CD3-specific antibodies: a portal to the treatment of autoimmunity

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Abstract | Targeted immunotherapies hold great promise for the treatment and cure of autoimmune diseases. The efficacy of CD3-specific monoclonal antibody therapy in mice and humans stems from its ability to re-establish immune homeostasis in treated individuals. This occurs through modulation of the T-cell receptor (TCR)–CD3 complex (also termed antigenic modulation) and/or induction of apoptosis of activated autoreactive T cells, which leaves behind 'space' for homeostatic reconstitution that favours selective induction, survival and expansion of adaptive regulatory T cells, which establishes long-term tolerance. This Review summarizes the pre-clinical and clinical studies of CD3-specific monoclonal antibody therapy and highlights future opportunities to enhance the efficacy of this potent immunotherapeutic.

Multiple sclerosis
A chronic inflammatory and demyelinating disease of the central nervous system. It is an autoimmune response against components of myelin, which is thought to contribute to disease pathogenesis. Self glycolipids are autoantigens that are recognized by T cells in this disease.

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Autoimmune diseases arise following a breakdown in the balance between autoregulatory immune pathways and pathogenic autoreactivity, which leads to aggressive antibody and T-cell-mediated reactions directed against antigens expressed by the host's own tissues. Over 80 diseases have been purported to have an autoimmune aetiology. Therefore, autoimmune-associated morbidity and mortality ranks third highest in developed countries (after cardiovascular diseases and cancer), and the frequency of these diseases has steadily increased over the last three decades. Present treatments are either substitutive (administration of insulin to patients with type 1 diabetes (BOX 1), thyroid hormones to patients with thyroiditis or vitamin B12 to patients with pernicious anaemia) or palliative (based on the chronic use of anti-inflammatory and/or immunosuppressive agents). Both of these types of treatment are often only partly efficacious and potentially toxic. Therefore, over the past decade a greater emphasis has been placed on inducing immune tolerance by taking advantage of the multiple immune pathways that are present in the periphery to avoid unwanted self-reactivity.

Achieving true immune tolerance will require a therapy of short duration that abrogates pathogenic reactivity to autoantigens but that leaves the patient with full capability to mount a normal immune response against foreign pathogens. One approach to induce immune tolerance takes advantage of autoantigens that have been identified in target tissues, such as insulin (in diabetes), acetylcholine receptor (in myasthenia gravis), desmoglein-3 (in pemphigus vulgaris) and myelin basic protein (in multiple sclerosis), to create therapeutic vaccines that tolerize antigen-specific immune responses. Although this approach was effective in experimental settings[1–12], it has been less successful when transferred to the clinic[13–16]. This failure is possibly due to several factors including: difficulties in translating the dose and timing used in animal models for human application; the late administration of the vaccine in the disease process, which might result in the exacerbation rather than the dampening of the immune response by the 'immunomodulatory' capacities of the autoantigens, as was the case in one clinical trial of multiple sclerosis treatment[16]; a lack of clear knowledge of the pathogenic epitopes on the targeted tissues at any given stage of disease (due to epitope spreading)[1,2,17,18] or of the HLA haplotype; and a lack of complete efficacy of the vaccines, as it is unlikely that the autoimmune response to all potential pathogenic autoantigens will be eliminated by the therapy (as bystander suppression might not be as effective as in the experimental setting)[19,20]. A second approach — pioneered by the development of the first mouse monoclonal antibody specific for human CD3 (known as orthoclone OKT3; Ortho Biotech; the first Food and Drug Adminstration approved monoclonal antibody for treatment in transplantation) — takes advantage of biological agents that promote immune tolerance, such as monoclonal antibodies and soluble ligands directed to functionally relevant receptors on immune cells. In fact, the use of therapeutic biological compounds has become the most successful therapeutic approach available to modulate the immune system to date, with

Epitope spreading
This term was used to describe how a self-directed immune response induced by a single peptide (or epitope) could spread to include other peptides (or epitopes) not only on the same autoantigen (intramolecular spreading) but also on other self-molecules clustered in close vicinity within the target cell (intermolecular spreading). A good example for epitope spreading is the *de novo* activation of autoreactive T cells by self-antigens that have been released after β-cell damage.

Bystander suppression
The extension of tolerogen-induced suppression to immune responses that are directed against antigens not structurally related to the tolerogen but expressed by the same target tissue.

Anergy
A state of unresponsiveness that is sometimes observed in T and B cells that are chronically stimulated or that are stimulated through the antigen receptor in the absence of co-stimulatory signals.

multiple drugs approved for therapy for a wide range of immune diseases, such as autoimmunity[21], allergy[22] and organ transplantation[23–25]. Therefore, it is not surprising that the first successful potential tolerogenic therapy in humans uses a biological product. In particular, this Review focuses on the clinical success of Fc receptor (FcR)-non-binding CD3-specific monoclonal antibodies that have been used to preserve endogenous insulin-secreting β-cell mass in patients recently diagnosed with type 1 diabetes[26–28]. The clinical studies showed that a short immune intervention with these antibodies rapidly arrested ongoing autoimmunity and had a long-term effect by altering fundamental immune processes towards durable immune tolerance[26–28]. Our goal here is to speculate on the future clinical use of CD3-specific antibodies for the treatment of type 1 diabetes, and to highlight its potential therapeutic role in other T-cell-mediated autoimmune diseases and solid-organ and bone-marrow transplantation.

