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Can we vaccinate against Type 1 diabetes?

Mark Peakman

Address: Department of Immunobiology, King's College London and National Institute for Health Research Biomedical Research Centre at Guy's & St Thomas' NHS Foundation Trust and King's College London, 2nd Floor, Borough Wing, Guy's Hospital, London, SE1 9RT, UK

Email: mark.peakman@kcl.ac.uk

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Abstract

Vaccination is the administration of antigenic material to stimulate the immune system to develop adaptive immunity to a disease. As the most successful prophylactic in medical history, there is now an emerging interest as to whether vaccination can be applied in autoimmune and inflammatory conditions. These are diseases of failed immune regulation; vaccination in this context aims to exploit the power of antigenic material to stimulate immune homeostasis in the form of active, adaptive, regulatory immune responses. Type 1 diabetes is an autoimmune disease that could benefit from the therapeutic potential of vaccination. The major conditions necessary to make prophylaxis feasible are in place; the self antigens are known, the failure of existing immune regulation has been demonstrated, early studies of vaccine approaches have proved safe, and the preclinical prodrome of the disease can be easily detected by simple blood tests. Challenges for future implementation include finding the best mode of delivery and the best blend of adjunctive therapies that create the favorable conditions required for a vaccine to be effective.

Introduction: autoimmunity and vaccination

In the autoimmune disease Type 1 diabetes, T-lymphocytes of the immune system kill the pancreatic islet β-cells that produce insulin [1], resulting in severe metabolic disturbance. In patients who develop the disease, the immune system, which is designed to recognize and react against infectious agents, becomes self-reactive and targets its damaging immune effector responses against the β-cells. The resulting progressive destruction of β-cells leads to loss of glucose homeostasis, which results in death if untreated. Even with exogenous insulin replacement, patients cannot control blood glucose perfectly and have chronic hyperglycemia. This often results in severe disease complications, such as blindness and kidney failure, which carry significant burdens of increased morbidity and early mortality.

Thus, the problem at the heart of Type 1 diabetes is loss of immunological tolerance to β-cell antigens. Immunologists have obtained good evidence that this arises, in part, because a whole arm of the adaptive immune system that deals with the regulation of anti-self responses is defective. Specifically, it is proposed that cohorts of regulatory T-lymphocytes (Tregs), which would ordinarily prevent autoimmunity in the pancreas, are absent or defective.

Vaccination has proved an extraordinary medical success story and is predicated on the ability of antigen to induce an adaptive immune response. In its most familiar form, the antigen used is a part of, or whole infectious pathogen, which is administered with a powerful pro-inflammatory adjuvant (an agent designed to boost the response). Vaccination originated in excess of 500 years ago and has been responsible for innumerable lives saved (e.g. for smallpox vaccine alone over 500 million). It has spawned countless heroes: the scourge of summer polio epidemics in mid-20th century North America, captured so vividly in Philip Roth's recent novel "Nemesis" and responsible for over 50,000 deaths per year, was ended by Jonas Salk's invention of a killed polio vaccine.

Thus, vaccination for infectious disease is the biggest success story in immunology and one of the biggest in medicine. The principle is that through a safe encounter with an agent that represents the disease-causing pathogen, a natural

immunity is built. Should the host ever be exposed to the real pathogen, the fore-arming is effective and disease is prevented. This principle has been adopted for cancer immunotherapy and the main thesis of this article is that it has many features that make it an attractive strategy for autoimmune diseases, such as Type 1 diabetes.

Immune regulation and Type 1 diabetes

Type 1 diabetes is one of a growing set of diseases that is believed to arise on a background of defective immune regulation. Immune homeostasis is maintained through multiple immune regulatory mechanisms: culling of potentially pathogenic T-lymphocytes with high affinity receptors for self-antigens through a process that takes place in the thymus and is termed negative selection, thymic *positive* selection of a cohort of Tregs with receptors for self-antigens, and the generation, maintenance, expansion and function of Tregs in the periphery [2]. Partial or complete failure of one or all of these mechanisms is required to allow a damaging autoimmune response against the β-cell to develop. There are several strands of evidence that indicate that such checkpoint failures can arise in individuals who are prone to Type 1 diabetes.

