Colloquium

Specific antigen vaccination to treat autoimmune disease
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Specific antigen vaccination by administration of the target antigen in aqueous solution has resulted in significant decreases of disease severity in animal models of experimental allergic encephalomyelitis, type I diabetes, and several forms of antigen-induced arthritis, even if administered after the initiation of symptoms. However, in experimental autoimmune encephalomyelitis (EAE) and type I diabetes in nonobese diabetic (NOD) mice, repeated administration of peptide fragments of target antigens in incomplete Freund’s adjuvant has resulted in severe anaphylactic reactions. Although these methods of administration are known to potentiate CD4 T helper 2 (Th2) responses, which is the goal of specific antigen vaccination, the risk of anaphylaxis raises a red flag concerning use of this therapy for diseases such as type I diabetes, where the survival time after onset is quite long. It is clear that specific antigen vaccination is effective in preventing several animal models of autoimmune disease, and in treating these diseases once the symptoms are overt. However, the risks of this therapy require serious consideration of alternative methods for down-regulation of the autoimmune process.

For many years immunologists have sought to develop methods for preventing and/or treating autoimmune diseases by identifying those self antigens that are the target of autoimmune processes resulting in tissue damage and clinical symptomatology (1). For the most part, these strategies have attempted to address autoimmune diseases in which the primary pathogenic mechanism and the principal effector mechanism seem to be the result of activated T cells inducing inflammation and damage in a variety of tissues (2). Much of this experimentation has dealt with experimental models of multiple sclerosis (MS) (experimental autoimmune encephalitis) (3, 4), rheumatoid arthritis (for which one experimental model is type 2 collagen-induced arthritis), and type I diabetes (TIDM) (5), in which the spontaneous disease in nonobese diabetic (NOD) mice is the primary model. Although in antibody-mediated diseases the target of the autoantibodies is frequently well defined, less effort has been directed toward developing specific antigen vaccination to treat the T cells activating these diseases.

On the other hand, the description of anergy induction in T cells by stimulation with the cognate peptide/MHC ligand (in the absence of costimulatory molecules) has stimulated many investigators to pursue the goal of “T cell tolerance.” Tolerance in this sense is used to refer to a number of mechanisms that lead to nonresponsiveness to a particular antigen, without true, deletional T cell tolerance. Thus, the principal goal of specific antigen vaccination is to either achieve specific T cell ablation (by activation-induced cell death); achieve specific T cell anergy; achieve the induction of regulatory T cells (primarily CD4+CD25+ regulatory T cells); or cause a shift in the predominant phenotype of the specific T cell anti-self response from a T helper (Th) 1 to a Th2 phenotype (3). With the exception of true T cell deletion (recessive tolerance), these dominant mechanisms are associated with detectable T cell responses to self antigens, which are controlled by one of the latter three mechanisms given above.

Because T cell anergy can be broken by exposure to high doses of IL-2; because regulatory T cells can be overwhelmed by a developing pathogenic T cell response; and because induction of a Th2 shift is not always a stable phenotype, the individual with one of these autoimmune diseases must be constantly monitored to ensure that the nonresponsive state has been adequately maintained. In addition, as will be seen below, the effort to induce a strong Th2 shift in the phenotype of antigen-specific T cells carries with it the risk of inducing immediate hypersensitivity.

Studies in Animal Models

In a number of induced autoimmune animal models, most of which require the induction of the anti-self response by immunization with self antigen in complete Freund’s adjuvant (CFA), it has been possible to both prevent and treat induced autoimmune diseases by administration of either the protein self antigen target, or peptide epitopes of the self antigen in aqueous solution, administered in a noninflammatory manner (i.e., without adjuvant or other stimulants in the therapeutic injections). Thus, it has been possible to develop successful immunotherapy of experimental autoimmune encephalitis (3, 4) and type II collagen and collagen-induced arthritis. In many of these models, administration of the immunodominant epitope of, for example, myelin basic protein, has been successful in preventing or stopping the progression of neurological symptoms in animals already developing the illness, even after immunization with whole spinal cord emulsified in CFA.

The drawback of these approaches is that it is impossible to induce these autoimmune diseases without the use of complete Freund’s adjuvant, and often without the required use of i.v. injection of pertussis bacilli to potentiate the autoimmune manifestations. Thus, the ease with which these disease models can be prevented and treated by injection of either the self proteins or peptides derived from them may be a less rigorous approach to treating autoimmune diseases by specific antigen vaccination.