CD3-specific antibodies in type 1 diabetes

Mechanisms and mode of action of CD3-specific monoclonal antibodies. Over the past decade or so, we have come to appreciate that FcR-non-binding CD3-specific monoclonal antibodies have multiple effects on T cells that lead to immune tolerance. These antibodies modulate the T-cell receptor (TCR)–CD3 complex resulting in the cells becoming 'blind' to antigen (a process that is also known as antigenic modulation) (FIG. 1a)[29]. In addition, the monoclonal antibody preferentially induces anergy or apoptosis of activated T cells through alterations in TCR-mediated signal transduction. TCR engagement by FcR-non-binding CD3-specific monoclonal antibodies leads to partial phosphorylation of the TCR complex and downstream targets, which results in blockade of interleukin-2 (IL-2) production and the subsequent inactivation of T helper 1 (T_H1) cells[30,31]. This biochemical cascade is the result of incomplete immunological synapse formation during the early stages of T-cell activation. Unlike T-cell activation by antigen–MHC complexes or multivalent binding by FcR-binding CD3-specific monoclonal antibodies, the bivalent FcR-non-binding CD3-specific monoclonal antibodies abrogate early signalling events in T-cell activation, such as calcium influx, unless the TCR complex is artificially crosslinked with a secondary immunoglobulin-specific antibody[30,32]. Moreover, ligation of the TCR outside of the immunological synapse leads to preferential activation of the SRC-family kinase FYN but not synapse-associated LCK, and this results in a partial signal that leads to the downstream inactivation of T cells and the inhibition of apoptosis induction[30,31]. This model fits with other data that implicate FYN and CBL (casitas B-lineage lymphoma) as crucial elements involved in the induction of anergy[33]. Induction of apoptosis in this system is partially mediated by CD95–CD95L (CD95 ligand) interactions with neighbouring T cells (a process known as activation-induced cell death). This may be a result of the induction of CD95 expression and enhanced CD95 DISC (death-inducing signalling complex) formation following engagement of CD3 by the antibody (FIG. 1a). These observations explain why effector T-cell death is most dramatic at the site of inflammation where the highest density of activated T cells exist (Q. Tang and J.A.B., unpublished observations). By contrast, forkhead box P3 (FOXP3)$^+$CD4$^+$CD25$^+$ regulatory T (T_{Reg}) cells are relatively more resistant to cell death induced by FcR-non-binding CD3-specific monoclonal antibodies compared with recently activated effector and naive T cells. This effect seems to be dose dependent, in that low doses of FcR-non-binding CD3-specific monoclonal antibodies (for example 10 μg per injection) selectively delete effector T cells, whereas higher doses (100 μg per injection) of the antibodies delete T_{Reg} cells, supporting the use of lower doses of the immunomodulatory drug for therapeutic applications (Q. Tang and J.A.B., unpublished observations).

Preclinical studies in mice. The first demonstration that CD3-specific monoclonal antibodies could affect autoimmune diabetes was published in the mid 1990's using the non-obese diabetic (NOD) spontaneous mouse model of type 1 diabetes (TABLE 1). A short, 5-day treatment at the time of diabetes onset was sufficient to reverse the disease, induce long-term remission, and prevent recurrent immune responses even towards transplanted syngeneic pancreatic islets[34]. A few weeks after the end of treatment these mice had a normal immune response to exogenous antigens, thus proving that immune tolerance to pancreatic islet antigens was selective. Such effects were seen with both FcR-binding and FcR-non-binding CD3-specific monoclonal antibodies (a whole 145-2C11 antibody to mouse CD3 or its F(ab′)$_2$ fragments)[34,35].

The FcR-binding CD3-specific monoclonal antibodies are potent mitogens, eliciting the release of mostly T-cell-derived cytokines following *in vivo* administration[36,37]. In sharp contrast, the FcR-non-binding CD3-specific monoclonal antibodies bivalently bind CD3, and this induces abnormal activating signals that abrogate

Box 1 | Pathogenesis of type 1 diabetes

Type 1 diabetes is an autoimmune disease ensuing from the selective destruction of pancreatic insulin-secreting β-cells by autoreactive CD4$^+$ and CD8$^+$ T cells. Therefore, there is great interest in T-cell-directed immunointervention therapies for the prevention and treatment of this disease. Autoantibodies to β-cell antigens are also detected in the serum of patients with type 1 diabetes but their production is thought to be secondary to the destruction of β-cells and they are not pathogenic. In patients with type 1 diabetes they are considered as markers of β-cell destruction.

Several candidate target autoantigens have been identified in type 1 diabetes, which mainly include the β-cell hormone proinsulin/insulin, glutamic-acid decarboxylase (GAD), the pancreatic islet tyrosine phosphatase islet-cell antigen 2 (IA2; also known as PTPRN2) and the islet-specific glucose-6-phosphatase catalytic subunit-related protein (IGRP; also known as G6PC2) that is a target for autoreactive CD8$^+$ T cells.

Pathogenic CD4$^+$ T cells express a T helper 1 (T_H1)-cell phenotype, producing interleukin-2 (IL-2) and interferon-γ (IFNγ)[98]. In non-obese diabetic (NOD) mice, disease is accelerated by the administration of IL-12 (REF. 99), which favours the differentiation of T_H1 cells. On the other hand blockade of T_H1-cell development, as afforded by the administration of a monoclonal antibody that neutralizes IFNγ (REF. 98) or IL-12 antagonists[100], effectively inhibits disease progression.

Disease development is under the control of heterogeneous CD4$^+$ regulatory T cells and includes thymus-derived naturally occurring regulatory T cells and adaptive regulatory T cells that originate from mature T cells in the periphery.