Particular interest has focused on post-thymic, peripheral Treg function. This is due in part to the fact that our understanding of the biology of these cells has expanded considerably in recent years, opening up new therapeutic options, and also in part to the fact that defects in thymic function occur very early in life and are therefore unlikely to be therapeutically tractable.

The most extreme example of an etiological link between Treg defects and Type 1 diabetes is seen in children born with mutations in the gene encoding the essential Treg transcription factor FOXP3, who develop β-cell autoimmunity and diabetes in association with marked abnormalities in Treg number and function [3]. Many other lines of evidence point to relative hypo-function of Tregs in classical autoimmune Type 1 diabetes; evidence of reduced suppressor function of various populations of Tregs in patients, especially those characterized as $CD4^+CD25^{hi}FOXP3^+$ [4-9], an association between Type 1 diabetes and the *IL2RA* gene (encoding the IL-2 receptor α-chain, CD25) [10], which is constitutively expressed at high levels on both naturally occurring and peripherally induced $FOXP3^+$ Tregs [11,12], experimental findings that suggest altered plasticity [13], defects in IL-2R signaling that diminish maintenance of FOXP3 expression [14] and increased apoptosis [6] in Tregs from Type 1 diabetes patients, and evidence from both mice and humans that IL-2 plays a key role in both the generation, function, survival and expansion of $FOXP3^+$ Tregs [15-19]. These findings indicate a strong likelihood that gene polymorphisms in the IL-2/IL-2RA pathway influence disease risk via effects on the functional ability of $FOXP3^+$ Tregs and our studies show that a major *IL2RA* susceptibility haplotype is associated with decreased signaling via the IL-2 pathway in Tregs, which is linked to diminished Treg function [20].

Antigen-based vaccination in human inflammatory disease

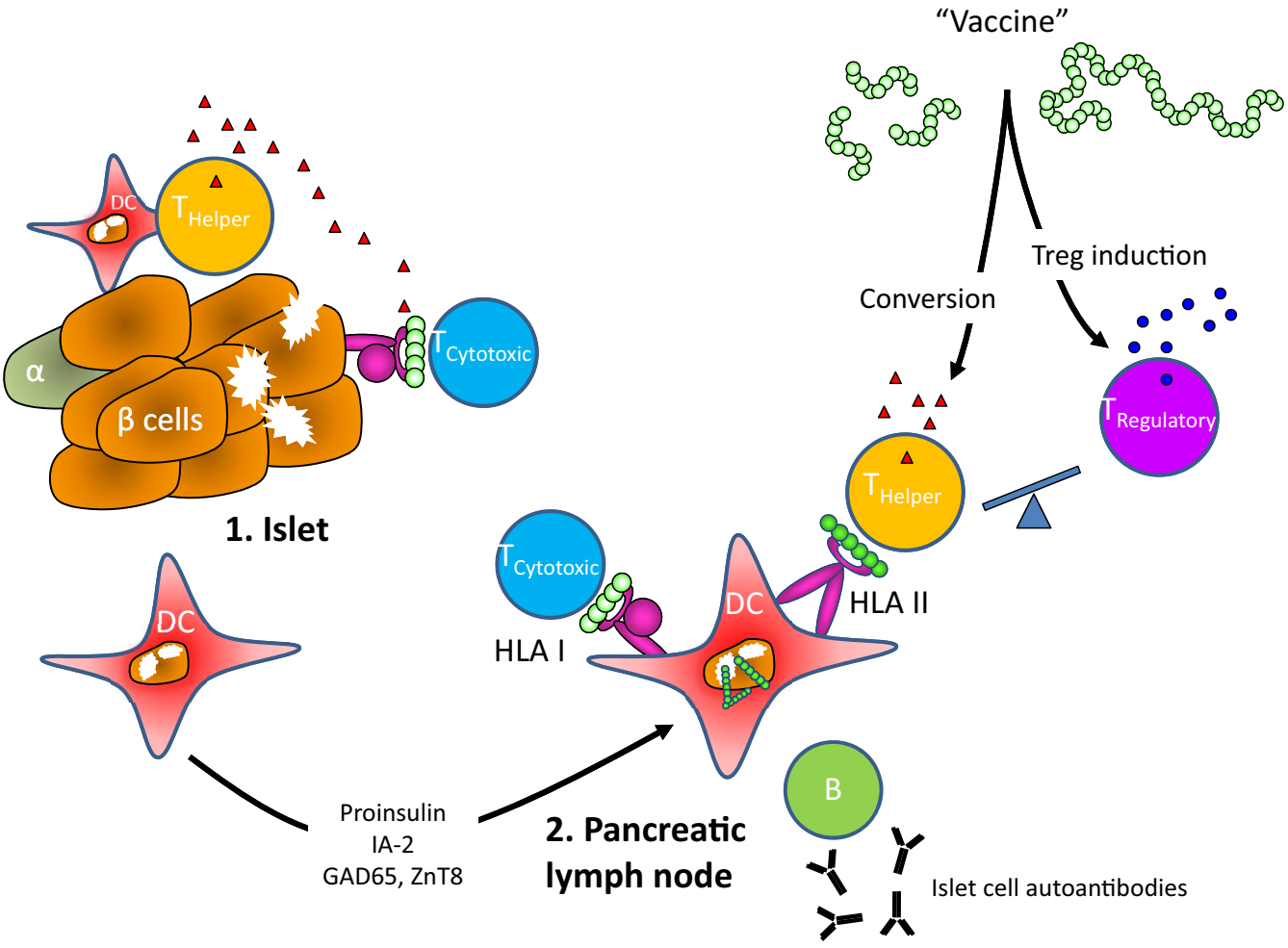
Armed with this knowledge, how might one devise therapeutic approaches for the disease? The link with vaccination comes from the observations made in many animal models in the last two to three decades that simple (no adjuvant) antigen administration via a variety of routes (oral or nasal, or by intravenous, intradermal, or subcutaneous injection) can render an animal resistant to subsequent attempts to induce an effector immune response (e.g. antibodies, effector T-lymphocytes) when the same antigen is administered with strong immunogenic adjuvants [21]. It has since become apparent that this antigen-dependent manipulation of the adaptive immune system (i.e. "vaccination") works via effects on several different components of the immune response; critically, these include the generation of antigen-specific Tregs with potent suppressive capabilities (Figure 1) [22], although there remains controversy as to whether this is *de novo* induction or conversion of effector T-lymphocytes [23]. It has also become clear that the same manipulation will work in the preventive stages of autoimmunity in animal models of Type 1 diabetes and in the intervention stage if complemented by adjunctive therapies [21].

