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Vaccine against autoimmune disease: antigen-specific immunotherapy[☆]

Robert P Anderson¹ and Bana Jabri²

¹ImmusanT, Inc., ImmusanT Inc., B2004 One Kendall Square, Cambridge, MA 02139, USA

²Department of Medicine, Pathology and Pediatrics, University of Chicago, Chicago, IL 60637, USA

Abstract

Recent interest in testing whether the success of antigen-specific immunotherapy (ASIT) for autoimmune diseases in mice can be translated to humans has highlighted the need for better tools to study and understand human autoimmunity. Clinical development of ASIT for allergy has been instructive, but limited understanding of CD4 T cell epitope/determinant hierarchies hampers the rational design and monitoring of ASIT. Definitive identification of pathogenic T cell epitopes as is now known in celiac disease and recent initiatives to optimize immune monitoring will facilitate rational design, monitoring and mechanistic understanding of ASIT for human autoimmune diseases.

Introduction

Genes encoding major histocompatibility (MHC) class II molecules are the strongest determinants of susceptibility to type-1 diabetes (T1D), rheumatoid arthritis, multiple sclerosis and many other autoimmune diseases. Consequently, CD4 T cells specific for immunodominant epitopes (see glossary) restricted by disease-associated MHC class II elements are ideal targets for immunomodulation using antigen-specific immunotherapy (ASIT) or more specifically with naked peptides in epitope-specific immunotherapy (ESIT) [1,2]. Extrapolating from mouse models, therapeutic vaccinations for human autoimmune disorders would elicit an immune response restoring tolerance by eliminating, modulating or blocking pathogenic immune responses. Vaccines would target disease-specific pathogenic T cells without inducing generalized immunosuppression. The selectivity of ESIT is possible by using T cell epitopes recognized by disease-causing CD4 T cells. Progress in developing this new therapeutic class has been hampered by the inability to define and monitor disease-specific pathogenic T cells. Here, we will examine why vaccines are pertinent to autoimmune disorders. The potential mechanisms underlying vaccine-induced tolerance will be reviewed including a discussion of the design of ASIT, ESIT and immune monitoring, and highlight celiac disease as an informative human ‘model’.

Support for vaccines to treat allergic and autoimmune disorders

Rigorous clinical trials of ASIT for allergic diseases confirm that long-term disease modification is possible for established pathological immune responses in humans [3].

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Corresponding author: Anderson, Robert P (bob@immusant.com, BAnderson99133@gmail.com).

Authoritative guidelines have summarized the level of evidence supporting the safety and efficacy of whole-protein allergen-based therapeutic vaccines for allergic diseases [4,5]. More recently, a vaccine formulation including allergen-derived peptides encompassing HLA-DR restricted epitopes from cat dander protein (Fel-d1) that target Fel-d1-specific CD4 T cells has also shown clinical efficacy in chamber studies of cat-sensitive allergic rhinoconjunctivitis [6,7]. However, despite many successful studies of ASIT in well-defined animal models of autoimmunity, translating the success of ASIT from human allergy to clinical autoimmunity has not been straightforward.

T1D exemplifies the clinical need for treatments that modify the natural history of chronic autoimmune disease without long-term systemic immunosuppression. However, development of ASIT for T1D has highlighted that the scarcity of autoantigen-specific CD4 T cells in fresh blood not only confounds the definition of critical immunodominant T cell epitopes, but also impacts on designing the composition, monitoring and understanding of ASIT [8–10].

Mechanisms underlying vaccine-mediated immune tolerance

Although pathogenic CD4 T helper 1 (Th1), 2 (Th2) and 17 (Th17) responses may not be as clearly demarcated in humans as they are in the mouse, allergic responses are typically associated with Th2 responses with high levels of IL-4, IL-5 and IL-13 [11]. In contrast, organ-specific autoimmune disorders are generally associated with pro-inflammatory Th1 and Th17 immune responses directed against self-antigens and high levels of IFN- γ and/or IL-17 production, respectively [12]. In principle, these T-cell mediated diseases could be effectively treated by either depleting the naïve T cell repertoire of all pathogenic T cells specific for the antigens driving the disease, or by dampening or blocking the pathological immune response directed by T cells specific for epitopes derived from the most important antigens (Figure 1).

