-disulfide linked form of the $\gamma\delta$ TCR encoded by Vδ1/Jδ1 gene segments (Halstensen *et al.*, 1989; Spencer *et al.*, 1989). Two particularly pressing questions are: what restriction elements are recognised by these IELs and what antigen or antigens are they responding to? The present literature is ambiguous as to whether $\gamma\delta$ T cells are restricted by class I or class II HLA molecules (Matis *et al.*, 1987; Kozbor *et al.*, 1989). Some studies suggest that $\gamma\delta$ 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 $\gamma\delta$ 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 (Pfeffer *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 $\gamma\delta$ T cells. They found that substituting one amino acid for another along the peptide-binding groove did not influence $\gamma\delta$ 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 distal to the peptide-binding groove. Based on these findings, Schild *et al.* (1994) speculated that $\gamma\delta$ TCRs may have similarities to immunoglobulins and may recognize antigens in the context of HLA class I or II molecules altogether differently from $\alpha\beta$ TCR^+ lymphocytes.

Dellabona *et al.* (1990) conducted similar studies on presentation of the superantigen, staphylococcal enterotoxin B (SEB), to T cells. Once again, they were able to substitute alanine for any one of 30 amino acids within the antigen binding groove of HLA class II molecules with negligible effects on antigen presentation of SEB. Presentation of conventional peptide antigens was, however, severely compromised by the same substitutions. This led Dellabona *et al.* (1990) to postulate that superantigens interact with HLA class II molecules at a site(s) away from the antigen binding groove.

Models for superantigen-HLA class II-T cell interactions proposed by Herman *et al.* (1991) and Fraser *et al.* (1993) predict that superantigens bind laterally to one side of HLA class II molecules. Current indications are that there are amino acid residues on both the superantigen and HLA class II molecule that are critical to T cell engagement (Herman *et al.*, 1991). HLA class II molecules are known to contain oligosaccharide residues. A common carbohydrate attachment site is residue 86 (residue 78 in HLA-DR molecules), located on the α 1 domain of class II molecules, found in X-ray crystallographic studies to be protruding away from the antigen binding grove (Brown *et al.*, 1988, 1993). This attachment site is close to the location predicted by Fraser for the binding of staphylococcal enterotoxin A to HLA class II molecules (Fraser *et al.*, 1993).

DeRitis and others have demonstrated that there are a number of peptides derived from the NH_2-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 (Kocna *et al.*, 1991) which conceivably might be important in ther purported interactions with the human immune system.

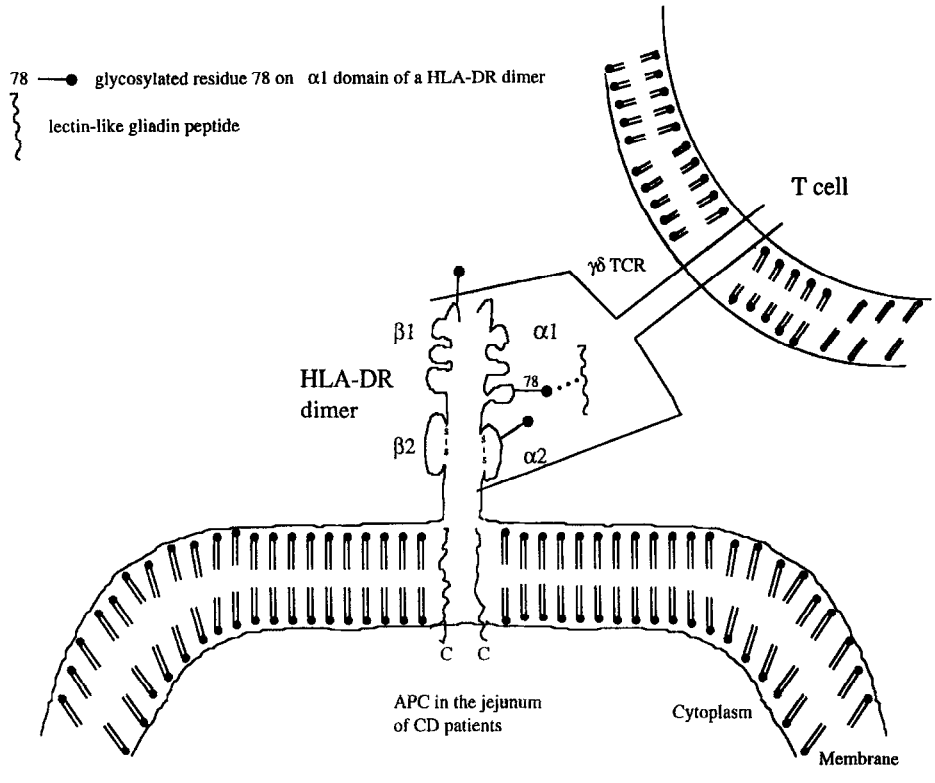
It occurred to us, after reviewing the models for superantigen-HLA class II-T cell interactions proposed by Herman and Fraser, that infiltration of elevated numbers of $\gamma\delta$ T cells into CD lesions, might be a result of molecular mimicry between one or more gliadin peptides and an unidentified 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 $\gamma\delta$ 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 $\gamma\delta$ TCR-bearing lymphocytes (see Fig. 1). Engagement by these $\gamma\delta$ 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 $\alpha\beta$ 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



