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COMMENTARY

A future for CD3 antibodies in immunotherapy of type 1 diabetes

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Abbreviations

AbATE	Autoimmunity-Blocking Antibody for Tolerance
MMTT	Mixed-meal tolerance test
PD-1	Programmed cell death 1

More than 30 years have passed since the first immunotherapy trials in autoimmune insulin-dependent type 1 diabetes were conducted [1]. At this time, it was already apparent, given the compelling observations of the central role of CD4 and CD8 autoreactive T cells in the destruction of insulin-secreting beta-cells, that the immune system of patients would be the most effective drug target, though few tools were available. Small molecule immunosuppressants, such as ciclosporin, were transforming treatment of organ transplant rejection as they proved to be more effective than the conventional therapies, corticosteroids and azathioprine. Based on these observations, ciclosporin was tested in patients with recently diagnosed type 1 diabetes. The results of these first trials provided a fundamental proof-of-concept: that it was possible to effectively treat patients with established hyperglycaemia because, contrary to the prevailing dogma, even after disease diagnosis, a significant mass of beta-cells remained such that recovery of metabolic control was possible if treatment was started early enough [2–4]. However, the other important conclusion that was immediately evident, and which explains why ciclosporin was not developed further, was that the associated side effects of the drug treatment meant that the risks outweighed the benefits. Indeed, any antigen non-specific immunosuppression strategy that required chronic administration to maintain effectiveness, as did ciclosporin in type 1 diabetes, exposed patients, including children and young adults, to unacceptable risks of over-immunosuppression and off-target toxicities.

New strategies quickly emerged that focused on inducing operational immune tolerance, i.e. a short treatment leading to long-term preservation of the functional beta-cell mass without chronic immunosuppression. This concept was based on robust data obtained in the NOD mouse, an experimental model that spontaneously develops the disease. In this model, administration of autoantigens or certain monoclonal antibodies directed against functionally relevant lymphocyte receptors made it possible to prevent or even reverse the development of type 1 diabetes, permanently in many cases, by altering the autoimmune response, inducing immune homeostasis [5, 6]. For example, in NOD mice with recent-onset hyperglycaemia, a short treatment of CD3-specific antibody therapy induced permanent remission of the disease [7]. Remarkably, this effect was indeed due to a restoration of immune tolerance against self-antigens, since the treatment of the NOD mice with CD3 monoclonal antibody did not result in long-lasting immune depression but, rather, a rapid recovery of general immune function within a few weeks after the end of treatment, leading to the ability of treated mice to respond normally to foreign antigens [7–10].

Many well-designed therapeutic trials with a number of different classes of therapeutic agents followed, and their findings led to three major conclusions.

First, the administration of beta cell autoantigen alone in type 1 diabetes with established hyperglycaemia, or even under 'pro-tolerogenic' conditions, such as in secondary prevention, cannot reverse diabetes over the long term [11–15].

Second, in contrast, in randomised controlled trials, multiple different biological products targeting T cells (CD3 antibodies, LFA3 antibodies, anti-lymphocyte serum) [16–21], B cells (CD20 antibodies) [22] and T cell-associated co-

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stimulation pathways [23, 24] improved beta cell function, although the magnitude and duration of the improvement varied among the products. Humanised Fc-mutated CD3 antibodies, extensively studied in multiple clinical trials, provided some of the best clinical results. Clinical trials on two distinct molecules of the same class, teplizumab and otelixizumab, independently showed efficacy in type 1 diabetes patients with recent onset hyperglycaemia. A short treatment (6–14 days), started 4–12 weeks after the beginning of insulin treatment in newly diagnosed patients consistently preserved C-peptide levels for 2 to 4 years [18–21]. Furthermore, when used at clinically effective doses, especially in the case of teplizumab, the treatment was safe, with only mild, transient adverse events reported (occurring at the time of drug infusion). One study showing the efficacy of teplizumab was the Autoimmunity-Blocking Antibody for Tolerance (AbATE) protocol, the fourth therapeutic trial using teplizumab in type 1 diabetes, the 2 year results of which were reported in 2013 [25]. The AbATE trial was a randomised, open-label study that was conducted between 2005 and 2011. The treatment group received a 14 day course of teplizumab at study entry and at 1 year. The design of AbATE set the ground for a Phase III trial, Protégé [26]. Protégé did not meet its primary endpoint, which was based on HbA_{1c} and insulin usage, but post hoc analyses of the data showed efficacy in terms of C-peptide preservation, especially among younger patients (8–17 years old) and those patients started on drug within 6 weeks of diagnosis (i.e. the start of insulin therapy) [26].

Third, it has become clear that the similar effects seen with T cell receptor-specfic, T cell co-stimulatory molecule-specific and, even, B cell-specific therapies suggest that type 1 diabetes is a complex disease with variable impact on different components of the immune system. Moreover, the disease is likely to be variable within the affected population, which means that patients can be stratified into multiple subgroups depending on disease pathophysiology. Thus, increasing efficacy and achieving a longer-term effect, a key goal, will depend on combination therapies.