Dendritic cells (DCs) are professional antigen presenting cells that function as key players during the induction phase of immune responses, directing the outcome toward either tolerance or protective immunity. The functional phenotype of the DC critically determines the fate of the naïve T cell: deletion, anergy, and differentiation, for example, into Th1, Th2, Th17 or 'tolerogenic' phenotypes [13]. The tissue environment and innate stimuli associated with a particular antigen determine the level of co-stimulatory molecule expression and the soluble factors produced by the DCs [14,15]. Hence the route of immunization, the mode of delivery, the presence or absence of adjuvants and the nature of the antigen all play a critical role in determining the nature of the T cell response first induced and later reactivated by a given antigen.

Deletion and anergy of cognate T cells can be achieved by DCs presenting (self) antigens in the absence of co-stimulation [16,17]. Vaccines for allergic and autoimmune disorders in humans targeting established pathogenic immune responses have generally been delivered subcutaneously or intradermally, in the absence or presence of adjuvants such as aluminum hydroxide (alum) [18–20], but the optimal administration regimens for ASIT to achieve these outcomes in humans are not yet defined. Furthermore, the degree to which deletion needs to be achieved to assure clinical efficacy of ASIT is not known and may be disease-specific.

Alternatively, vaccines could result in blocking pathological effector T cell responses by promoting regulatory immune responses specific for the antigens driving the disease. The term regulatory, in this case, encompasses the induction of regulatory Foxp3⁺ T cells, IL-10 producing regulatory type-1 (Tr1) cells and T helper effector cells that may have the ability to block the pathogenic immune response (e.g. Th2 can block differentiation of Th1 and

conversely Th1 cells can block Th2 cell differentiation) [21]. Foxp3⁺ T cells are classically generated in the presence of TGF- β and the vitamin A metabolite, retinoic acid [22]. Inducible regulatory Foxp3⁺ T cells exert their immune regulatory functions through the production of TGF- β and potentially IL-10, as well as other properties such as the ability to eliminate effector T cells by cytotoxicity [23,24]. Regulatory type-1 (Tr1) cells are T cells producing high levels of IL-10 and TGF- β in the absence or low levels of Th1, Th17 and Th2 cytokines [25,26]. A potential disadvantage of reliance on Foxp3⁺ regulatory T cells alone for therapeutic tolerance is that they may not maintain a stable phenotype [27] and they may be ineffective in an inflammatory environment such as that encountered in the inflamed target organ of an autoimmune disease. For instance, it was shown that IL-15, a cytokine upregulated in celiac disease and various autoimmune disorders [28], renders effector T cells resistant to the regulatory effects of Foxp3⁺ T cells [29,30]. In contrast, Tr1 cells and their secretion of IL-10 seem to be stable and able to mediate immunoregulatory functions in the presence of proinflammatory effector cytokines [25].

