Safely targeting autoimmunity in type 1 diabetes: the MonoPepT1De trial

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Current treatment of type 1 diabetes (T1D) centres on exogenous insulin to replace the deficit due to autoimmune destruction of β-cells. Despite considerable advances in insulin therapy and delivery, patients still have the daily concern of glucose excursions which lead to the serious complications of hyperglycaemia, risks of hypoglycaemia and the significant psychological impact of the disease. Immunotherapy for diabetes aims to target the disease at its core, halting autoimmune destruction of β-cells and removing dependence on exogenous insulin.

Early studies in the 1980s provided proof of principle that immunosuppression with agents such as cyclosporine,[1] azathioprine and prednisolone[2] could lead to remission of β-cell destruction and metabolic improvements, at least in the short term. More recent studies show varying degrees of success in prolonging β-cell function by targeting different aspects of the autoimmune response via drugs such as anti-CD3[3,4] and anti-CD20[5] monoclonal antibodies and CTLA-4 fusion protein.[6] Yet this type of immunosuppression/immune modulation remains generalised and continues to raise concerns over side effects of non-specific targeting of the immune system. Ideal timing for immunotherapy is at or preferably before clinical onset, the point at which there is greatest scope for β-cell salvage. Such early treatment targets a young age group leading to greater concerns over the potential impact of long-term risks such as toxicity, viral reactivation and malignancy, especially as current insulin treatment is relatively simple and safe.

Antigen-specific immunotherapy (ASI) offers a safer targeted approach to modulating the autoimmune process towards a tolerogenic response instead of global suppression. The aim is to re-establish tolerance by administration of an autoantigen, which can be in the form of a whole protein or as peptide immunotherapy (PIT), using short peptides derived from key β-cell autoantigens. The use of ASI has been successfully pioneered in clinical allergy[7–10] with interest fuelling research across autoimmune and inflammatory diseases (Figure 1). Use of peptides has potential benefits over whole antigen administration such as ease of use, enabling a pure highly concentrated epitope to be delivered at lower cost than whole antigen without the risk of complications of biological activity.[11] In PIT, natural processing of the antigen is bypassed compared to the use of whole antigen-based approaches, in which antigen-presenting cells (APCs) cleave the protein into peptides for loading onto the major histocompatibility complex (MHC). Therefore, in PIT it is important to utilise peptides that are biologically valid[12] for presentation to T cells. One way this has been tackled is by eluting peptides from MHC molecules on APCs which have been exposed to whole antigen and screening the subsequent panel of epitopes for T-cell responses[13] to confirm the biological validity.

ASI appears to induce tolerance through a number of mechanisms which vary in relevance depending on the route of administration and dosing. One of the main mechanisms is thought to be the induction of T cells with a regulatory phenotype (Tregs)[9,14] specific for the autoantigen.