Spontaneous Disease Models

The two principal spontaneous autoimmune disease models in the mouse, among other variants, are, first, the murine model of systemic lupus erythematosus represented by the (NZB × NZW) F1 mouse and its various derived related strains (e.g., NZM 2410), (an excellent model of a spontaneous, antibody-mediated immediate hypersensitivity).

Abbreviations: NOD, nonobese diabetic; Th, T helper; GAD, glutamic acid decarboxylase; TCR, T cell antigen receptor; IFA, incomplete Freund’s adjuvant; MS, multiple sclerosis.

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autoimmune disease), and the spontaneous model for T1DM, which develops in the NOD mouse. Because a well defined group of autoantigen targets of the autoimmune response in the (NZB × NZW) F1, mouse does not exist, this model is less amenable to T cell-targeted immunotherapy. Therefore, this discussion will focus primarily on the NOD mouse model.

These mice were originally discovered in Japan, in the course of inbreeding an outbred strain derived from the Swiss–Webster outbred line. One subline was found to have glycosuria, and on investigation, to have inflammatory lesions in the islets of Langerhans. Further examination has revealed that this spontaneous model in the NOD mouse has many characteristics similar to T1DM in humans (see references in ref. 5). These mice develop glycosuria and hyperglycemia with an 80% incidence in females and a 20–40% incidence in males by 30 weeks of age. The disease begins shortly after puberty, at 4–5 weeks of age and is manifested at this stage primarily by infiltration of lymphocytes into and around the islets. This process then progresses to severe insulitis by 12–15 weeks of age, and finally overt destruction of β-islet cells by 15–20 weeks of age, at which time the first clinical manifestations of classical T1DM are manifest (6–8, †). The disease can be transferred by purified lymphocytes but requires both CD4 and CD8 splenic T cells for efficient transfer of disease. The disease can also develop spontaneously in μ-membrane −/− mice who have no B cells, thus establishing that the disease is entirely a function of the development of autoreactive T cells. Susceptibility is strongly linked to the major histocompatibility complex, with the I-Ag7 allele of the NOD mouse being the strongest susceptibility factor. The β chain of this molecule lacks the usual proline and aspartic acid at positions 56 and 57 in the β chain, which are replaced by histidine and serine. The cellular infiltrate in the islets is predominantly lymphocytes (80%), with mostly CD4+ and some CD8 T cells and an admixture of macrophages and dendritic cells. Initially, this lymphocytic infiltrate has T cells producing both Th1 and cytotoxic T cell phenotype in the later stages (6–8, †).

Several of the autoantigen targets in the NOD mouse have been identified over the past 10–15 years. These targets include insulin, itself, glutamic acid decarboxylase 65 (GAD 65), heat shock protein 60 (HSP 60), and several unknown target antigens that nonetheless are effective targets, because T cells directed against these antigens can induce diabetes. One of the most interesting of these proteins is the (as yet unknown) islet cell protein that is the target of the T cell receptor response in the BDC 2.5 T cell antigen receptor (TCR) transgenic mouse. The BDC 2.5 TCR transgene on the NOD background results in only 20–30% development of diabetes in females by 30 weeks of age. However, on the C57BL/6 background, this T cell receptor results in diabetes in 50% of the animals at a relatively early age. The availability of the NOD model, the BDC 2.5 T cell receptor transgenic mouse line [originally developed by Mathis, Benoist, and colleagues (19)], and a mimotope that effectively stimulates T cells with this receptor makes this an excellent model for studies of pathogenesis and therapy of T1DM.

There are a bewildering number of various immune system manipulations that can prevent T1DM in this mouse model (5). In sum, these various effects indicate that, to develop T1DM, the NOD mouse must have effective functioning of all arms of the immune response, with the exception of the B cell line, because μ-membrane −/− mice can still develop T1DM on the NOD background.

### Specific Antigen Immunotherapy

In recent years, there have been a number of experiments showing that administration of islet cell proteins, or peptides derived from them, which are targets of the T cell immune response in the NOD mouse, can decrease the incidence of diabetes and prevent onset during the period that the antigens are administered. Thus, injection of insulin peptide B-(9-23) in aqueous solution, and in incomplete Freund’s adjuvant (IFA), and insulin β chain administered intranasally can all prevent the onset of T1DM during the period of administration of the peptide (6–9, †). Thus, administration of a peptide derived from insulin (and in a few experiments insulin itself) can decrease the immune response or alter it sufficiently to interfere with the diabetic process and prevent onset of severe β cell destruction, as long as these peptides are administered.