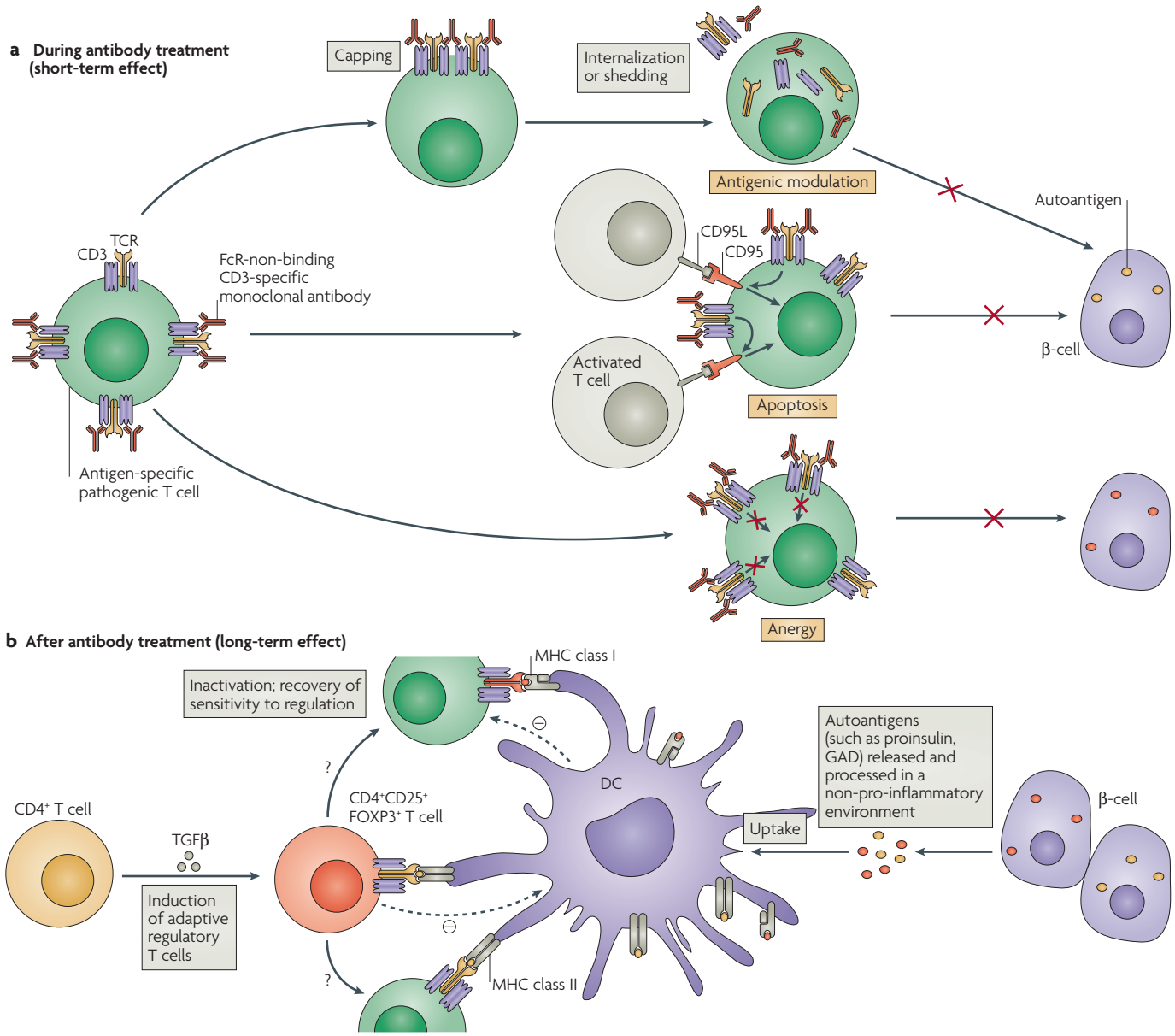

Figure 1 | **Postulated mode of action of FcR-non-binding CD3-specific monoclonal antibodies in type 1 diabetes.** A short course treatment with FcR-non-binding CD3-specific monoclonal antibodies induces remission of overt autoimmune diabetes. This figure represents the different immune mechanisms that have been proposed to explain the therapeutic effect of CD3-specific monoclonal antibodies. Two phases can be distinguished: the first phase covers the period of antibody administration (short-term effect) and the second phase evolves after the end of the treatment (long-term effect). **a** | During antibody treatment, FcR-non-binding CD3-specific antibodies act on T cells through three distinct non-mutually exclusive mechanisms: antigenic modulation of the T-cell receptor (TCR)–CD3 complex, through the redistribution at the cell membrane of complexes formed by the receptors bound to the antibody (capping) followed by their internalization or shedding from the cell surface; the induction of apoptosis that preferentially affects activated T cells, possibly through the ligation of CD95 by its ligand (CD95L) expressed by neighbouring T cells; or the induction of anergy in T cells. These mechanisms operate while the antibody is present in the environment and ensure a combined physical and functional 'debulking' of pathogenic T cells, which explains the rapid clearance of insulitis and reversal of established disease. But as these effects are transient they cannot explain the long-term efficacy of CD3-specific antibody treatment. **b** | After treatment, the operational immunological tolerance is of the 'active' type implicating a specialized subset of adaptive regulatory T cells, which effectively control pathogenic effectors, are transforming growth factor-β (TGFβ)-dependent and are derived from $CD4^+CD25^-$ peripheral T-cell precursors. During this phase the infiltration of the target organ recurs as a non-invasive and/or non-destructive insulitis suggesting that autoantigens are still presented locally. There is compelling evidence to show that regulatory T cells exert their control on pathogenic T cells through an indirect effect involving dendritic cells (DCs) rather than from a direct T-cell–T-cell interaction. The immunoregulatory cytokine TGFβ, which in this model is not only produced by regulatory T cells but potentially by DCs and other stromal cell types, appears as an ideal candidate to favour the maintenance of a non-pro-inflammatory environment through its potential action on T cells (effector T cells and/or regulatory T cells) and/or on DCs. GAD, glutamic-acid decarboxylase.

Immunological synapse
A region that can form between two cells of the immune system in close contact. The immunological synapse originally referred to the interaction between a T cell and an antigen-presenting cell. It involves adhesion molecules, as well as antigen receptors and cytokine receptors.

Activation-induced cell death
A pathway of T-cell apoptosis that often involves the upregulation of CD95 ligand that binds to the cell-death receptor CD95.

Table 1 | **Therapeutic CD3-specific monoclonal antibodies in type 1 diabetes**

	Model	Antibody	Refs
Preclinical	Reversal of established diabetes in NOD mice	Parenteral 145-2C11 (a hamster monoclonal antibody specific for mouse CD3)	34
	Reversal of established diabetes in NOD mice	145-2C11 $F(ab')_2$	35
Clinical	US-based Phase I and II clinical trial	Teplizumab	26,28
	European-based Phase II, placebo-controlled clinical trial	ChAglyCD3	27

NOD, non-obese diabetic.

the release of most cytokines. Moreover, the abnormal signals delivered by FcR-non-binding CD3-specific monoclonal antibodies are responsible, at least in part, for their tolerogenic effect on T cells, as the induction of tolerance was completely blocked by co-administration of cyclosporin[35], a potent calcineurin inhibitor, which impaired downstream transcriptional signals crucial for the induction of tolerance[38]. FcR-binding CD3-specific monoclonal antibodies (such as OKT3) induce flu-like symptoms (due to their mitogenic activity) in treated patients[39–41]. Thus, the reduced release of cytokines following the administration of FcR-non-binding CD3-specific monoclonal antibodies has propelled this class of antibodies into the clinical arena.

FcR-non-binding CD3-specific monoclonal antibodies were most effective when given to NOD mice once ongoing pancreatic-islet-cell destruction was apparent and even once disease was diagnosed[35]. This effect has been confirmed in other models of autoimmune diseases, such as experimental autoimmune encephalomyelitis (EAE) in rats[42] and in mice[43]. This enhanced antibody activity at the height of autoimmune destruction is a unique and highly desirable characteristic of this antibody therapy with important clinical implications. The identification of individuals with autoimmune diseases occurs in most clinical settings once significant tissue destruction has occurred after the onset of disease.