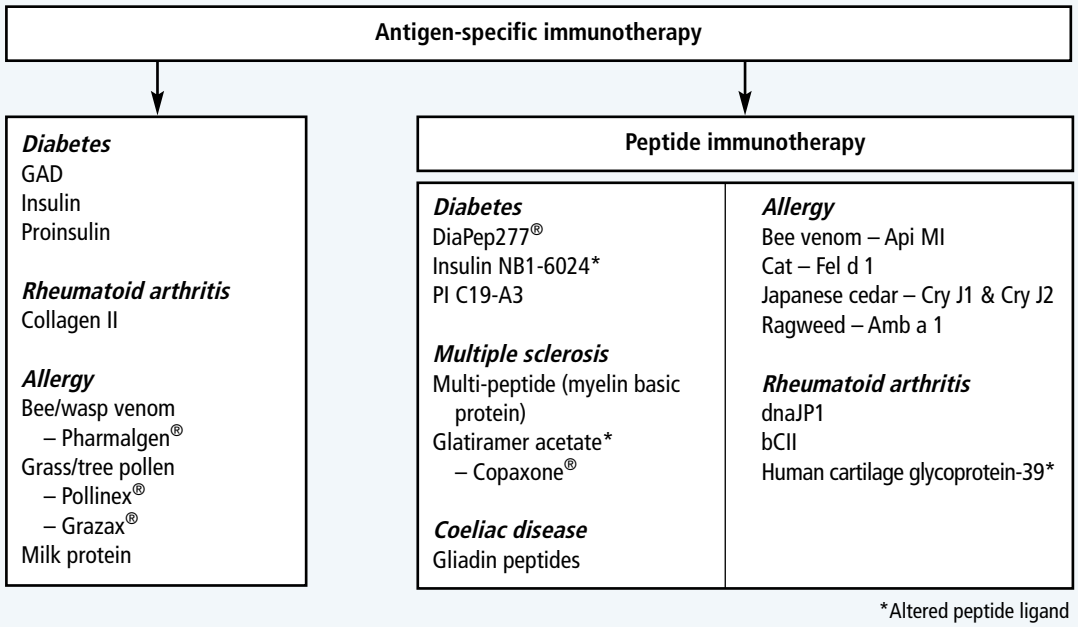

Figure 1. Examples of antigen-specific immunotherapy and peptide immunotherapy studies in human disease

This may lead to deviation of an autoimmune response from a pro-inflammatory T-helper (Th)1 phenotype to a regulatory phenotype with induction of signature anti-inflammatory cytokines such as interleukin (IL)-10.[10,11,15,16] Deletion of effector T cells through activation-induced cell death has also been observed.[17] Stimulation of Treg cells and IL-10 production allows bystander suppression of autoreactive CD4+ T cells involved in the autoimmune response but recognising other target epitopes. With treatment stimulating an antigen-specific proliferation of Treg cells, these mechanisms are finely targeted to the antigen of choice and theoretically bypass the pitfalls of immunosuppression.

Antigen-specific immunotherapy in type 1 diabetes

The use of ASI in T1D offers the hope of a 'vaccine' treatment which switches immune attack to immune tolerance. In doing so, β-cell function could be salvaged if treatment were started early enough, potentially reducing or eliminating exogenous insulin needs. Key studies using whole antigen[18–21] and peptide immunotherapy[15,16,22,23] in T1D have established that this approach is safe, showing no evidence of notable adverse events or disease acceleration. A peptide of heat-shock protein 60 named DiaPep277® showed initial promise in a phase 2 study,[15] preventing C-peptide decline against placebo. A further large-scale (n=457) phase 3 study using subcutaneous DiaPep277 in newly-diagnosed adults was recently reported to have reached its primary endpoint of C-peptide changes compared to placebo at the end of the 24-month study period.[24] A secondary endpoint of glycaemic control was also met with 45.5% of the treatment group reaching an HbA1c of <7% (53mmol/mol) compared to 35.7% of placebo (p=0.035). Full results of this study are still awaited. Other ASI studies have ultimately shown disappointing results after preliminary successes. Most notably, GAD antigen had shown C-peptide preservation on mixed-meal tolerance test (MMTT) in children treated within six months of diagnosis,[25] but a larger study failed to reproduce these findings.[26]

The MonoPepT1De trial

The MonoPepT1De trial is a multicentre placebo controlled, double-blind, clinical phase 1b trial currently investigating the effect of proinsulin peptide immunotherapy using the peptide C19-A3 (PI C19-A3) in individuals newly diagnosed with T1D. Subjects are adults aged 18–45 years and within 100 days of diagnosis who are screened for the HLA *DRB1*0401* allele (required for presentation of the proinsulin peptide) and a peak C-peptide of >200pmol/L after a mixed-meal tolerance test before inclusion. The goal is to randomise 24 patients into three treatment groups of eight patients: a high frequency group receiving 10μg of intradermal PI C19-A3 fortnightly, compared with a low frequency group who receive 10μg PI C19-A3 monthly interspersed with placebo, and a third group which receives placebo at every dose (Figure 2). Post-treatment subjects are monitored for a further six months. The primary endpoint is assessment of safety of PI C19-A3 in the context of new-onset T1D, with secondary endpoints assessing stimulated C-peptide production, HbA1c, mean glucose excursions, quality of life scores and islet cell autoantibody biomarkers of β-cell specific immune responses.