In addition to insulin, GAD 65 and its peptides have also been used to prevent the onset of T1DM in NOD mice. Tisch et al. (10, 11) used intrathyemic injection of baculovirus derived GAD 65 in intrathyemic injections at 3 weeks of age to down-regulate the subsequent development of T1DM. In later experiments (11), i.v. injection of four doses of 200 μg GAD 65 over a 1-week period at 12 weeks of age (when insulitis and T cell responses to GAD 65 and insulin are well established) resulted in a marked decrease in the incidence of diabetes at 30–36 weeks (18% vs. 78% in controls). In the latter experiments, the splenic T cells from GAD 65-treated mice produced predominantly IL-4 and very little IFN-γ in response to stimulation with GAD 65. Further, splenic T cells from GAD 65-treated mice (treated at 12 weeks of age) were able to prevent transfer of diabetes from recently diabetic female mice in a standard transfer system (11). Thus, as with insulin, GAD 65 protein is quite capable of inducing a predominant shift to a Th2 phenotype, resulting in reduction in the incidence of diabetes and the ability to block transfer of diabetes into NOD.scid recipients.

In more recent experiments, Tisch et al. (12) were able to prevent T1DM in mice treated with GAD peptides, although, when the treatment was begun at 12 weeks of age, only two of the collection of GAD 65 T cell epitopes, p217 and p290 together, were able to reduce the incidence of diabetes at later ages from 80% in controls to 20–25% in treated mice. Thus, insulin, GAD 65, and their peptides are capable of inducing a significant shift of islet cell-specific T cells to a predominant Th2 phenotype, resulting in the prevention of development of T1DM in the NOD mouse. These results would suggest that a similar approach in prediabetic individuals could succeed in delaying the onset of T1DM in patients with autoantibodies to insulin, GAD 65, and/or IA-2 and a permissive HLA genotype. However, as will be seen below, such an approach is not without the risk of inducing excessive Th2 stimulation, resulting in signs of anaphylaxis.

### Untoward Side Effects of Specific Antigen Immunotherapy

Initial attempts to further refine the use of peptides as immunotherapeutic agents in NOD T1DM resulted in the development of a very high degree of anaphylactic sensitization, leading to acute death in the majority of treated mice. In these initial experiments, the peptides were emulsified in IFA, (primarily to permit this vehicle to serve as a depot for the gradual release of peptides) to optimize the effect of these peptides in preventing the activity of diabetogenic T cells. To this end, 12-week-old NOD mice were initially treated with 200 μg of three GAD 65 immunodominant epitopes in IFA, or control peptides injected intraperitoneally. The protocol then required monthly booster injections of peptides in IFA to maintain the Th2 phenotype. However, at the first monthly injection, the majority of animals treated with the three peptides 206-220, 221-235, and 286-300 suffered the prompt onset of an immediate hypersensitivity

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Table 1. Characteristics of GAD 65-specific TCR transgenic mice: 286–300 and 206–220 TCR transgenic lines

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<thead>
<tr>
<th>GAD 286–300 and 206–220 TCR transgenic NOD mice do not develop diabetes.</th>
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<tr>
<td>Transgenic T cells respond well to cognate peptide/MHC stimulation.</td>
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<td>Transgenic T cells escape thymic negative selection by expressing a second TCR α chain and down-regulating the introduced TCR transgenes.</td>
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<tr>
<td>Transgenic T cells express low levels of IFN-γ.</td>
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<td>Transgenic T cells protect against transfer of diabetes.</td>
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Mechanism of Diabetes Protection by GAD 65-Specific T Cells