As a consequence of these findings, a debate has emerged as to whether the power of using antigens to stimulate adaptive immune responses, as seen in classical vaccination, can be effective in an entirely different setting: that of autoimmune disease. The principle is the same: induction of an adaptive immune response using antigen. The difference lies in the type of immune response engendered. In classical vaccination, a powerful protective immune response is induced, with generation of pathogen-specific antibodies, T-lymphocytes that secrete inflammatory cytokines such as interferon-γ (known as T helper 1 or T_H1 cells) when pathogens are encountered, and T-lymphocytes that kill pathogen-infected cells (cytotoxic T-lymphocytes, [CTLs]). In vaccination for autoimmune disease, the converse is the aim: to induce self-reactive T-lymphocytes that have potent immune regulatory properties, such as the secretion of anti-inflammatory cytokines like IL-10 [24,25] and transforming growth factor (TGF)-β.

Key questions for clinical translation of vaccination for Type 1 diabetes

Five key questions arise in the debate: what is the evidence that vaccination can restore immune tolerance in human

Figure 1. Depiction of molecular and cellular events in the immunopathogenesis of Type 1 diabetes



β-cells are damaged as a result of an environmental insult, and with concomitant inflammation, activated dendritic cells (DC) migrate to the local lymph node, carrying β-cell remnants that include the key autoantigens (pre)proinsulin, glutamic acid decarboxylase (GAD65), islet-associated tyrosine phosphatase (IA-2) and the zinc transporter 8 (ZnT8). *En route*, the autoantigens are processed and presented to T cells by DCs, leading in susceptible individuals (who have the appropriate HLA molecules and hypo-functioning β-cell-specific immune regulation) to the priming of pro-inflammatory helper T-lymphocytes and cytotoxic T-lymphocytes, both of which are capable (in *in vitro models* [1,61]) of mediating β-cell damage, further accelerating the destructive cycle. Regulatory T-lymphocytes (Tregs) may limit this effector activity, and this balance may be critical in determining outcome in susceptible individuals. The balance can be favorably skewed by provision of β-cell autoantigens in a vaccine form (either as whole proteins or peptides), which may induce Tregs *de novo* or promote conversion of effector T-lymphocytes towards a regulatory phenotype. Abbreviations: DC, dendritic cell; GAD65, glutamic acid decarboxylase; HLA, human leukocyte antigen; IA-2, islet-associated tyrosine phosphatase; Treg, regulatory T lymphocyte; ZnT8, zinc transporter 8.