Enrichment for Foxp3⁺ regulatory T cells has additionally been demonstrated upon oral vaccination of rheumatoid arthritis patients with dnaJp1 peptide [2]. Subcutaneous immunization of T1D patients with insulin peptide and incomplete Freund's adjuvant [20] or alum-formulated glutamic acid decarboxylase 65 (alum-GAD65) [18,31•] also results in a relative increase in Foxp3⁺ regulatory T cells. Although the mechanism or mechanisms by which Tr1 cells are induced *in vivo* remain poorly understood, it has been shown that repetitive activation with low concentrations of antigen and IL-10 or CD2 co-stimulation [25] and cytokines such as IL-27 promote their differentiation [32]. Certain adjuvants such as TLR2/6 ligands promote differentiation of tolerogenic DCs and induction of Tr1 cells in mice *in vivo* [33]. Tr1 cells are the dominant subset specific for environmental antigens in healthy individuals, whereas IL-4 secreting Th2 cells are observed at high frequencies in allergic individuals [34]. Tr1-like cells are induced in patients with cat-sensitive allergy following administration of low doses of peptides containing T cell epitopes from Fel d1 [35] in humanized HLA-DR1 transgenic mice [36••] after allergen-specific immunotherapy [37•] and in T1D patients receiving low dose insulin peptides [38•]. In contrast to Foxp3⁺ regulatory T cells and Tr1 cells, Th2 cells can induce disease and are regulatory only in the sense that they can block differentiation of Th1 cells [39]. Induction of Th2 cells was shown in T1D patients receiving heat shock protein peptide 60 [40] and alum-GAD65 [41,42•].

The potential advantage of active immunization with induction of T cells having regulatory properties is their ability to mediate both linked epitope suppression and infectious tolerance whereby DCs presenting vaccine-derived epitopes promote induction and also perpetuation of regulatory T cells specific for other epitopes within the same antigen and other epitopes presented on its surface [36••,43,44]. By implication, linked and infectious tolerance might be triggered by ASIT potentially overcoming the need to administer all pathogenic T cell epitopes in a therapeutic composition, and also for an effective ASIT to eliminate all pathogenic T cells [45,46] (Figure 1).

Unlike naïve T cells, memory T cells have less stringent co-stimulatory requirements for activation and are not readily deleted upon activation in the absence of co-stimulation [47,48]. However, there is evidence that memory T cells can be tolerized by systemic administration of antigen and low density of peptide-MHC ligands [49,50] (Figure 1). Furthermore, studies in mice suggest that the functional status of a memory T cell pool at commencement and during ASIT may participate in shaping the functional phenotype of DCs [15,44,51]. Therefore the longevity and size of the cognate memory T cell pool could have an impact on initial responses to ASIT. The full therapeutic benefit of ASIT may only be revealed after disappearance of a pathogenic memory T cell pool. It could therefore be anticipated that a vaccine capable of both deleting the cognate memory T cell pool and

expanding the regulatory T cell pool may be most effective in restoring and maintaining immune tolerance and health.

Celiac disease is a disease prototype for the development of vaccines against autoimmune disorders

Celiac disease is defined by the presence of small intestinal inflammation that improves or normalizes with exclusion of dietary gluten derived from foods including wheat, barley and rye. Celiac disease is one of a cluster of diseases associated with autoantibody production and T-cell mediated organ-specific immunopathology that are strongly associated with HLA-DR3-DQ2 and DR4-DQ8 haplotypes [52–54]. In fact, celiac disease is effectively ruled out in patients lacking HLA-DQ2 or HLA-DQ8. However, unlike classical autoimmune diseases, in celiac disease it is an exogenous antigen, dietary gluten, that is recognized by pathogenic CD4 T cells [55]. The immunogenicity of native gluten in celiac disease is enhanced by post-translational modification mediated by intestinal transglutaminase-2 activity. Transglutaminase-2 selectively deamidates particular proline-rich and glutamine-rich gluten peptides enhancing binding to HLA-DQ2 and/or HLA-DQ8 and their recognition by CD4 T cells [56,57].