The mechanism of this diabetes protective phenotype in these TCR transgenic mice is as yet not completely understood. The fact that two of two GAD 65-specific TCR transgenic mice show a decidedly protective phenotype, and that very few other reports of pathogenic T cells specific for GAD 65 have been presented (9), suggests that the immune response to GAD 65 is in some way protective for most of the epitopes recognized by the T cell response. Evaluation of both GAD 65 TCR transgenic lines shows that they have a very similar cytokine phenotype, namely, production of low amounts of IFN-γ and low levels of IL-10, IL-2, and TNF-α. Extensive studies (15) have shown that this protective phenotype is not due to low level expression of GAD 65 protein in β islet cells, or to increased numbers or activity of CD4+CD25+ regulatory T cells (15). Because the production of IL-10 in these transgenic T cells is quite low, it is probable that IL-10 is not mediating the protective effect observed in these mice. On the other hand, the consistent low to moderate levels of IFN-γ produced upon stimulation of GAD 65 TCR transgenic T cells with peptide, with a drop in body temperature, decreased respiratory excursions, and death in the majority of animals within 15 min (13). [The latter two of these peptides are the same ones used in Tisch’s experiments (12) to prevent T1DM with the injection of aqueous peptides.] Studies in laboratories of Steinman and of Eisenbarth (cited in ref. 13) also showed that acute anaphylactic reactions could be induced in NOD mice with insulin peptides, and in C57BL/6 mice with myelin basic protein peptides (14). Thus, the phenomenon is not limited by the nature of the immunizing peptide mixture, nor by the genetic background of the recipient strain.

These observations are of great importance in pursuing the possibilities of specific antigen immunotherapy in T1DM. It has already been shown that peptide immunotherapy can induce immediate type hypersensitivity in patients treated with peptides, as reported by Steinman and colleagues (14) in a trial by using an altered peptide ligand of myelin basic protein to treat patients with MS. Approximately 10% of these patients developed signs of local immediate hypersensitivity, although none of them developed severe systemic hypersensitivity. Clearly, the use of antigen-specific immunotherapy needs to be considered in the context of the disease under study. Whereas a low incidence of immediate hypersensitivity reactions can be tolerated in a trial context of the disease under study. Whereas a low incidence of immediate hypersensitivity reactions can be tolerated in a trial context of the disease under study. Whereas a low incidence of immediate hypersensitivity reactions can be tolerated in a trial context of the disease under study.
cognate peptide/MHC ligands raises the possibility that IFN-γ in low levels may be capable of having down-regulatory effects. Although this down-regulation is contrary to the generally accepted role of IFN-γ, i.e., as an inflammatory cytokine, there are numerous recent reports that administration of low doses of IFN-γ is protective, and blockade of IFN-γ or the introduction of an IFN-γ receptor null mutant results in increased severity of autoimmune manifestations in murine experimental autoimmune encephalitis and collagen II-induced arthritis models (see references in ref. 16). Further, recent studies comparing the pro- and antiinflammatory roles of IL-23 and IL-12 point to a similar result (16). In these studies, introduction of a null mutation of the p35 chain (unique to IL-12) in this same model resulted in increased production of IL-17, IL-1β, IL-6, and IL-17 and increased severity of arthritis findings in affected joints. The possibility that low-level IFN-γ may be protective in these TCR transgenic mice through an antiinflammatory effect needs to be investigated by treating the G286 and G206 TCR transgenic mice with blocking doses of anti-IFN-γ from birth to 10 weeks of age.

The question then arises as to the possible mechanisms of antiinflammatory effects produced by low-level IFN-γ. Recent evidence has shown in several studies (17, 18) that IFN-γ can induce IDO (indoleamine deoxgenase). This enzyme destroys tryptophan and is markedly immunosuppressive for the inflammatory effects of T cells, which require this amino acid for their proliferation and function. This possibility will be examined in the near future.

Summary

1. Diabetes in NOD mice can be treated or prevented by “vaccination” with β islet cell proteins and/or peptides, usually by inducing a Th1 to Th2 shift.

2. The response rate is <100% (only a variable proportion of mice are protected from developing T1DM) and of uncertain duration in the limited protocols used to date.

3. Anaphylactic sensitization is a real risk in using GAD 65 immunization to prevent T1DM. This is of course a serious consideration because (unlike MS) patients with early or impending T1DM have a 40- to 50-yr 50% survival time and are often first seen in the childhood years. This population should not be subjected to any significant risk of anaphylaxis.

4. Protective (GAD 65) as well as pathogenic (insulin) proteins and peptides can treat and/or delay the diabetic process.

5. Specific antigen vaccination trials in a silent autoimmune disease such as T1DM require a surrogate marker for therapeutic effect, which is currently not available.

6. It may be necessary to consider alternate methods for down-regulating the diabetic process. These methods include antigen-pulsed immature dendritic cells.

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References