disease; is there any reason it will not work in Type 1 diabetes; what antigens could be of use in a diabetes-centric approach; what are the current experiences; and how can they be built upon and optimized?

Currently, the lead setting for using antigens to impact upon an inflammatory disease in man is clinical allergy. This was the setting for the very first recorded description of the approach, in 1911 [26], and it has now entered mainstream clinical practice (as "allergic desensitization" or "allergen-specific immunotherapy"). The antigen(s) (allergen extract) are injected as simple solutions into the subcutaneous tissue, typically in the clinical setting of prevention of intractable seasonal pollen allergy or anaphylactic responses to wasp and bee stings. Treatment is typically undertaken for a 1-2 year period, during which the majority of patients show sustained clinical benefit. Numerous mechanisms of action have been described, including the generation of Tregs and the production of IL-10 [27-29]. New developments include the

administration of allergens, via the sub-lingual route, and the use of short peptide sequences that represent the key regions (termed "epitopes") within an allergen molecule that are presented to, and recognized by, T-lymphocytes [30,31]. The latter ("peptide immunotherapy") has the advantage that only the relevant parts of the allergen need be used in the vaccine, avoiding the risk of antibody-allergen complex formation that can precipitate anaphylactoid responses. Peptide selection can also be tailored to a particular immune response, thus introducing a step towards personalized medicine. This could be in the form of selection of patients by human leukocyte antigen (*HLA*) genotype (the *HLA* genes encode HLA class II molecules that present peptide epitopes to T-lymphocytes), or in the avoidance of CTL epitopes to limit any risk of disease exacerbation [32]. Thus, the clinical allergy experience is that this simple vaccination-like manipulation can bring significant symptom relief, reduced inflammation and reduced dependence on drug therapy to many thousands of patients worldwide.

As has been noted already, there is a considerable body of evidence that Treg function is defective in patients with Type 1 diabetes, and several of the underlying diabetes-associated single nucleotide gene polymorphisms identified in genome-wide studies are considered likely to impact upon immune regulatory pathways [33]. This highlights the question as to whether, if the immunological brakes have a design fault, there is any prospect that just pumping the brake pedal harder will achieve any notable effect. In other words, can functional antigen-specific Tregs be induced in patients with Type 1 diabetes? To counter this argument, there are several indications that other aspects of immune regulation may be amenable to a vaccine approach. We have reported for example, that there are non-classical islet autoantigen-specific CD4+ Tregs, characterized by IL-10 production, that are present and functional in many adult patients with Type 1 diabetes, their presence being associated with a later age of onset of disease [34]. These cells potently regulate pro-inflammatory T cell responses [35]. Unpublished work in our laboratory shows that cells of this phenotype are also a frequent finding in autoantibody-negative (i.e. low risk) unaffected siblings of Type 1 diabetes patients, hinting at a role in active regulation of islet autoimmunity. Our interpretation of these data is that this pathway of immune regulation can be functional on a genetic background predisposing to Type 1 diabetes, but that its development may require environmental cues that may be stochastically missing or insufficient in those developing the disease. Intriguingly, it is widely thought that one of the pathways of therapeutic effect of the vaccine approach is induction of IL-10 secreting antigen-specific CD4+ T cells. Future studies will need to address the question of whether these induced regulators, the naturally-arising IL-10+ regulatory cells that we have described, and so-called Tr1 cells [36] are all one and the same.

To address the third key question, it is clear that selection of the appropriate antigen(s), around which to build the vaccine, is a key step. Tregs utilize a conventional T-cell receptor for antigen, through which signaling is required to initiate suppressor functions. Thus, a successful vaccination will be one which yields Tregs present at the site of inflammation that can be activated by the antigens being presented. In essence, this implies that the vaccine should be based on the same autoantigens that are the target of the inflammatory autoimmune response. Much progress has been made in the last two decades in defining these β-cell molecular targets, and, in several cases, the immunodominant peptides recognized by T-lymphocytes are also known [37]. This provides options to use whole antigens or peptides as the vaccine with the caveat that autoantigens that are also bioactive molecules, such as insulin, may be limited to certain routes of administration.