(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

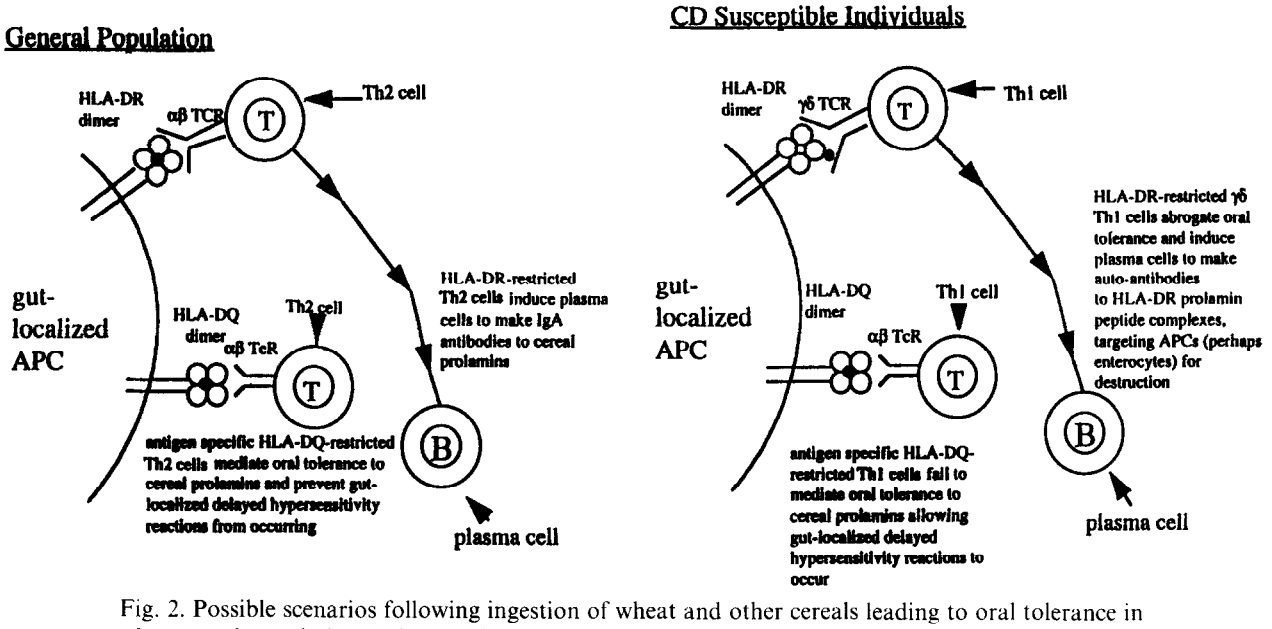


Fig. 2. Possible scenarios following ingestion of wheat and other cereals leading to oral tolerance in the general population and to gut-localized delayed hypersensitivity in CD-susceptible individuals.

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 insufficent to trigger CD and that disease pathogenesis ensues only when these peptide–HLA complexes are recognized and bound by $\gamma\delta$ TCR-bearing lymphocytes. Is there any evidence suggesting that $\gamma\delta$ T cells play a role in CD pathogenesis and for the idea that $\gamma\delta$ 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 $\gamma\delta$ TCR-bearing $CD3^+$, $CD4^-$, $CD8^-$ T cells. This phenomenon also occurs in C3H/HeN mice where Fujihashi and coworkers have shown that $\gamma\delta$ T cells abrogate oral tolerance and $\alpha\beta$ T cell provide helper function to B cells (Fujihashi *et al.*, 1992). No human studies have been reported in this area. Thus, no direct evidence exists for $\gamma\delta$ 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 $\times$ 0501 and DQB1 $\times$ 0201 genes inherited either in the *cis* or the *trans* position that encode for a particular HLA-DQ heterodimer (Mazilli *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 (α1 $\times$ 0501, β1 $\times$ 0201) restricted T cells have been isolated from jejunal biopsies of CD patients (Lundin *et al.*, 1993). Disease susceptiblity cannot be confined to a combination of DQA1 $\times$ 0501 and DQB1 $\times$ 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 gliadin peptide-binding affinities among HLA class II molecules both in CD patients and in non-susceptible individuals, and that one has a strong likelihood of eventually being inflicted with CD if they have inherited HLA haplotypes that code for not one but at least two distinct $\alpha\beta$ class II dimers.

According to our hypothesis, one or more of the putative gliadin peptides must bind to an, as 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 (α1 $\times$ 0501, β1 $\times$ 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 (α1 $\times$ 0501, β1 $\times$ 0201) restricted T cells exhibited strong proliferative responses to gluten, they failed to respond to overlapping peptides from the celiac active, NH_2-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 suppostion 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 occuring 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 (α1 $\times$ 0501, β1 $\times$ 0201) negative and appear to share a second CD associated DQ (α1 $\times$ 0301, β1 $\times$ 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 (α1 $\times$ 0501, β1 $\times$ 0201) and DQ (α1 $\times$ 0301, β1 $\times$ 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 $\gamma\delta$ T cells to a specific set of HLA-DR-restricted gliadin peptides. Reactive subsets of $\gamma\delta$ 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 $\gamma\delta$ T cells but $\alpha\beta$ T cells recruited to jejunal lesions from the underlying lamina propria (Griffiths *et al.*, 1988; Kutlu *et al.*, 1993). This implies that these $CD4^+$ $\alpha\beta$ 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 $\gamma\delta$ T cells. There is evidence for "crosstalk" between $\alpha\beta$ and $\gamma\delta$ T cells, possibly via $\gamma\delta$ 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 biposies 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 unneccessary 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.

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