Throughout these preclinical studies, it became increasingly apparent that the long-term therapeutic effect of FcR-non-binding CD3-specific monoclonal antibodies in NOD mice could not be explained solely by their capacity to eliminate and/or inactivate pathogenic T cells. Although complete clearing of insulitis occurred within the first 24–48 hours of treatment, concomitant with a rapid return to normal glycaemia, it was not long-lasting[34,44]. Two weeks after the last injection, immune-cell infiltration of the pancreas recurs but the cells remain confined to the periphery of the pancreatic islets, with no clear signs of active destruction of β-cells[34,44]. A transient polarization towards T_H2 cells is observed during this first phase of cell re-infiltration. This is in keeping with our previous *in vitro* studies showing that FcR-non-binding CD3-specific monoclonal antibodies have a differential effect on T_H1 cells compared with T_H2 cells. As mentioned above, the partial signalling induced in T_H1 cells by an FcR-non-binding CD3-specific monoclonal antibody results in unresponsiveness. By contrast, treatment of T_H2 cells with the antibody does not render them unresponsive; they secrete IL-4 and are able to proliferate[31]. However, this transient T_H2-cell polarization is not essential for the long-term antigen-specific effect of the FcR-non-binding CD3-specific antibody, since antibody-induced disease remission was observed in NOD mice deficient in IL-4[45]. By contrast, increasing evidence suggests that the long-term effect of FcR-non-binding CD3-specific antibody therapy results as a consequence of a significant increase in the number of transforming growth factor-β (TGFβ)-dependent adaptive regulatory T cells, as well as an increased sensitivity of pathogenic T cells to the effects of regulatory T cells[45–47] (FIG. 1b).

It is important to stress that the effect of FcR-non-binding CD3-specific antibodies can be seen effectively as a *de facto* antigen-specific therapy as it acts selectively on autoantigen-specific activated T cells while sparing and/or inducing antigen-specific regulatory T cells at the site of inflammation. Of course, there is the increased risk of untoward side effects during the therapeutic window if the patients are exposed to invading pathogens, especially in the target tissue during the antibody treatment.

Disease progression in humans. Therapeutic interventions in type 1 diabetes would be more effective if we better understood the differences in the progression of disease among individuals. It is likely that disease progression in type 1 diabetes is not a linear process like in other autoimmune diseases (FIG. 2). Therefore, in some individuals, the disease may progress from a benign stage with self-limiting β-cell destruction to an overwhelming destruction of β-cells. In other individuals, the disease may take on a relapsing–remitting phenotype that may or may not lead to significant destruction of the target β-cell mass and ultimate disease manifestation (overt hyperglycaemia). Alternatively, the autoimmune response may be chronically progressive. Thus, it will be essential that we have a better understanding of the disease progression to enhance the 'therapeutic window' for developing drugs such as FcR-non-binding CD3-specific monoclonal antibodies. For instance, our data from studies in NOD mice showed that FcR-binding CD3-specific antibodies were most effective at the time of disease onset and not in very young 4–8 week-old mice, in which there are no signs yet of a destructive cell infiltration[35]. However, this may not translate similarly to humans, as pancreatic-islet-cell destruction may proceed for years before clinical diagnosis, making treatment in pre-diabetic individuals more likely to be successful. Therefore, there is an urgent need to develop better imaging techniques and biomarkers to detect autoimmune β-cell destruction as soon as it occurs to allow for earlier intervention when the greatest pancreatic islet mass exists.

Human clinical trials. In the year 2000, two trials were launched using two different humanized FcR-non-binding antibodies specific for human CD3 (REFS 48,49) that constituted an important example of successful clinical

translation. In the US-based Phase I and II clinical trial, patients with type 1 diabetes received an FcR-non-binding CD3-specific monoclonal antibody, known as hOKT3γ1 Ala-Ala (teplizumab), for 2 weeks at the time of disease onset[26,28]. In this trial, teplizumab, which was administered without other immunosuppressants, halted the progression of disease for more than 1 year in most of the patients in the trial, and even 3 years after the treatment patients that received the antibody showed a continued benefit in terms of connecting-peptide (C-peptide) production and use of insulin compared with the control group[28]. Significant increases in the amount of IL-10 and IL-5 in the serum were observed in approximately two-thirds of treated patients. Both IL-10-producing $CD4^+$ and $FOXP3^+CD8^+$ regulatory T-cell populations were observed *in vivo* after drug treatment[50].

Another multicentre Phase II placebo-controlled clinical trial based in Europe included 80 patients and used the FcR-non-binding CD3-specific antibody ChAglyCD3 (REF. 27). Eight milligrams of ChAglyCD3 or placebo were administered daily to 40 patients per group for 6 consecutive days. ChAglyCD3 treatment preserved

