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REVIEW ARTICLE

NON-FcR-BINDING ANTI-CD3 MONOCLONAL ANTIBODIES FOR THE TREATMENT OF TYPE 1 DIABETES

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SUMMARY

Teplizumab and otelixizumab are non-FcR-binding anti-CD3 monoclonal antibodies (mAbs) that have been tested in early clinical trials for the treatment of recent-onset type 1 diabetes. They act to arrest the autoimmune destruction of pancreatic islet β-cells, which leads to the preservation of endogenous insulin production. Clinical trials of teplizumab and otelixizumab are ongoing to investigate their use for maintaining insulin responses. It is hoped that in the future non-FcR-binding anti-CD3 mAbs may be used as single agents or in combination with other immunomodulating therapies with insulin at the onset of hyperglycemia to improve clinical outcomes.

INTRODUCTION

Type 1 diabetes is one of the most common severe, chronic autoimmune diseases, characterized by the loss of self-tolerance to pancreatic islet β-cells, leading to the insidious loss of β-cells, resulting in hyperglycemia at diagnosis and, eventually, absolute insulin deficiency (1). In the U.S. there is an annual incidence of 30,000 new cases with a prevalence of 1 in 300 children and 1 in 100 adults (2). The incidence of type 1 diabetes is rising rapidly in children, with a predicted increase of 70% over the next 15 years in Europe. The age of onset is also decreasing, with a predicted doubling of cases in children under the age of 5 years during the same period (3). Current therapy with insulin has significantly improved since first introduced 88 years ago with the development of human insulin analogues, insulin pumps, continuous glucose monitoring and educational programs. At the same time, cardiovascular risk modification, close follow-up and treatment of early diabetic eye and kidney disease have improved outcomes (4). Despite these interventions, type 1 diabetes remains a lifelong chronic disease that reduces life expectancy by 10-15 years due to microvascular and macrovascular complications (5).

Over the last decade considerable clinical and experimental progress has been made in the understanding of the immunopathogenesis of type 1 diabetes (6). The etiology of insulin deficiency in type 1 diabetes is a loss of self-tolerance to pancreatic β-cells with the emergence of autoreactive immune cells. Treatment with non-FcR-binding anti-CD3 monoclonal antibodies (mAbs) aims to restore self-tolerance by downregulation of autoimmune responses to pancreatic islets (2). This knowledge of the mechanisms of disease and the effects of anti-CD3 mAbs has now been translated initially into phase I/II clinical trials to determine the efficacy of non-FcR-binding anti-CD3 mAbs in patients with type 1 diabetes, as well as a placebo-controlled phase II trial (7, 8). The success of this treatment in the preservation of β-cell function has led to the development of phase III trials, the DEFEND-1 and Protégé trials (9, 10). These trials of otelixizumab and teplizumab, respectively, in recently diagnosed diabetes have now completed accrual. This review focuses on our current understanding of the immunopathogenesis of type 1 diabetes and the use of non-FcR-binding anti-CD3 mAbs for treatment.

IMMUNOPATHOGENESIS OF TYPE 1 DIABETES

The development of type 1 diabetes is thought to be a result of environmental exposures combined with inherited susceptibility. Recent longitudinal studies of a large cohort of monozygotic twins initially discordant for type 1 diabetes has found that 65% of twins ultimately develop type 1 diabetes, and with even longer follow-up, the proportion of discordant twins becomes even smaller, demonstrating that previous studies have underestimated the genetic contribution to the development of the disease (11). Genetic susceptibility to diabetes is largely conferred by the inheritance of the HLA-DR and DQ

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haplotypes located within the major histocompatibility complex (MHC) on chromosome 6p21. These genes encode the MHC I and II molecules that present to T cells (12). From genome-wide association studies, a further 25 non-HLA-associated loci have been identified that contribute additional susceptibility to diabetes. The vast majority (n = 22) of these genes encode proteins that are involved in immune function and regulation (13). The large number of modifier genes means that, apart from monozygotic twins, the likelihood that any two patients with diabetes share identical susceptibility traits is quite low. This also raises the likelihood that there are several different autoimmune pathways that may lead to the final loss of tolerance to pancreatic islet β-cells in humans (14). This has important implications for the development of strategies to target immunomodulating therapy to individuals with autoimmune type 1 diabetes, since it may be necessary to target multiple immune pathways and a single approach may not be effective in all patients.

In humans, the diagnosis of type 1 diabetes is usually preceded by a chronic autoimmune prodrome that may be monitored by autoantibody responses (15). The autoantibodies themselves are not directly pathogenetic, but are biomarkers of ongoing autoimmunity (16). The development of insulin antibodies in susceptible individuals may be detected in individuals as young as 6-12 months of age and may predate the development of type 1 diabetes by up to 10 years (17). The age of development of autoimmunity in type 1 diabetes is related to the degree of genetic susceptibility, with individuals with a high genetic susceptibility developing autoimmunity prior to the age of 5 years (18). Progression of the autoimmunity leads to the evolution of autoimmune responses, with the emergence of antibodies to 65 kDa glutamic acid decarboxylase (GAD-65), tyrosine phosphatases, islet cell antibody-2 (IA-2), insulin autoantibodies (IAAs) and zinc transporter 8 (ZnT-8) (19-21). The risk of developing diabetes is related to the number of autoantibodies, suggesting that progression of the disease is associated with spread of the autoantigenic repertoire (22, 23). In a large prospective trial of diabetes prevention, individuals positive for a single antibody had approximately a 6% 5-year risk for the development of type 1 diabetes, while those with four antibodies had a > 90% 6-year risk of diagnosis (22, 24). The combination of metabolic and autoantibody tests can now identify individuals with a 75% or greater 6-year risk for the development of type 1 diabetes in relatives of patients with the condition (25-28). More recently, additional anti-islet autoantibodies have been identified, such as anti-ZnT-8, which may further refine predictive algorithms (21).

