
Development of a Vaccine for Celiac Disease

R.P. Anderson

Autoimmunity and Transplantation Division, Walter and Eliza Hall Institute of Medical Research, Parkville, Victoria, Australia

Abstract

Celiac disease is the result of an immune response to gluten. Gluten exclusion removes the antigen that stimulates CD4 T-cell-mediated tissue damage in the gut, but does not remove the immune response. In fact, gluten exclusion may heighten the immune response stimulated by gluten since regulatory T cells are typically not maintained unless antigen exposure continues. This may explain why gluten exposure triggers more dramatic symptoms following adoption of a gluten-free diet than during chronic gluten exposure associated with untreated celiac disease. Peptide-based therapeutic vaccines aim to strengthen the antigen-specific regulatory T-cell response to suppress proinflammatory adaptive and innate immunity in an antigen-specific and nonspecific fashion. Peptide-based therapeutic vaccines require detailed understanding of the peptides derived from pathogenic antigens that stimulate pathogenic CD4 T cells. The present knowledge of gluten peptides recognized by CD4 T cells largely derives from T-cell clones and lines isolated from celiac-disease-affected intestinal tissue. In this chapter, it is argued that T cells mobilized into blood by acute gluten exposure facilitate a more reliable mapping of gluten peptides recognized by relevant CD4 T cells. Understanding not only the specificity, but also the hierarchy (immunodominance) of peptides is critical to the practical design of a peptide-based therapeutic vaccine. The effort is worthwhile and the debate important since peptide-based therapeutic vaccines offer the possibility of a qualitative change in the pathogenic immune response to gluten and for patients to return to a virtually normal lifestyle.

Copyright © 2008 S. Karger AG, Basel

In principle, any human allergic or autoimmune disease for which there is a known causative antigen could be amenable to peptide-based or antigen-specific immunotherapy. Whole-antigen immunotherapy provides long-term remission in allergic diseases such as hay fever [1], while a prototype vaccine using allergen-derived (Fel d 1) peptides recognized by CD4 T cells in cat-sensitive asthma shows efficacy in phase II clinical trials [2]. Provided that suitable immunodominant gluten peptides can be selected, peptide-based therapeutic vaccines may be ideally suited to the treatment of celiac disease. In contrast to other proposed nondietary therapies [3], peptide-based therapeutic vaccines would specifically modify the pathogenic T-cell response rather than reduce the amount of gluten peptide presented to the T cell or compromising other aspects of the immune system. This overview addresses the development of a peptide-based therapeutic vaccine for HLA-DQ2 (*HLA-DQA1*05* and *-DQB1*02*)-associated celiac disease. HLA-DQ8-associated celiac disease will not be discussed.

Peptide-based therapeutic vaccines utilize immunodominant peptides derived from defined environmental or self-antigens that trigger disease by activating pathogenic T cells, a small fraction of

the body's total T-cell population [4]. In common with traditional whole-antigen immunotherapy, peptide-based therapeutic vaccines delivered in multiple small doses over a course of injections or mucosal applications can induce immune tolerance not only to the selected immunodominant epitopes or protein, but also potentially spreading to involve other subdominant pathogenic epitopes [5].

Whole-antigen immunotherapy for allergic diseases carries a small risk of triggering clinically significant anaphylaxis. Anaphylaxis occurs because the administered antigen/allergen is sufficiently large to allow cross-linking of membrane-bound IgE on mast cells [6]. Peptides less than 20 amino acids in length are sufficiently small to avoid IgE cross-linking and promise to be safer than protein-based strategies [6].

In celiac disease, a T-cell- rather than IgE-mediated disease, it is the insolubility of gluten proteins and their requirement of selective deamidation by tissue transglutaminase (tTG) to facilitate T-cell recognition [7] that makes whole-protein immunotherapy unattractive. In celiac disease, peptide-based therapeutic vaccines would be anticipated to minimize the risk of anaphylaxis and allow selection of soluble peptides with immunological properties suitable for a pharmaceutical agent with predictable efficacy and safety.

Peptide-Based Therapeutic Vaccine – Proof of Principle

In contrast to celiac disease, there are no strong HLA associations in allergic diseases such as asthma, and the epitopes recognized by allergen-specific T cells are rather inconsistent between individuals [4]. In cat-sensitive asthma caused by skin dander protein (Fel d 1), rather than use defined CD4 T-cell epitopes, a successful peptide-based therapeutic vaccine has been designed according to the binding affinity of Fel d 1 16-mers for various common HLA-DR molecules [2]. Intradermal escalating doses (0.1, 1, 5, 10, 25,

50 and 100 μg) of peptide cocktail administered every 3–7 days leads to clinical nonresponsiveness and abolishes cutaneous CD4 T-cell-mediated late-phase reactions to Fel d 1 [2, 5]. This protocol using Fel d 1 16-mers does not cause acute anaphylaxis.