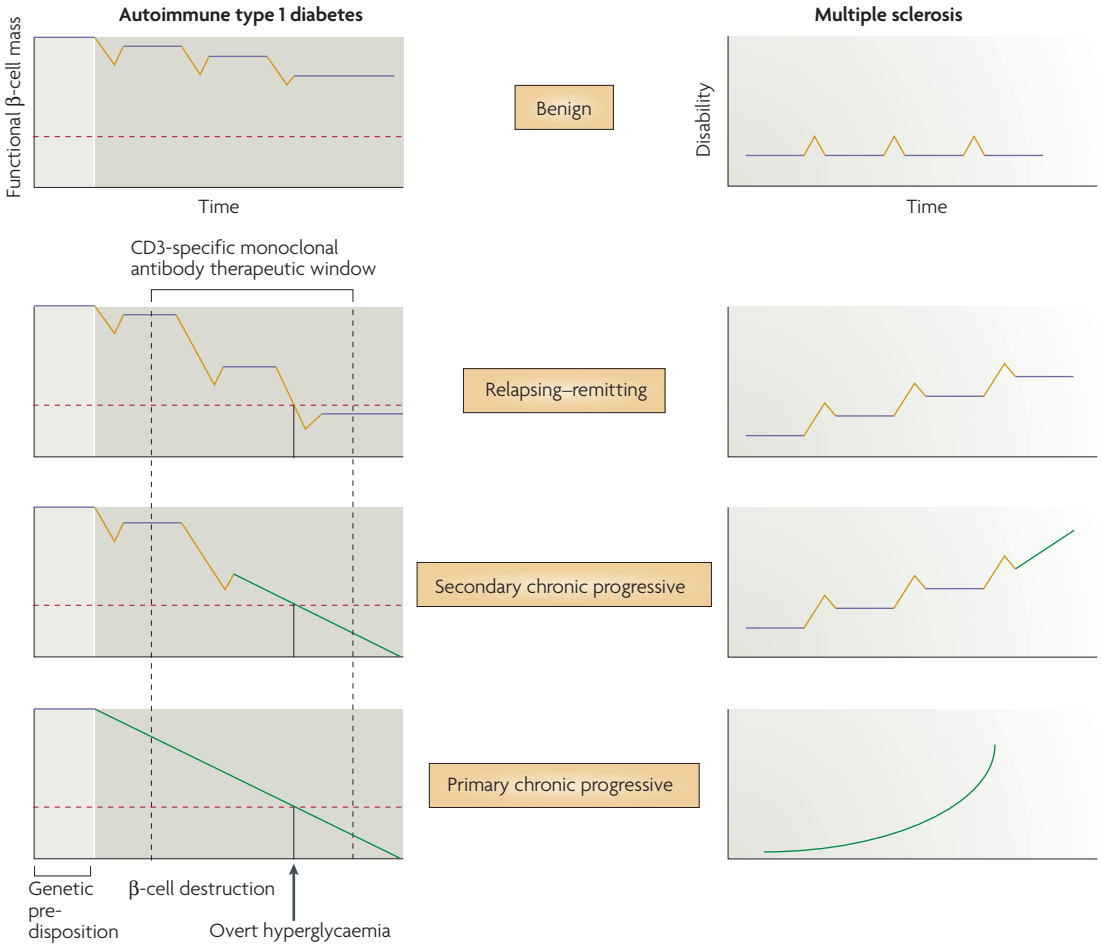

Figure 2 | **Potential therapeutic window for CD3-specific monoclonal antibody in type 1 diabetes.** Autoimmune type 1 diabetes is a chronic disease leading to massive destruction of insulin secreting β-cells. It is well established that once hyperglycaemia and glycosuria appear, about 70% of the functional β-cell mass has been destroyed[97]. This ultimate stage is preceded by a relatively long phase, which is usually termed 'pre-diabetic' and characterized by the presence of β-cell-specific autoantibodies, which although non-pathogenic are good markers of ongoing β-cell destruction. Multiple models of progression towards the stage of overt metabolic disturbance may be envisioned, although the lack of reliable techniques to quantify β-cell mass prevent us from obtaining direct proof for the existence of such different patterns. By analogy to what is known for multiple sclerosis, the immunological insult may occur according to relapsing–remitting kinetics or may be present as a chronically progressive form. Within such heterogeneity one may contemplate that some individuals with type 1 diabetes, in whom the immunological attack(s) is(are) self-limiting, will present a clinically 'benign' form of the disease and never progress to the point of irreversible metabolic dysfunction. Intervention with a CD3-specific monoclonal antibody would be best initiated at the time of disease flare — during tissue destruction — when functional β-cell mass preservation (and therefore good metabolic reconstitution) can be maximized. This is in keeping with our data in non-obese diabetic (NOD) mice in which CD3-specific monoclonal antibody treatment was most effective at the time of ongoing β-cell destruction and was totally ineffective in 4–8 week-old NOD mice that showed peripheral non-invasive or non-destructive insulitis[35]. The red dotted line indicates the level of β-cell mass under which overt hyperglycaemia will occur.

Connecting peptide
(C-peptide). Insulin is synthesized by β-cells as a hormone precursor pro-insulin. When released from the pancreas into the blood pro-insulin is cleaved into insulin and a small peptide known as C-peptide. C-peptide can be used as a measure of endogenous insulin secretion (one C-peptide is released for each insulin molecule secreted).

β-cell function very efficiently, maintaining significantly higher levels of endogenous insulin secretion (as assessed by C-peptide levels after controlled intravenous glucose stimulation) compared with the placebo group at 6, 12 and even 18 months after treatment[27]. Importantly, this led to a significant decrease in the insulin requirement of the patients for at least 18 months after the single course of ChAglyCD3 administration[27]. 75% of the subset of patients with the high β-cell mass (higher than the median value of the whole population) required insulin doses of ≤ 0.25 U per kg per day at 18 months — a dose requirement that is compatible with clinical insulin independency[27].

Improving CD3-specific antibody therapy

Improving safety. FcR-non-binding CD3-specific monoclonal antibodies that are presently used in the clinic exhibit dramatically reduced side effects induced by T-cell activation after the first dose, as compared with FcR-binding CD3-specific antibodies such as OKT3. However, some degree of T-cell activation is still observed that leads to limited cytokine release and, therefore, to minor acute side effects[26,27,51,52]. The symptoms mainly include moderate fever, headaches and self-limiting gastrointestinal manifestations, all of which are amenable to palliative treatment. In addition, these symptoms did not hamper normal enrolment of the patients in the trials or necessitate a particular pretreatment other than the antihistamine diphenhydramine hydrochloride (Benadryl; Pfizer, Inc.) and/or the anti-inflammatory agent ibuprofen. However, for obvious reasons, as the therapeutic opportunities expand it may be important to completely prevent the cytokine-related symptoms. It is possible that other variant CD3-specific antibodies may be developed that can reduce the T-cell activation even further or that pre-medication, such as a single administration of high-dose corticosteroids or tumour-necrosis factor (TNF)-specific antibodies might be beneficial[53–56]. However, experimental data suggest that some level of T-cell activation may be essential for efficacy and corticosteroids (or other immunosuppressives that directly affect TCR signalling) may interfere with the tolerogenic effects of these biological agents[35].

In the European trial, in patients who had already experienced a primary infection with Epstein–Barr virus (EBV) a transient viral reactivation was observed 10–20 days after the first CD3-specific monoclonal antibody injection (as assessed by an increase in EBV copy number in peripheral-blood mononuclear cells)[27]. In terms of safety, it is important to highlight that this viral reactivation was transient and did not involve viruses other than EBV and never recurred in any of the patients who had follow-up check-ups (4–7 years). In all patients EBV copy numbers returned to normal baseline pretreatment numbers owing to an efficient humoral and cellular immune response specific for EBV that developed within 1–3 weeks following the end of the antibody treatment. In particular, a significant proportion of $CD8^+$ T cells that were specific for peptides expressed during the lytic cycle of EBV were detected in conjunction with a $CD8^+$ lymphocytosis that peaked by 3–4 weeks after the first antibody injection and then progressively decreased. Although the mechanisms responsible for this EBV reactivation are still ill-defined, there is some evidence to suggest that they may be related to the dose of the antibody used, as EBV reactivation was only reported in the European-based clinical trial that used higher cumulated doses of the CD3-specific monoclonal antibody than the US-based clinical trial did. These results suggest that the effect of CD3-specific monoclonal antibody therapy in patients is autoantigen-specific and that it does not suppress protective immunity to at least some viral antigens. Therefore, due to the fact that the therapies that use FcR-non-binding CD3-specific monoclonal antibodies as single agents are very short, over-immunosuppression is not a safety concern, at least at present. It is relevant to highlight this point as it differs completely from the broader experience in transplantation settings with FcR-binding CD3-specific monoclonal antibodies such as OKT3 that are used together with broad spectrum immune suppression, and in all the protocols presently used in autoimmunity, which include biological agents administered either on a more long-term basis or in repeated courses. However, this situation may change as combination strategies are envisaged.