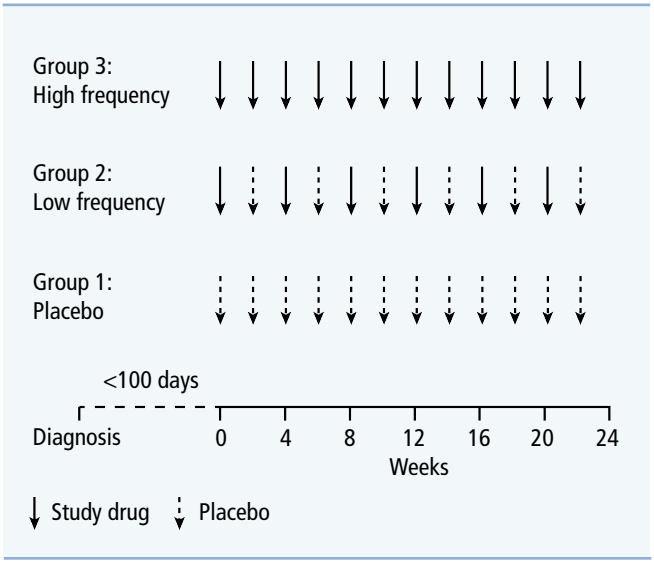

Figure 2. MonoPepT1De study dosing regimen

Why proinsulin C19-A3?

Proinsulin C19-A3 (PI C19-A3) is an HLA-DR4 *(DRB1*0401)* restricted 18 amino acid peptide which was identified as an epitope naturally processed and presented by this MHC class II molecule after presentation of proinsulin to antigen-presenting cells and subsequent elution of bound peptides.[13] This process of elution gave strength to the potential biological validity of PI C19-A3 as an antigen in T1D which was reinforced through *in vitro* ELISpot studies showing PI C19-A3 elicits pro-inflammatory interferon γ (IFNγ) responses from newly-diagnosed T1D patients compared to IL-10 responses in controls. Importantly, since proinsulin is generally not a secreted protein, PI C19-A3 as an autoantigen is largely confined to the islets. From a safety aspect this specificity is reassuring, is unique among other ASI antigens in T1D and should allow for focused immunoregulation within the islets and local lymph nodes. The intradermal route of administration of the PI C19-A3 in this trial differs from many other ASI trials, but aims to exploit the natural abundance of antigen-presenting cells for peptide presentation in the epidermis and upper dermis compared to the subcutaneous route.

A previous phase 1 study using intradermal PI C19-A3 in established T1D patients examined the effect of using three intradermal injections of the peptide given monthly at a dose of either 10μg or 100μg.[16] This revealed that favourable *in vitro* cytokine responses, which were predetermined as a fall in IFNγ or rise in IL-10 response at three months, could be seen more commonly in the lower-dose group (Figure 3). However, these responses were not sustained after withdrawal of the peptide. Interestingly, there was a small but significant improvement in glycaemic control in the low-dose group, shown by a fall in HbA1c by 0.23% (95% CI -0.42 to -0.03, p=0.02). The two dosing arms of the current MonoPepT1De study may reveal whether the beneficial cytokine changes seen are sustainable with more frequent dosing.