Three to six hours after administration, the Fel d 1 peptide-based therapeutic vaccine is occasionally followed by bronchospasm. The delayed reaction is readily controlled and typically occurs after the first administration of peptide or with dose escalation. This delayed reaction is due to activation of effector T cells in the lungs [2, 6, 8]. Such T cells are likely to be 'effector memory' T cells. Upon activation by cognate antigen, effector memory T cells are characterized by cytokine secretion rather than proliferation [9].

In contrast, activation and proliferation of central memory T cells residing in secondary lymphoid organs are dependent upon antigen carried and presented by tissue-derived dendritic cells [9]. Amplification of T-cell responses by antigen-driven proliferation is due to central memory T cells. Following proliferation, relevant T cells exit secondary lymphoid tissue and travel via lymphatics to eventually appear in peripheral blood in the days after antigen encounter. Both effector and central memory T cells are present in blood.

Indeed, peripheral blood T cells are qualitatively altered by Fel d 1 peptide immunotherapy. Regulatory CD4+CD25+ T cells in blood 1 week after Fel d 1 peptide therapy effectively suppress Fel d 1-stimulated proliferation of peripheral blood T cells drawn before therapy, and in vitro Fel d 1-stimulated γ -interferon (IFN- γ) is reduced but interleukin (IL) 10 secretion is increased [5, 10]. Interestingly, regulatory T cells are capable of suppressing established asthma in rats and require ongoing allergen exposure both to suppress disease and for their own maintenance [11]. Consistent with this observation, tolerance induced by peptide and allergen-based immunotherapy is durable for weeks or months, but is not indefinite

[1, 4]. Hence, without some form of maintenance, immune tolerance induced by peptide-based therapeutic vaccines is likely to be reversible and to require ongoing monitoring utilizing a marker of disease remission and/or immune tolerance.

Relevant T-Cell Epitopes in Human HLA-Associated Disease

Celiac disease and many autoimmune diseases are strongly associated with MHC class II molecules, HLA-DR3-DQ2 and/or HLA-DR4-DQ8 [12]. These consistent associations suggest that specific CD4 T-cell epitopes presented by HLA-DR3 and/or -DR4, or HLA-DQ2 and/or -DQ8 are critical to initiation or maintenance of these diseases. However, the identity and hierarchy of epitopes for pathogenic autoreactive CD4 T cells in human autoimmune diseases are poorly defined, handicapping the rational design of peptide-based therapy.

Celiac disease does have a known causative antigen, gluten, and characterization of T-cell epitopes is well advanced. Could a peptide-based therapeutic vaccine be designed for celiac disease?

T Cells and the Immunopathogenesis of Celiac Disease

Celiac disease is unequivocally an immune disease caused by dietary gluten. Almost all individuals with celiac disease possess genes encoding either HLA-DQ2 or HLA-DQ8. Gluten-specific T-cell clones and lines have been successfully isolated from intestinal biopsies and expanded in vitro using gluten combined with various mitogens. Almost all such T-cell clones are HLA-DQ2 or -DQ8 restricted and secrete Th1-associated cytokines dominated by IFN- γ [13, 14]. Their presence supports the contention that gluten-specific CD4⁺ T cells play a central role in celiac disease.

T-Cell Expansion in vitro: Gold Standard or Contrivance?

Despite gluten-specific CD4⁺ T cells isolated from disaggregated intestinal tissue proliferating when incubated with growth factors and gluten in vitro, 24-hour incubation of celiac intestinal tissue with gluten stimulates expression of the nuclear proliferation-associated marker Ki-67 in intraepithelial CD8⁺ γ/δ T cells but not lamina propria CD4⁺ T cells [15]. Although gluten does not stimulate proliferation, it increases expression of the activation marker CD25 in celiac lamina propria CD4⁺ T cells [16]. In vivo, ingestion of gluten is followed by crypt hyperplasia, villous atrophy and intraepithelial lymphocytosis in the small intestine within 4–6 h [17], yet ex vivo incubation of celiac intestinal biopsies with gluten does not cause crypt hyperplasia but increases the density of intraepithelial lymphocytes [16]. In other words, there is no compelling evidence that lamina propria gluten-specific CD4 T cells proliferate in intestinal tissue. But in vitro, various protocols utilizing gluten and nonspecific lymphocyte growth factors do drive CD4 T-cell proliferation and allow expansion of polyclonal gluten-specific T-cell lines from which monoclonal T cells can be isolated, further expanded and characterized.