After treatment with an FcR-non-binding CD3-specific monoclonal antibody about 40% of the patients develop anti-idiotypic antibodies[26–28]. In most patients these antibodies disappear by 6 months and only a small percentage of patients continue to produce them long term. Despite their potential neutralizing capacity, anti-idiotypic antibodies do not represent a problem for CD3-specific antibody treatment, as they appeared by 2–3 weeks after the last dose of drug was injected. However, as we shall discuss later, anti-idiotypic antibodies may represent a potential problem if repeated treatment is needed.

Compensating for initial absence of or insufficient response to treatment. Although the CD3-specific antibody therapy was effective overall in preventing or even reversing established diabetes progression, the effects of the therapy varied among individuals. Some patients failed to recover endogenous C-peptide secretion from the very beginning of the treatment, whereas in other patients the initial response was not sustained. In the European-based clinical trial, the patients who exhibited the best response were those that had a higher functioning β-cell mass at the beginning of treatment (4 weeks post-diagnosis)[27]. It is well known that T-cell-mediated autoimmune aggression begins months to years before the appearance of overt diabetes (that is when hyperglycaemia and glycosuria appear due to approximately 70% of the β-cell mass having been destroyed or rendered non-functional). Therefore, drug treatment earlier in the course of the disease would probably result in improved outcomes (FIG. 2). Unfortunately, routine assays to detect pathogenic islet-specific T cells or to quantify β-cell mass using non-invasive methods are still lacking. However, epidemiological studies have shown that autoantibodies in siblings of patients with type 1 diabetes reflect ongoing β-cell destruction in these siblings, with

Lymphocytosis
An increase in the number of lymphocytes in the blood, which is usually associated with chronic infections or inflammation.

Anti-idiotypic antibody
An antibody that is directed against the antigen-specific binding site of an immunoglobulin or a T-cell receptor and therefore may compete with antigen for binding.

60–80% of the subjects that have at least two circulating autoantibodies progressing to overt diabetes within 5–7 years[57]. Therefore, while the field awaits better surrogate T-cell and β-cell markers to allow for earlier intervention, continued treatment of new disease onset and high-risk pre-diabetic patients should be encouraged.

Recurrence of β-cell dysfunction. Although the effectiveness of FcR-non-binding CD3-specific monoclonal antibody therapy in preventing or even reversing disease progression is clear, the effect may not be permanent in many individuals. In the European-based clinical trial, detailed analysis of the data collected after 18 months showed a continued stability of insulin production. However, in the US-based clinical trial, although patients exhibited a statistically significant improvement in insulin secretion even years after treatment compared with pretreatment levels, a significant percentage of patients showed a decline in insulin production by 2 years[28]. The difference between the two studies may reflect the doses used or the fact that the US-based clinical trial included a majority of 'younger' patients, who generally exhibit more aggressive disease progression and are likely to have a more functional thymus that is able to regenerate an autoimmune repertoire. Therefore, it may be important in the future to better adapt the treatment based on the age of the patients; in younger subjects the treatment may need to be instituted earlier than in adult subjects.

One other way to potentially overcome the decline in insulin production is to repeat the CD3-specific antibody treatment. However, caution is needed owing to neutralizing anti-idiotypic antibodies that develop after the first course of CD3-specific monoclonal antibody, even if they have disappeared at the time of re-treatment. Perhaps a short course of conventional immunosuppressants might be given in association to the first course of monoclonal antibody to efficiently block sensitization. A clinical trial to test re-treatment with CD3-specific monoclonal antibodies is currently underway through the support of the Immune Tolerance Network.

In addition to the possible recurrence of autoimmunity, data suggest that patients with a limiting functional β-cell mass may experience an increase in their insulin needs in spite of continued endogenous insulin-secreting capacity. This may occur due to increased insulin resistance, as previously reported in cyclosporin-treated patients in whom hyperglycaemic episodes led to a progressive decline of C-peptide levels owing to glucose toxicity of the β-cells[58,59]. Therefore, an aggressive strategy to achieve optimal metabolic control using intensive insulin treatment and drugs aimed at decreasing insulin resistance (regularly used for the treatment of type 2 diabetes) is likely to improve the efficacy of FcR-non-binding CD3-specific antibody therapy. In addition, newer drugs that promote β-cell survival and growth, such as incretin hormones — glucagon-like peptide-1 (GLP1) or its related mimic, exendin-4 (Byetta; Amylin Pharmaceuticals, Inc. and Eli Lilly and Company) — may have additive or synergistic effects[60]. These drugs augment glucose-stimulated insulin release, decrease levels of glucagon, and may increase β-cell replication, decrease apoptosis of β-cells, and stimulate β-cell neogenesis. Indeed, Ogawa *et al.* found that treatment of mice with a combination of anti-lymphocyte serum (ALS) and exendin-4 enhanced the reversal of diabetes in hyperglycaemic NOD mice, compared with ALS treatment alone[61]. Similarly, the combination of exendin-4 and CD3-specific monoclonal antibody therapy led to a more effective reversal of spontaneous diabetes in NOD mice by enhancing the insulin content of pancreatic β-cells and their response to insulin secretagogues, as compared with treatment with either the agent alone (K. Herold, J.A.B. and colleagues, personal communications). These promising results have led to support for a Phase II clinical trial, sponsored by the National Institutes of Health, USA, in which patients with newly onset type 1 diabetes will be treated with a combination of exendin-4 and the FcR-non-binding CD3-specific monoclonal antibody teplizumab.

Combinations with other immunotherapies

One of the lessons learned from the development of cancer therapies is the value of combination therapies. Therefore, it is possible that combination immunotherapies might be the most effective therapy to reverse autoimmunity and maintain immune tolerance by taking advantage of synergistic effects between the therapies, which may allow for a decrease in the dosage of each individual drug used. Of course, important prerequisites for establishing combination regimens will be to define the optimal and suboptimal antibody dose and the dose regimen. In addition, it will be crucial to use the panels of early efficacy biomarkers and of prognosis markers to define the optimal therapeutic windows for administration of the drugs, which will allow for optimal effectiveness with minimal side effects.

The options are virtually unlimited, reflecting the increasing knowledge of the crucial importance of the multiple arms of the immune system in autoimmunity and the recent explosion of new drug entities as potential immunotherapeutics. A few candidates that are either already approved for use in therapeutic treatment and that are being tested in clinical trials of type 1 diabetes or show promise as combination therapies, are described here.

