More than 50 years have passed since Dicke clearly demonstrated that removal of gluten from the diet caused the symptoms and signs of celiac disease (CD) to disappear. The term gluten is currently applied to the storage proteins of wheat grain, which on the basis of their different alcohol solubility can be divided into gliadins and glutenins. At present, the pathogenic effect of wheat gliadin on the small bowel mucosa of genetically susceptible individuals is undeniable and much interest is focused on the identification of the putative epitope(s).

Earlier attempts to characterize a short peptide sequence toxic for CD focused on the amino terminal region of α-gliadin and led to the identification of overlapping sequences, i.e., the residues 31-55 and 31-49, whose detrimental properties were demonstrated both in an organ culture assay and in vivo feeding experiments.

More recently, 2 different groups have found that the region of α-gliadin corresponding to amino acids 57-75 is able to induce immunological activation of HLA DQ2 positive gut-derived and peripheral T cells from adult CD patients and becomes a potent, immunodominant epitope when a single glutamine residue (Q65) is deamidated by tissue transglutaminase (tTG) to glutamic acid. Interestingly enough, there are noticeable contradictions between results obtained with these different in vitro systems, since the capacity of gliadin peptides to induce toxicity in an organ culture model of CD does not correspond to that of stimulating T cells, and vice versa.

However, this discrepancy is not completely unexpected because gluten may have an early T cell unrelated effect.
on celiac intestinal mucosa, eventually leading to a subsequent T-cell activation.

Many points still await clarification: gliadin peptides 57-68 and 62-75 failed to stimulate T-cell clones derived from celiac children and, on the other side, a DQ8-restricted glutenin-derived T-cell epitope has been preliminarily identified.

In this issue of GASTROENTEROLOGY, Vader et al. have elegantly characterized the T-cell response toward several gluten (gliadin and glutenin) peptides, trying to address some of the questions still unsolved. Looking at the T-cell profile in 16 DQ2-positive CD patients not older than 12 years of age, they surprisingly found that the repertoire of gluten peptides able to induce a HLA-restricted T-cell activation is wider and more heterogeneous than previously reported in adults. In fact, it seems to be also driven by 6 novel additional gliadin and glutenin peptides characterized in the study. Moreover, only half the pediatric (and even adult) patients responded to the putative immunodominant epitopes (gliadin 57-68 and 62-75), whereas in children, but not in adults, a repetitive glutenin sequence seems to be the most suitable stimulatory peptide, being the only one to be active in the youngest patient studied. Finally, the finding that T-cell responses to 3 of the novel peptides are deamidation-independent suggests that in pediatric CD T-cell responses can be initiated toward native gluten peptides. In this context, it should be stressed that the necessity of tTG-mediated deamidation to increase the peptides’ avidity to the HLA molecule has been demonstrated for gliadins but does not seem so critical for glutenins.

The evidence of such an important heterogeneity between children and adult patients and the possibility that different epitopes might be involved in different stages of CD sounds fascinating and opens a new scenario in the immunopathogenesis of this condition. The authors hypothesize a multistep sequence in which in an early phase (childhood) the immune response is directed to a wide array of native gluten peptides and the presence of the same highly repetitive sequence (PQQPYQPQPQ) in both glutenin and gliadin molecules represents the most likely mechanism of this epitope spreading. In a more advanced phase (adulthood), the immune response is polarized to immunodominant peptide(s) capable of stronger binding affinity to DQ2 molecules and of more vigorous T-cell stimulatory effects. Gluten peptide deamidation, catalyzed by tTG increasingly released from cytoplasmic stores as a consequence of increasing tissue injury, represents the most likely mechanism of this epitope focusing.

Recent evidence from animal models of autoimmune disease points to epitope spreading as a crucial mechanism in development of autoimmunity, relapse, and disease progression. Intra- and intermolecular epitope spreading refer to the acquired recognition of new determinants distinct from the primary disease-inducing epitope, and it has been invoked in the pathogenesis of multiple sclerosis, type 1 diabetes, autoimmune arthritis and dermatitis herpetiformis, a gluten-sensitive condition strictly related to CD. Unlike these disease states, CD is also characterized by the deviation from glutenin-gliadin spreading to a dominant epitope. We can further speculate that secondary spreading of other molecules may be of additional importance in increasing the risk for autoimmune diseases associated with CD (Figure 1), which may reach 35% after 20 years of gluten exposure.

It is conceivable that in the course of CD progression a cascade of further epitopes, such as biological, nutritional, or endogenous functional/structural proteins may contribute to the development of secondary autoimmunity. Among the mechanisms that could expose and spread cryptic epitope(s) in CD, Th1 cytokine up-regulation in the gut mucosal milieu, by modifying cleavage of proteins during antigen processing and by recruiting nonprofessional antigen-presenting cells characterized by specific cleavage patterns, may play an important role. Enzymatic (tTG-mediated), posttranslational protein modification, and generation by apoptosis of usually cryptic epitopes have been indicated as other candidate mechanisms.

Clearly, the implications of epitope spreading in terms of future treatment strategies of CD are of great relevance; at this point, peptide immunotherapy or selective T-cell deletion might appear too elementary approaches. However, dominant T-cell epitopes have also been shown to be the most potent tolerogens when ingested by alternative routes. Data from murine allergy models

**Figure 1.** Epitope spreading and focusing in CD. The steps inside the continuous lines are supported by the results of Vader et al. It is hypothesized that the steps inside the broken lines may lead to secondary autoimmunity in CD.
indicate that treatment with the immunodominant peptide may induce tolerance to the whole antigenic molecule, thus generating an intramolecular epitope suppression. Therefore, the real challenge is understanding the hierarchy of the spreading process in CD, as it could be helpful in the design of stage-specific therapies. In non-obese diabetic mice, autoimmunity spreads in a predefined way regulated by the frequency and avidity of autoreactive T-cell repertoires, and this predictable sequential pattern can constitute the basis for the induction of peptide-specific tolerance. Alternative strategies may be represented by the DNA vaccine technology, that will permit the construction of plasmids containing multiple epitopes, by the blockade of the CD28/B7 co-stimulatory pathway, and mainly by the use of antigen-presenting cells genetically engineered both to present the dominant gluten peptide and to express Fas ligand to target Fas positive antigen-specific celiac T cells and induce their apoptosis.

In conclusion, there is no doubt that the novel results by Vader et al. will provide important tools for future studies aimed to expand our knowledge about pathogenesis, and perhaps treatment, in CD. In particular, the temporal variations in immune responses of autoreactive T cells toward gluten peptides should be more carefully clarified. To this purpose, by analogy with preclinical type 1 diabetes, we suggest that potential-latent CD represents a model more appropriate than childhood CD to study the early phase of epitope spreading in CD.

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