Celiac disease is unique among autoimmune diseases because the pathogenic T cell population can be mobilized in blood following *in vivo* antigen challenge (oral gluten intake) and can also be isolated from the target organ and expanded after *in vitro* antigen challenge with semi-purified wheat gluten. Epitope mapping using T cells in blood after oral gluten challenge screened by overnight interferon- γ ELISpot assays, or intestinal biopsy-derived T cell lines in proliferation or interferon- γ release assays identifies the same immunodominant α -gliadin sequence [58,59]. Oral challenge with other cereals toxic in celiac disease also mobilizes gluten-reactive T cells that can be enumerated by interferon- γ ELISpot assay. T cells in blood collected after oral wheat, barley and rye challenge have been screened with a comprehensive gluten epitope library that has defined a consistent T cell epitope hierarchy in patients who possess the immune response genes *HLA-DQA1*05* and *HLA-DQB1*02* (HLA-DQ2.5⁺) but not *HLA-DQA1*03* and *HLA-DQB1*0302* (HLA-DQ8⁻); three peptides encompassing five HLA-DQ2.5-restricted immunodominant epitopes consistently account for the majority of gluten-reactive T cells [60••]. This identification has facilitated the rational selection of gluten peptides for inclusion in ESIT as well as assays to monitor T cells. At present, peptide-based ESIT for HLA-DQ2.5⁺ celiac disease is under investigation in Phase 1b clinical trials. When compared with other autoimmune indications such as T1D, clinical development of ASIT/ESIT for celiac disease is also facilitated by ready access to the target organ and the ability to monitor relevant T cells in blood mobilized after oral gluten challenge. Clinical development of ASIT/ESIT for celiac disease may inform a development pathway more relevant for HLA-determined autoimmune disease than ASIT/ESIT for allergy.

Design of antigen-specific immunotherapy

Although advances in adjuvants, delivery systems, and optimized administration schedules influence the efficacy of ASIT in animal models, the indivisible, final active moieties of ASIT are linear peptides of 9–10 amino acids that comprise immunodominant epitopes recognized by pathogenic human CD4 T cells. Such epitopes may be delivered in whole protein antigens, encoded by DNA or RNA, or as short linear peptides [61]. In principle, individual peptides or mixtures of small numbers of peptides may be simpler to manufacture and formulate, but require a clear and complete understanding of immunodominant peptides. In contrast, compositions with whole proteins or plasmid DNA do not require such detailed understanding of the discrete epitopes, but still require knowledge of the disease-causing

antigen and may include epitopes recognized by both CD4 and CD8 T cells that induce a broader immune response than desired [62]. Furthermore, antigenic peptides with more than 20 amino acids have the potential to cross-link IgE and trigger anaphylaxis [62].

A major limitation in the development of ASIT has been the inability to identify immunodominant disease-driving epitopes. Rational selection of epitopes for inclusion in ASIT or ESIT to maximize the responding T cell population requires analysis of the weight and quality of experimental evidence supporting epitopes as truly being disease relevant. The difficulty to identify the correct epitopes by establishing T cell lines from peripheral blood is illustrated by the observation that gluten-reactive intestinal T cell lines from HLA-DQ2.5⁺ celiac disease donors are almost exclusively HLA-DQ2.5-restricted and recognize gluten peptides deamidated by transglutaminase-2 [56,63], whereas peripheral blood T cell lines raised against native gluten are not consistently HLA-DQ2.5-restricted and do not show preference for deamidated gluten [56,64,65]. Table 1 summarizes the reliability of various approaches developed to test the toxicity gluten-derived peptides and ultimately define immunodominant T cell epitopes responsible for celiac disease. It is pertinent that in a recent study to identify T cell epitopes relevant to T1D, even with state-of-the-art T-cell detection methods using HLA-DR4-peptide tetramers, Yang et al. were unable to convincingly distinguish between the fine-specificity of short-term peripheral blood T-cell lines raised against GAD65 from HLA DR4⁺ T1D and healthy donors [66].

Pharmacogenetics is also important when selecting epitopes for inclusion in ASIT and ESIT. In the example of celiac disease, gluten epitope hierarchy is well conserved in the 80% of patients who are HLA-DQ2.5⁺ [60•]. However, in patients who carry both HLA-DQ2.5 and HLA-DQ8, gluten-reactive T cells not only recognize epitopes restricted by HLA-DQ2.5 and HLA-DQ8, but they also respond to epitopes presented by transdimers encoded by *HLA-DQA1*05* and *HLA-DQB1*0302* and by *HLA-DQA1*03* and *HLA-DQB1*02* [67,68].