Antigen-based therapy. As previously discussed, tolerogenic administration of autoantigens, such as insulin or myelin basic protein, would represent the ideal means to re-establish self-tolerance. The multiple activities of FcR-non-binding CD3-specific monoclonal antibody treatment suggest that combining this therapy with the administration of an autoantigen might be a robust approach to induce antigen-specific tolerance. First, the capacity of CD3-specific antibodies to rapidly 'freeze' the ongoing autoimmune process may lead to pro-tolerogenic effects. When antigen-specific pathogenic T cells come in contact with the immunomodulatory antibodies, the TCR–CD3 complex undergoes rapid internalization from the cell surface. Upon TCR re-expression and exposure to the autoantigen, an 'altered' TCR signal causes the T cells to die, become anergic or even change from pathogenic to regulatory T cells. Moreover, the suppressive milieu

Secretagogues
Molecules, often peptides, that stimulate the secretion of a variety of substances including hormones and enzymes.

created by CD3-specific therapy (such as the production of 'suppressive' cytokines and the enhancement of regulatory T cells around the target tissue) may ensure fidelity of tolerance induction by the autoantigen or even redirect antigen-specific responses towards tolerance (FIG. 1). Based on these hypotheses, a study by Von Herrath, Herold, Bluestone and colleagues examining the treatment of mice with an FcR-non-binding CD3-specific monoclonal antibody together with intranasal proinsulin peptide has been carried out[62]. The results of this study showed that the combination therapy reversed recent onset diabetes in two mouse models of diabetes with higher efficacy than monotherapy with suboptimal doses of CD3-specific antibody or with antigen alone. Moreover, the activity of insulin-specific $FOXP3^+CD4^+CD25^+$ regulatory T cells was strongly enhanced and transfer of these cells resulted in dominant tolerance in immune competent recent-onset diabetic recipients[62]. These cells were found to secrete IL-4, IL-10 and TGFβ, consistent with the induction of an antigen-specific adaptive regulatory T-cell subset. This strategy is now being pursued clinically in a trial sponsored by the Juvenile Diabetes Research Foundation with the goal of enhancing specificity and reducing the risk for side effects in immune interventions in recent-onset diabetes.

Targeting B cells. B cells have emerged as an important target for the treatment of autoimmune diseases, fuelled by the recent success of CD20-specific monoclonal antibody rituximab (Rituxan; Genentech, Inc. and Biogen Idec) therapy for rheumatoid arthritis. The rationale for this treatment approach was based on the hypothesis that B-cell depletion using rituximab would remove the pathological autoantibodies and eliminate a potentially potent antigen-presenting cell population. Testing rituximab, as well as drugs that target other pathways that regulate B-cell survival, such as B-cell-activating factor (BAFF; also known as BLYS), may be another means to induce B-cell tolerance. These drugs include the BAFF-specific antibodies belimumab (LymphoStat-B; Human Genome Sciences), BR3-Fc (Genentech Inc.) and atacicept (formerly known as TACI-Ig; ZymoGenetics). Based on the complementary nature of B-cell and T-cell activities in autoimmunity (the privileged role of B cells as autoantigen-presenting cells is now well established[63]), combination therapy with CD3-specific monoclonal antibodies and B-cell targeting drugs will probably synergize for the induction of immune tolerance. Needless to say, particular caution will be needed for these types of association trials, as one cannot certainly minimize the potential impact resulting from the combined targeting of B cells and T cells. Combinations in which a very objective definition of the optimal and suboptimal doses and regimes to be used for each of the antibodies, which may vary depending on the autoimmunity condition being treated, will be key in determining success.

Co-stimulation blockade. Stimulation of T cells through the TCR complex in the absence of co-stimulatory factors, most prominently represented by the CD28–B7 interactions, results paradoxically in T-cell inactivation, death or altered function[64]. Aside from the crucial biological implications of co-stimulation, the identification of co-stimulatory signals had important implications for clinical intervention, as the effects of co-stimulation blockade would be restricted to only those T cells of which the antigen-specific receptors have been engaged (that is T cells that have already received the first activation signal (signal 1)). Most interestingly, the CD28 homologue cytotoxic T-lymphocyte antigen 4 (CTLA4) is a high-affinity receptor of the same gene family that provides a potent downregulatory signal to T cells. Based on these basic concepts, the first approved co-stimulatory blocking agent abatacept (Orencia; Bristol–Myers Squibb) was developed, which is a CTLA4–immunoglobulin fusion protein that is efficacious in treating psoriasis[65,66] and refractory rheumatoid arthritis[67–70]. Many of the signalling and functional attributes of abatacept complement CD3-specific monoclonal antibody immunotherapies, as the combination would interfere with both pathways induced by signal 1 and signal 2. Moreover, as discussed earlier, one of the main effects of CD3-specific antibodies is the induction of adaptive regulatory T cells. This inductive capability has been observed in CD28-deficient mice[47], suggesting that it is independent of CD28–B7 co-stimulation and, thus, the combination therapy of CD3-specific antibody and abatacept is likely to be complementary.

The CD40–CD154, OX40–CD134 and inducible T-cell co-stimulator (ICOS)–ICOS-ligand pathways are all potential candidates for combination therapy with CD3-specific antibodies, although to date none of these co-stimulation pathways have been successfully developed clinically.

Pro-inflammatory antagonists. Although not directly tolerogenic in their own right, a number of anti-inflammatory therapies may be helpful in promoting tolerance induction by CD3-specific monoclonal antibodies or other similar agents. The ability of any drug to induce tolerance is likely to be affected by the cytokine, chemokine and cellular milieu at the site of inflammation. Multiple studies have shown that induction of tolerance is most difficult during inflammatory responses. Thus, drugs such as TNF antagonists, developed for the treatment of autoimmune diseases including rheumatoid and psoriatic arthritis, ankylosing spondylitis and Crohn's disease[71], may enhance the efficacy of CD3-specific antibody therapies. Similarly, an IL-1 receptor antagonist[72,73], Toll-like receptor antagonists and other anti-inflammatory agents, such as α_1-antitrypsin[74] and prostaglandin E_2 (REF. 75), may be useful adjuvant therapies. The mechanism of action of these drugs include the blocking of cell-migration to inflammatory sites in response to soluble mediators and the enhancement of the numbers and function of regulatory T cells at these sites, which makes these adjunctive therapies attractive in augmenting and/or sustaining CD3-specific monoclonal antibody activity. One other effect of these therapies might be to reduce cytokinaemia, fever and rashes that are observed when the highest doses of CD3-specific antibodies are used[55,56]. However, these non-specific drugs also have there own toxicities, including increased

Rheumatoid arthritis
An immunological disorder that is characterized by symmetrical polyarthritis, often progressing to crippling deformation after years of synovitis. It is associated with systemic immune activation, with acute-phase reactants being present in the peripheral blood, as well as rheumatoid factor (immunoglobulins specific for IgG), which forms immune complexes that are deposited in many tissues.