Currently we are at an early stage in developing vaccines/antigen-specific immunotherapies in Type 1 diabetes, but several developments are noteworthy. First, where patients have been given autoantigens as part of a vaccine strategy, there have been few, if any, safety concerns [38]. There has, for example, been no evidence of hypersensitivity induction. Acceleration of disease is also a theoretical concern, but it has been observed only rarely in animal models (in comparison to the number of studies conducted) [39-41], although it may have been a factor in the relapse of a minority of patients treated with altered peptides in patients with multiple sclerosis [42,43]. This experience suggests that care must be exercised in relation to dose (lower doses being better) and in relation to any attempt to enhance immunogenicity by deviation from the wild-type autoantigen sequence. Nonetheless, there has been no specific observation that antigen-induced disease acceleration is a risk in the Type 1 diabetes setting, even when there is very clear evidence that immunization has been achieved, as occurred for example in the studies of glutamic acid decarboxylase-alum immunization [44]. A second noteworthy point is that a sub-study of oral insulin administration to at-risk first-degree relatives (the Diabetes Prevention Trial-1) demonstrated a significant delay of progression to diabetes in those subjects with high levels of insulin autoantibodies [45], suggesting that a therapeutic effect is achievable, and again that appropriate immunological staging of participants is a key component of trial design. Further encouragement is derived from unpublished reports that the heat shock protein-derived peptide 277 (Diapep), a putative diabetes autoantigen [46], has beneficial metabolic effects when

administered parenterally in the context of a recent phase III study [47]. A third point is that proinsulin and insulin peptides have also proved safe at early stages of clinical development, opening up the potential for epitope-based vaccines [48,49]. These positive interpretations must be balanced, however, by other notable failures of antigen-specific immunotherapy. Nasal insulin has not proved effective in a large study of at-risk children in the prevention setting [50] and alum-conjugated glutamic acid decarboxylase-65 (GAD65, a known islet autoantigen in Type 1 diabetes [51]) therapy failed in separate studies at phase II conducted by Diabetes TrialNet [52] and phase III conducted by Diamyd [53]. There may, however, be important clues and lessons in these failures. Nasal insulin was given daily, a strategy which is known to be sub-optimal for Treg induction and might even be counter-productive by favoring deletion of Tregs, a well-described effect of abundant or frequent antigen administration [54]. In addition, our own unpublished mechanistic studies on samples from the TrialNet study of GAD-alum show that it is a potent inducer of GAD-specific pro-inflammatory CD4 T cells, which may not be the required phenotype for a vaccine for autoimmune disease.

The final question is the key one: what are the translational steps that are currently required to realize the potential of vaccine-based therapeutics for autoimmune diseases, such as Type 1 diabetes, and avoid the pitfalls? A series of research imperatives can be outlined to address this.

First, the clinical activity needs to be maintained and extended. It is important that studies in man continue to be conducted, but if these are in the setting of recent-onset diabetes then the expectations of funders, patients, investigators and journal reviewers and editors will need to be carefully managed. The success of these studies should be judged by the development of robust data regarding safety and the identification of biomarkers of vaccine administration, with preservation of C-peptide (a measure of residual β-cell function) a secondary end-point. Thus, there should be a clear delineation of what "success" looks like for an early stage vaccine study in new-onset disease. Thereafter, it should be a relatively straightforward progression of a vaccine approach to move to the next stage. This will either be as a mono-therapy in a secondary prevention setting (i.e. subjects are identified as being at risk of progression to diabetes using autoantibodies; the best current example of this approach being oral insulin conducted by Type 1 diabetes TrialNet) or as a combination approach (see below), either in secondary prevention or as an early intervention after diagnosis.