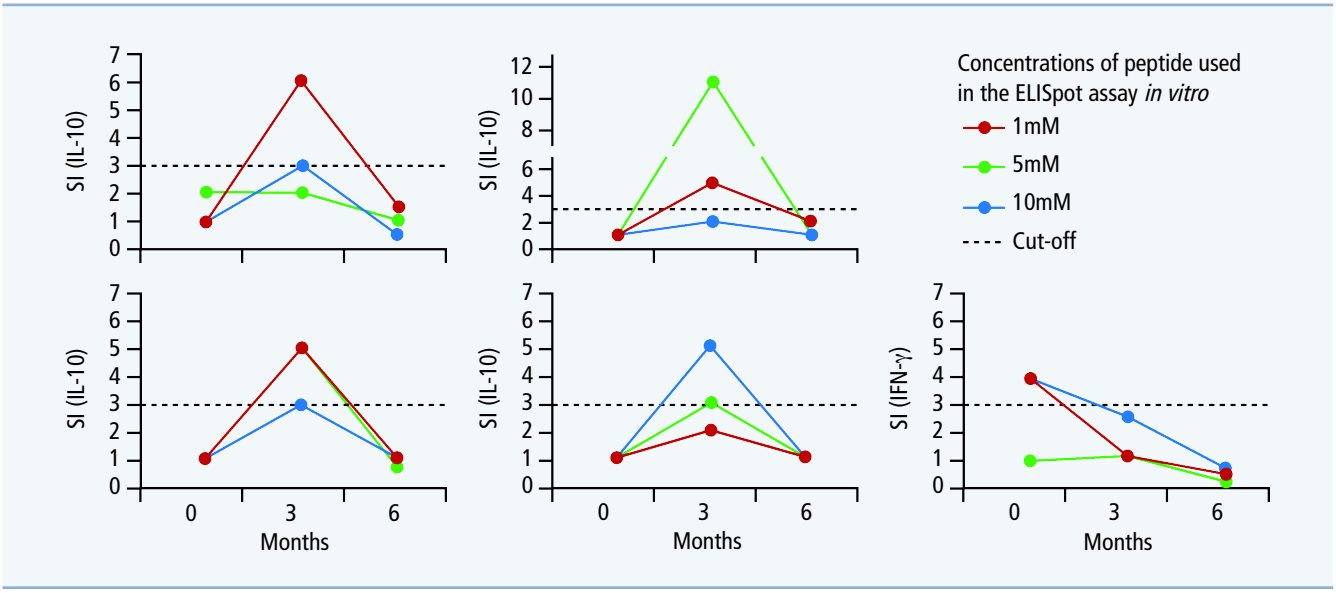

Figure 3. Favourable responses, from Thrower *et al.*'s representative example of 5 patients, seen in ELISpot assay during phase 1 trial of proinsulin C19-A3 peptide. The graphs show ELISpot interleukin (IL)-10 and interferon γ (IFN-γ) responses to peptide at 0, 3 and 6 months measured by stimulation index (SI) in the 10µg treatment group. (SI derived as number of spots in test wells/number in saline wells). (From: Thrower *et al. Clin Exp Immunol* 2009;155:156–65.[16] Reproduced with permission of Wiley-Blackwell, © 2008 British Society of Immunology)

The future of PIT in type 1 diabetes

The results of the MonoPepT1De trial will establish the safety of intradermal PI C19-A3 in newly-diagnosed T1D patients as well as providing important evidence towards ideal dosing of such therapy. Current ASI trials are diverse in their design, often with significant variability in patient population, course of disease, dosing, and route of administration. The efficacy of PI C19-A3 may lie in targeting the optimal patient population, not only in terms of genotype but also in the stage of disease, as well as in using tailored dosing. As we are treating in a tertiary prevention setting, our patient cohort – despite being within a tight window from diagnosis – have already presented with clinical disease. In this situation, the true benefits of PIT may be outweighed by advanced islet destruction which precedes clinical presentation for many years. Such islet destruction may have led to release of multiple diverse autoantigens for T-cell attack, a process that spreads and broadens the autoimmune response, making it hard to halt. There are already plans underway for multiple peptide immunotherapy which would allow for attenuation of autoimmune attack against a number of antigens simultaneously. Trials in at-risk groups identified by having family history and high risk genes, possibly in combination with other peptides or immune therapies, could be the key to building tolerance to key β-cell epitopes, which may protect individuals from triggers of disease. As response to epitopes can vary considerably due to a number of factors including genotype, the choice of therapy for a subject at risk or recently diagnosed will have to be finely tailored. There are indications that the course of disease in adults versus children differs due to variations in the immune response. Therefore, there must be caution before extrapolating any results from adults to children, who will ultimately form the majority of recipients for such therapy.

Summary

PIT is a safe approach to immunoregulation in T1D with PI C19-A3 having unique properties which may be the key to switching immune attack to tolerance. In HLA DR4 positive individuals, PI C19-A3 PIT may form part of the strategy to enhance regulatory mechanisms, although full arrest of the autoimmune attack in T1D may hinge upon optimising timing, dosing and potentially amplifying response with additional peptides. The MonoPepT1De trial aims to further establish the safety profile of this treatment and elucidate on some of these issues.

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