Immune monitoring of vaccine efficacy

The frequency and functional phenotype of relevant antigen-experienced T cells have the potential to provide surrogate endpoints and mechanistic insights for clinical trials of ASIT and ESIT [69]. However, if auto-antigen-specific T cells cannot be detected in blood without expansion *in vitro* [66,70,71], monitoring such rare T cell populations in clinical trials will also be challenging. In autoimmune diseases such as T1D, variable reproducibility of assays for antigen specific T cells has compromised efforts to monitor ASIT and to define immunodominant epitopes [72,73].

Several factors contributing to variability in T cell readouts have been identified and formally addressed in recent publications, mostly from centers involved in cancer immunotherapy. For example, standardization of what constitutes a ‘positive’ T cell response was addressed by Moodie and colleagues by establishing an online tool that is now available for ELISpot readout analysis [74•]. Olson and colleagues formally tested conditions for shipping fresh blood to be used in T cell assays at central labs and found that maintaining specimen temperature above 22 °C or preferably near 30 °C was optimal [75]. Filbert et al. defined methods for cryopreservation that yielded optimal T cell responses in subsequent ELISPOT analysis [76]. And perhaps, most importantly, the reporting of immune readouts has been addressed by the publication of minimal information about T cell assays (MIATA) in an effort to harmonize assays between laboratories [77•]. Even when these methodological issues have been addressed there remains the biological question whether clinical outcomes of ASIT/ESIT will consistently correlate with the phenotype and function of antigen-specific T cells in blood. For example, this was not the case in a recent

study using *in vitro* allergen stimulation and MHC class II/peptide tetramer-positive CD4 T cells in blood after whole protein allergen therapy [78•].

Conclusions

There is now strong evidence that ASIT is safe. The challenge that remains, particularly in organ-specific autoimmune disorders, is to establish that vaccines constitute an effective treatment. A major limitation to date has been to perform ASIT in the context of diseases for which immunodominant CD4 T cell epitopes responsible for pathology are not well defined and the tools to accurately monitor the vaccine-associated immune response do not exist. Defining robust surrogate immunological outcomes that accurately and consistently predict clinical tolerance will accelerate development of this class of therapy for T1D and other autoimmune diseases that so far have relied upon clinical primary endpoints in trials. Celiac disease constitutes a unique ‘human model’ to study induction of tolerance with ASIT composed of immunodominant peptides. Indeed, with advances in understanding pathogenic immunodominant epitopes in human autoimmune diseases and development of peptide-based ASIT it may be time to refer to this emerging therapeutic class as ‘epitope-specific immunotherapy’ or ‘ESIT’. Clinical trials of ESIT for celiac disease should provide the opportunity to optimize delivery and dose regimens, and assess the potential role of adjuvant-epitope compositions.

Glossary

Antigen-specific immunotherapy (ASIT)	is a composition that is intended to modify the natural history of a disease, for example cancer, allergy or autoimmunity, through inducing, enhancing, or suppressing a specific immune response.
Epitope-specific immunotherapy (ESIT)	for autoimmune diseases is a composition including peptides (<20 amino acids minimizes the risk of allergenicity) encompassing immunodominant epitopes for disease-specific CD4 T cells that is intended to modify the natural history of a disease by suppressing or deleting a pathogenic immune response.
T cell determinant or T cell epitope	terms used interchangeably, and refer to the minimal (linear) 8–11 amino acid peptide that is required for activation of a specific T cell receptor.
T cell epitope/determinant hierarchy	refers to the relative response elicited by distinct epitopes in an antigen (e.g. A-gliadin in celiac disease) or a group of associated antigens (e.g. all gluten proteins from wheat, barley and rye) as measured by the frequency of T cells in a polyclonal population, or by the magnitude of a functional readout such as a secreted cytokine, or proliferation.
Immunodominant epitope/determinant	refers to the epitope in an antigen that elicits the strongest response or a response above an arbitrary, relative threshold (e.g. 70% of the most active epitope) as measured by the frequency of T cells in a polyclonal population, or by the magnitude of a functional readout such as a secreted cytokine, or proliferation.
T cell clone	a population of T cells expressing identical T cell receptors (TCRs), and for epitope mapping, have usually been expanded

T cell line

in vitro over weeks or months from a solitary parent T cell using repeated TCR stimulation and growth factors.