Polyclonal intestinal T-cell lines and clones from celiac donors raised against gliadin or gluten commonly recognize certain epitopes such as DQ2- α I (PFPQPELPY) [18]. Cognizant of the artifacts that may result from in vitro expansion, biological relevance of epitopes characterized using T-cell lines and clones is established by demonstrating that the same epitope is also recognized by T cells from tissue or blood that have not been expanded in vitro. MHC peptide tetramers directly identify epitope-specific T cells. However, the frequency of DQ2- α I-specific CD4 T cells in freshly disaggregated celiac intestinal biopsies is below the level of detection for MHC tetramers, yet the same DQ2- α I MHC

tetramer detects T cells from polyclonal lines expanded from the same tissue [19]. The scarcity of gluten-specific T cells in celiac intestinal tissue and their failure to proliferate in situ upon gluten stimulation emphasizes the nonphysiological measures that are required to expand these cells. In the absence of information regarding fresh unmanipulated T cells, immunodominance of epitopes inferred using T-cell lines and clones should be interpreted with caution. Unless efforts are made to select antigen-experienced cells, the relevance of T-cell clones is further compromised by the possibility that naïve gluten-responsive T cells may be activated, expanded and cloned.

Gluten Epitopes of Intestinal T-Cell Lines and Clones

Notwithstanding these reservations, intestinal T-cell clones or lines specific for either of the overlapping α -gliadin epitopes DQ2- α I (PFPQPELPY) or DQ2- α II (PQPELPYPQ) are isolated from half of Dutch children and adults [20] and all Norwegian adults with HLA DQ2-associated celiac disease [18]. The observed inconsistencies between the Dutch and Norwegian studies may be due to differences in methodology. Although immunodominance is less clear-cut in Dutch studies, intestinal T-cell lines raised against gliadin from Norwegian celiac donors respond equally well to deamidated gluten as to the α -gliadin 33-mer [21, 22] that encompasses serial overlapping versions of DQ2- α I, DQ2- α II and a variant of DQ2- α I (PYPQPELPY, referred to as DQ2- α III) [23]. In addition, the majority of intestinal T-cell lines raised against deamidated wheat gluten also recognize deamidated secalin and hordein peptides homologous to DQ2- α I or DQ2- α II (PFPQPELPY and PQPELPYPQ) and T-cell clones specific for DQ2- α I or DQ2- α II also respond to deamidated secalin, hordein and γ -gliadin sequences (e.g. PFPQPQQT) [23, 24].

Although DQ2- α I and DQ2- α II are the epitopes most commonly recognized by celiac intestinal T-cell lines raised against gluten, the first gluten peptide to be identified as an HLA-DQ2-restricted epitope was α -gliadin p31–47, albeit for a single peripheral blood T-cell clone [25]. At that time, this peptide had recently been shown to cause intestinal damage in vivo [26] but is now implicated as an innate immunostimulatory peptide [27].

The second HLA-DQ2-restricted gluten epitope reported, recognized by intestinal T cells from 3 celiac donors, and the first to be widely replicated was the γ -gliadin peptide PQQSF-PQQQ (DQ2- γ I) with glutamines at positions 7 and 9 deamidated to glutamate by the action of tTG [28]. Indeed, the majority of T-cell epitopes relevant to celiac disease are deamidated rather than wild-type gluten sequences, deamidation most likely to be due to intestinal mucosal tTG activity [29]. However, proliferative responses to DQ2- γ I by gluten-specific intestinal T-cell lines are less frequent and substantially weaker than to DQ2- α I or DQ2- α II [22].

After discovery of DQ2- α I and DQ2- α II published in 2000 by Arentz-Hansen et al. [18], other HLA-DQ2-restricted mostly deamidated wheat gluten epitopes have been reported. DQ2- γ II (IQPQQPAQL), DQ2- γ III (QQPQQPYPQ) and DQ2- γ VI (QQPFPQQPQ) are generally recognized by fewer than half of celiac intestinal T-cell lines, and rarely do responses match those to the α -gliadin 33-mer encompassing DQ2- α I, DQ2- α II and DQ2- α III [20, 22, 23]. Intestinal T-cell lines infrequently recognize other γ -gliadin epitopes, DQ2- γ IV (SQPQQQFPQ) and DQ2- γ VII (PQPQQQFPQ) [22, 23]. HLA-DQ2-restricted T-cell clones and lines also occasionally recognize the low-molecular-weight glutenin epitopes Glt-17 (QQPPFSQQQQQLPQ) and Glt-156 (PFSQQQSPF), the α -gliadin Glia- α 20 (PFRPQQPY PQPQPQ), and the gluten sequences Glu-21 (QSESQQPFQPQ) and Glu-5 [Q(I/L)PQQPQ QF] [20].