A post hoc analysis of the AbATE trial identified metabolic and immunological variables that differentiated individuals who responded to treatment with teplizumab from the non-responders [25], with treatment response defined as loss of <40% of baseline C-peptide at the 2 year follow-up. Approximately 45% of the drug-treated patients were classified as responders, and these individuals had lower HbA_{1c} and lower insulin requirements prior to treatment, suggesting the presence of a functionally larger beta cell mass at the time of treatment initiation [25]. Importantly, this result is fully consistent with those seen in the Phase III Protégé trial, which found a greater effect of teplizumab on C-peptide preservation in patients included earlier after diagnosis, no later than 6 weeks after the start of insulin therapy [26].

In this issue of *Diabetologia*, an article by Perdigoto et al provides further insight into the use of teplizumab for the treatment of type 1 diabetes, presenting the long-term follow-up (7 years on average, range up to 9 years) results of the AbATE trial [27]. This new study was designed at the conclusion of AbATE (2 years post-treatment) and data were available on 43 (56%) of the 77 participants in AbATE (31 in the treatment group and 12 in the control group). Patients with detectable C-peptide responses in a 4 h mixed-meal tolerance test (MMTT) at year 2 of AbATE were asked to return and underwent another 4 h MMTT (follow-up visit 1). If they had detectable levels of C-peptide at that visit, they returned 1 year later for a second follow-up visit and repeat tests. The definition of 'responders' differed from that in AbATE; it was arbitrarily defined as the absence of change in C-peptide between visit 1 and 2, i.e. <7.5% difference between the two values, as used previously in some clinical studies. The authors found that, overall, C-peptide levels were not significantly different between drug-treated patients and control participants during the MMTT; however, C-peptide levels were higher in drug-treated responders (identified at 1 year) than in control participants and drug-treated non-responders [27]. The question of the real clinical meaning of this difference in C-peptide is important given that it was not associated with better glycaemic control or a decrease in insulin requirements. Similar trials with such a long-term follow-up will need to be conducted using a well-defined protocol with a definition of responder and non-responder subgroups established at the initiation of the trial, not post hoc, to provide a definitive answer.

Interestingly, immunological changes were observed that appeared to be associated with clinical response during the first 2 years of the AbATE study [28–30]. Thus, drug-treated responders showed a significant increase in a subset of $CD8^+$ T cells that have been described elsewhere as 'exhausted' PD-1^+KLRG1^+CD57^- and 'anergic' PD-1^+KLRG1^-CD57^- T cells (where PD-1 is the receptor programmed cell death 1 and KLRG1 is killer cell lectin like receptor G1). These $CD8^+$ T cells expanded after treatment and their levels peaked at 3–6 months [28, 29]. Yet, even 7 years after the end of treatment, increased frequencies of PD-1^+CD8^+ T cells, including exhausted and anergic $CD8^+$ T cells, were observed in the responder group. It will be extremely important to explore whether these results can be replicated in future trials to validate their potential as markers of response to teplizumab treatment. Moreover, a role for these specialised T cell subsets in the effect of the treatment should be evaluated. It has been established in experimental models that $CD4^+$ and $CD8^+$ T cells presenting an 'anergic/exhausted' T cell phenotype mediate antigen-specific unresponsiveness (i.e. immune tolerance). Fife et al have shown that CD3 antibody-induced

remission of type 1 diabetes in NOD mice is fully reversed by in vivo administration of an antibody to PD-1 [31]. In the same vein, in an experimental mouse model where fully mismatched allogeneic islets were implanted in CD3 antibody-treated recipients the administration of an antibody to PD-1 fully reversed the immune tolerance and precipitated graft rejection [32, 33].

To date, nearly 800 patients have received teplizumab, including 166 young children aged 8–11 years, and 308 adolescents aged 12–17 years. The TrialNet group has recruited 76 children to a placebo-controlled study designed to test the ability of teplizumab to delay onset of insulin therapy in at-risk individuals presenting with at least two autoantibodies and an abnormal OGTT but no hyperglycaemia. Importantly, it would not have been possible to allow such a large number of patients to have undergone this therapy had it not been for the favourable safety profile of teplizumab, a major feature that is confirmed by the results of the 7 year follow-up study described here [27].

The field of diabetology is facing the harsh reality that the incidence of type 1 diabetes is increasing dramatically in industrialised countries [34–36], and affecting an increasingly younger patient population. In these patients, palliative treatment with insulin therapy, even if implemented with the latest delivery devices, is not sufficient to prevent frequent episodes of hypoglycaemia and the long-term degenerative side effects of the disease linked to a 'not ideal' metabolic control. In this context, the reality is that, unfortunately, there is not a single immunotherapy available on the market. Although new and improved insulins, pumps and monitors have reduced the frequency and severity of diabetes complications, the lack of approved therapies that target the cause of the disease, autoimmunity, rather than the symptom, hyperglycaemia, remains an unfortunate gap that the community must continue to tackle by actively supporting efforts to accelerate the study of therapies, such as CD3 monoclonal antibodies and combination studies, through Phase III trials.

Discussed above are hopes for the future based on combination therapies. The list of candidates gets longer every day, going from conventional chemical immunosuppressants to new biologicals, autoantigens and cell therapy (i.e. regulatory T cells). It is important to note, however, that several of the proposed combination therapies include a CD3 antibody [37–40].

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