Crohn's disease
A form of chronic inflammatory bowel disease that can affect the entire gastrointestinal tract, but is most common in the colon and terminal ileum. It is characterized by transmural inflammation, strictures and granuloma formation, and is believed to result from an abnormal T-cell-mediated immune response to commensal bacteria.

Adjuvant
An agent that is mixed with an antigen for the purpose of increasing the immune response to that antigen following immunization.

Table 2 | **Therapeutic CD3-specific monoclonal antibodies in other immune-mediated diseases**

Disorder	Preclinical			Clinical		
	Model	Antibody	Refs	Trial	Antibody	Refs
Inflammatory bowel disease	TNP–KLH-induced colitis in mice	Parenteral 145-2C11	85	Treatment of Crohn's disease	Visilizumab	88
Immune-mediated neurological diseases	EAE in rats	Parenteral G4.18 (a mouse monoclonal antibody specific for rat CD3)	42	No clinical trials have been carried out to date		
	EAE in mice	Parenteral 145-2C11 or 145-2C11 F(ab′)$_2$	43			
		Oral 145-2C11 or 145-2C11 F(ab′)$_2$	83			
Immune-mediated inflammatory arthritis	Collagen-induced arthritis in mice	Parenteral 145-2C11 F(ab′)$_2$	84	Treatment of psoriatic arthritis	Teplizumab	86
Organ transplantation rejection	Induction of tolerance to heart transplants in rats	Parenteral G4.18	81,82	Treatment of acute renal allograft rejection	Teplizumab	51
				Prevention of pancreatic islet allograft rejection	Teplizumab	94
				Treatment of acute renal allograft rejection	ChAglyCD3	52
GVHD	Prevention of GVHD	Parenteral 145-2C11 or 145-2C11 F(ab′)$_2$	95,96	Treatment of steroid-refractory acute GVHD	Visilizumab	89

EAE, experimental autoimmune encephalomyelitis; GVHD, graft-versus-host disease; TNP–KLH, 2,4,6-trinitrophenol-conjugated keyhole limpet haemocyanin.

susceptibility to infections, which must be taken into account when considering combining multiple immunosuppressive therapies.

Rapamycin. Rapamycin (Sirolimus; Rapamune; Wyeth) binds to FK506-binding protein 12 (FKBP12), a cytosolic protein that regulates the mTOR (mammalian target of rapamycin) pathway, which is a crucial regulator of IL-2 signalling leading to T-cell proliferation but not to T-cell apoptosis[76]. It has been speculated that rapamycin might shift T-cell activation from the induction of proliferation to the induction of apoptosis, thereby deleting autoreactive T-cell clones and preventing development of type 1 diabetes[77]. A number of studies have also reported that regulatory T cells are uniquely resistant to rapamycin, such that combination therapy of rapamycin and regulatory T-cell activating therapy could act synergistically[78,79]. This has led to the hypothesis that a combination of CD3-specific monoclonal antibodies and rapamycin would delete T_H1-cell-like β-cell-specific autoreactive T cells and simultaneously promote β-cell-specific regulatory T-cell development. A clinical trial sponsored by the Immune Tolerance Network, is underway to test a combination of rapamycin and IL-2, on the basis of promising preclinical experimental data[80]. The completion of these studies might provide safety data for combining a CD3-specific monoclonal antibody and rapamycin in the future.

CD3-specific antibody therapy for other diseases

T-cell-dependent autoimmune attack has been shown to contribute to several autoimmune and inflammatory diseases. Therefore, it is not surprising that CD3-specific monoclonal antibodies are being tested in a variety of diseases other than type 1 diabetes (TABLE 2). In mouse models, CD3-specific antibodies have been used to prevent heart allograft rejection[81,82], acute graft-versus-host disease[95,96], EAE[43,83] and collagen-induced arthritis[84] and induce specific tolerance in several settings. Encouraging data have also been reported in inflammatory bowel disease (2,4,6-trinitrophenol-conjugated keyhole limpet haemocyanin (TNP–KLH)-induced colitis)[85]. Also in this model, as in diabetic NOD mice, protection was abolished by *in vivo* blockade of TGFβ using a specific neutralizing antibody; TGFβ-producing T cells were present in the intestinal mucosa of protected mice[85]. In humans, a Phase I and II clinical trial of patients with psoriatic arthritis showed that the FcR-non-binding CD3-specific monoclonal antibody teplizumab reduced joint inflammation and pain in patients with advanced disease[86]. In addition, a trial is ongoing in patients with ulcerative colitis and there are planned trials in patients with multiple sclerosis and psoriasis and in organ transplantation. Finally, Plevy and colleagues[87] described remarkable initial results with the administration of the CD3-specific monoclonal antibody visilizumab (Nuvion; PDL BioPharma Inc.) to patients with severe, steroid-refractory ulcerative colitis[88]. Visilizumab was also reported to be effective in treating steroid-refractory acute graft-versus-host disease[89]. Most importantly, serious side effects or infectious complications were not observed in these trials. Future trials will continue to explore optimal dosing regimens, long-term efficacy, and the potential risks of CD3-specific monoclonal antibody therapies.

Concluding remarks

In recent years, several potentially important biological agents have been used in the treatment of clinical autoimmune diseases. However, in several cases continuous treatment was required to maintain efficacy, which thereby prolonged exposure of patients to the risks of overuse of immunosuppressants, leading to increased risk of infection — such as, the occurrence of mycobacterial infections with TNF-antagonist therapy[90,91] and JC virus infection with natalizumab (Tysabri; Biogen Idec and Élan) treatment[92,93].

At variance with these therapies, CD3-specific antibodies afford long-term effects following a short administration — a capacity that is directly linked to their tolerogenic properties in the experimental setting. The present challenge is to build on that experience, first, to set the use of CD3-specific monoclonal antibodies as an established therapy in well-selected subsets of patients with autoimmune diabetes; second, to adapt CD3-specific monoclonal antibody treatment to other autoimmune disorders in which it could also prove beneficial; and third, to use CD3-specific monoclonal antibody therapies in combination with other treatments for increased efficacy. Learning how to combine autoantigen therapy or adjunct pancreatic-islet-cell growth and survival factors are just two potential avenues to allow a safe and prolonged CD3-specific monoclonal antibody therapeutic effect.

Finally, OKT3 was originally developed for the treatment of kidney allograft rejection. The opportunities to treat organ transplant recipients with this safer therapy, especially patients highly sensitized to a target organ, are realistic. In these instances, we may settle for a more operational definition of disease 'cure' — the low-risk induction of immune homeostasis to control the pathological process at a late stage of rejection.

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Competing interests statement
The authors declare competing financial interests: see web version for details

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