Second, it will benefit the field if experimental models can be refined to examine vaccine approaches in a humanized setting, using the same agents as those that will go into man. To date, for example, there is no report of a humanized mouse that regulates glucose via synthesis of endogenous human preproinsulin, whilst preproinsulin is the basis for several vaccine approaches. These models could be particularly useful for understanding dose/dose frequency, route of administration and biomarkers, which will otherwise limit development of vaccine-based strategies. Third, there are considerable basic research questions about the fate of exogenous antigen administered to man, and its interaction with the immune system, that will need to be addressed. And finally, there is the question of whether antigen-alone vaccines should be used in combinations and with what additional immune modulators. Finding the best combination or adjunct therapy will be challenging. Preclinical models suggest that combinations of antigen and anti-CD3 monoclonal antibody therapy can synergise to induce sustained disease protection via induction of immune regulatory pathways [55,56]. Theoretically, any manipulation that reduces inflammation has the potential to aid the development of a regulatory response to antigen, and thus combinations of antigens plus biologics that mediate cytokine blockade or cell depletion might be effective. This may present a conundrum to the field, however, should these single agents prove ineffective on their own; at this stage, preclinical models of antigen combinations will be an important part of the puzzle. In addition, there is the possibility that highly safe and moderately effective single agent biologics (in particular co-stimulatory blockers, such as CTLA-4Ig [57]) will be counter-productive in combinations because they block pathways required by both effector and regulatory cells alike. Finally, scant attention has been paid in the past to "regulatory adjuvants", but this is an area that may expand as commercial interests focus on therapies designed for autoimmune and inflammatory diseases, such as Type 1 diabetes.

Vaccination for Type 1 diabetes: opportunities and developments

The roadmap for the next 2-5 years in this field is outlined above. What should our priorities and focus areas be during that period to build on the hoped-for successes? There are certainly some new opportunities emerging. One example is a novel approach to antigen delivery, using nanoparticles coated with peptide-major histocompatibility complex (MHC) complexes [58], a technology that appears to work through induction of T cells with regulatory phenotypes. Another is the use of greater refinement in antigen or peptide selection. The use of naturally processed and presented epitopes, for example, is a strategy that avoids potential induction of neo-responses due to presentation of peptides that are otherwise cryptic, which can lead to the complication of priming new cohorts of

inflammatory T cells. This enables the peptides presented to interact with the existing and responsive T cell repertoire, which is then diverted towards a regulatory phenotype. The importance of the nature of peptide presentation and T cell repertoire has been highlighted in recent work in a preclinical model, in which sequence modifications of an insulin B chain sequence were required to ensure binding into the MHC class II molecule pocket in the correct "natural" register to enable a therapeutic effect [59]. Our own work has focused on the natural peptide repertoire, and a further refinement will be the use of multiple peptides assembled into bespoke cocktails suited to particular HLA genotypes – a form of personalized medicine. Finally, there are new strategies to deliver antigens via the oral route, which is known to favor tolerance induction. A particularly novel approach is the delivery of antigen plus IL-10 using *Lactococcus* lactis as the "Trojan horse", which has shown considerable promise at the preclinical stage [55].

A second area of potential advance is in the identification of biomarkers for successful vaccination. Unlike general immunomodulators, antigen-specific medicines lend themselves to biomarker development because there is potential specificity in the assay read-out (e.g. antigen-reactive T cells, antibodies) [60]. The current state-of-the-art is probably the use of ELISPOTs or ELISAs, looking for a shift towards a favorable response (e.g. induction of IL-10 or TGF-β) or away from a disease-associated response (e.g. interferon (IFN)-γ, IL-17) in response to peptide challenge *in vitro*. One can envisage such assays becoming flow-based using activation markers to identify the antigen-reactive T cells, enabling further regulatory markers to be examined (e.g. CD25, FOXP3) and gene expression analysis to be performed, or T cell receptor clonotyping to establish whether vaccine approaches operate via changes in the peripheral antigen-specific repertoire. A related question is whether there are biomarkers that might predict responsiveness. This could be, for example, a specified response to the antigen or peptide used in the vaccine, a concept that receives encouragement from the Diabetes Prevention Trial-1 findings discussed earlier.

Abbreviations

CTL, cytotoxic T-lymphocyte; GAD65, glutamic acid decarboxylase; HLA, human leukocyte antigen; IFN, interferon; MHC, major histocompatibility complex; TGF, transforming growth factor; Treg, regulatory T lymphocyte.

Competing interests

The author declares no competing interests.

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