**T cell epitope/
determinant mapping**

a population of T cells expressing diverse TCRs that have been expanded *in vitro* by pulsing with growth factors and a single stimulatory peptide, protein or antigen mixture, for example protease-digested wheat gluten, or peptide. Lymphocytes proliferate approximately every 12 hours for 5–20 cell divisions, then stop and die over a period of weeks. Cells that proliferate and are expanded *in vitro* may be antigen-experienced from a ‘relevant’ memory T cell population, or be naïve and not have previously been exposed to the test antigen giving rise to experimental artifacts when mapping disease-relevant epitopes.

refers to screening T cells isolated directly from blood or tissue, expanded in lines or as clones using panels of peptides typically encompassing all potential epitopes from a given protein antigen or mixture. Scanning peptide libraries often consist of 15mers overlapping by approximately 11 amino acids to identify both MHC class I and class II restricted epitopes recognized by CD8 and CD4 T cells, respectively. Screening with peptides longer than 15mers is less efficient for detection of CD8 T cell responses.

**Fine epitope/
determinant/
specificity mapping**

refers to isolating the minimal 8–11mer amino acid sequence required for T cell activation using truncations and amino acid substitutions in a longer peptide identified by scanning whole protein antigens with overlapping peptides.

**Infectious tolerance
[43]**

describes a self-sustaining process by which a population of tolerant immune cells can pass on that tolerance to naive cells.

**Linked epitope
suppression [36••]**

a process through which cells rendered tolerant to one epitope suppress the function of T cells specific for other epitopes within the same molecule.

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Figure 1. Mechanisms underlying vaccine-mediated immune tolerance

1. ASIT may act at the level of naïve T cells either to induce deletion of naïve T cells specific for the peptides composing the vaccine or induce differentiation of T cells with regulatory properties. These regulatory T cells have the potential to inhibit pathogenic inflammatory (Th1) memory T cells and exert linked suppression by blocking the differentiation of naïve T cells that recognize self-antigens released as a result of tissue damage (epitope spreading). 2. ASIT may act directly at the level of memory T cells to promote anergy and cause deletion.

Table 1

Strategies to define CD4 T cell epitopes in celiac disease ^a	
1	Antigen selection
**	Protein mixtures, for example protease-digested wheat gluten
**	Representative pure proteins, for example wheat A-gliadin, and recombinant gliadins
***	Peptide libraries including all 12mers from wheat, barley and rye prolamins
2	Prediction based on biochemical characteristics
*	Biochemical peptide-MHC Class II binding assays
*	Purification of peptides modified by transglutaminase and resistant to proteases
*	<i>In silico</i> scan for peptides binding to HLA-DQ2, DQ8 or DQ2/8 transdimers
*	<i>In silico</i> scan for post-translationally modified peptides binding to HLA-DQ2
3	<i>In vivo</i> gluten antigen administration
	Immunization of wild-type mice with purified A-gliadin
*	Immunization of HLA-DR3-DQ2 transgenic mice with peptide
*	Direct intestinal instillation of synthetic peptide in patients
4	<i>Ex vivo</i> organ culture
	Intestinal biopsies incubated with peptides — epithelial changes as readouts
5	Epitope screening in functional T cell assays
*	Peripheral blood (polyclonal) T cell lines and clones
**	Intestinal T cell lines and clones
***	^b Fresh peripheral blood T cells after 3-day oral gluten food challenge

^aThe number of stars reflects the relative ability to identify immunodominant peptides in a reproducible manner.

^bThree-day challenge allows recirculation of gut-homing gluten-reactive T cells in peripheral blood.