HUMAN DIABETOGENESIS

At diagnosis, the immune-mediated destruction within the islet is highly specific, only affecting the pancreatic islet β-cells and not the α- and δ-cells (29). In contrast to mouse models of type 1 diabetes, the insulitis in humans always appears destructive (30). The insulitis in patients with recently diagnosed type 1 diabetes is characterized by a predominance of $CD8^+$ cytotoxic T cells and macrophages (31). With progression of the insulitis, $CD20^+$ B cells are recruited, but the vast majority of these cells do not secrete antibodies (32, 33). Once the islet β-cells have been destroyed, the immune infiltrate resolves (34). One to two years after diagnosis of type 1 diabetes, the autoimmune response in the pancreatic islets appears to regress once the active pathogenetic process of β-cell destruction has been completed (35). However, autoreactive T memory cells may still be present in circulation, as evidenced by the immune-mediated destruction of islet grafts in humans (36, 37).

IMMUNOTHERAPY TREATMENT STRATEGIES AND OUTCOME MEASURES

Immunoprevention aims either to prevent the emergence of autoimmunity (primary) or to reverse it (secondary) prior to the development of symptomatic type 1 diabetes. Primary immunoprevention involves the identification of individuals who are predisposed to the development of type 1 diabetes prior to the initiation of immune activation to pancreatic islet antigens. This is identified in humans by autoantibodies. Clinical trials of primary immunoprevention have involved the protective immunization with antigens or supplementation of nutrients that are thought to be important for the development of autoimmunity, often in individuals with high-risk HLA types (38). Secondary immunoprevention trials also involve the identification of individuals with high-risk HLA types, but also with evidence of islet autoimmunity, as confirmed by autoantibodies (39). Antigen-specific therapies are considered suitable for secondary immunoprevention trials since they have a suitable risk profile in asymptomatic participants (40).

Immunoreversal aims to intervene at the time of diagnosis to reverse autoimmunity and preserve residual β-cell function (41). The desired outcome of both strategies is to induce remission from autoimmunity by reestablishing therapeutic tolerance to pancreatic β-cells. Therapeutic tolerance refers to a state where autoimmunity is curtailed without the need for continuous immunosuppression (42, 43). Ideally, immunomodulating therapy would induce a prolonged remission from type 1 diabetes and achieve independence from exogenous insulin administration (44). A current, more realistic primary endpoint is to demonstrate prolongation of endogenous insulin production, as measured by C-peptide responses, given the evidence that this improves clinical outcomes in type 1 diabetes (45, 46).

The choice of agents to modify autoimmunity is determined by the disease stage of the individual balanced against the risk of side effects. Low-risk strategies such as the avoidance of cow's milk, gluten, vitamin D or a diet with n-3 fatty acids may be tested to prevent the primary emergence of autoimmunity, but their lack of potency as an immunomodulatory strategy suggests that they are not likely to be effective in reversing disease once hyperglycemia has appeared (47-50). Their safety would allow testing in a large population with some increased risk for disease, but would also be applicable to the general population. Medium-risk treatments could be tested for their ability to reverse established autoimmunity prior to the onset of diabetes. Examples that might be considered include protective immunization with antigens or brief treatment with immunomodulators that cause transient changes with lasting effects. Higher-risk treatments, such as non-FcR-binding anti-CD3 or anti-CD20 mAbs, have generally been reserved at the onset of diabetes to reverse the autoimmunity and preserve residual β-cell function (6). However, clinical experience with the use of non-FcR-binding anti-CD3 mAbs at diagnosis combined with the ability to predict the development of type 1 diabetes suggests that treatments that are effective at the time of onset will be tested in the near future to reverse autoimmunity prior to the development of diabetes (51).

ANTI-CD3 mAbs – TEPLIZUMAB AND OTELIXIZUMAB

Anti-CD3 mAb therapy is unique among immunomodulatory therapies in type 1 diabetes since it has the potential to reintroduce tolerance to pancreatic islet β-cell antigens after administration by expanding a population of T regulatory cells (Tregs) (52).

Preclinical studies in the NOD mouse demonstrated that treatment with a brief course of anti-CD3 mAb at the onset of diabetes induced a lasting remission by inducing tolerance (53, 54). In the NOD mouse, the immunomodulatory effects of anti-CD3 mAb treatment are biphasic. The first phase is of short duration and occurs at the commencement of treatment. There is a partial depletion of pathogenetic T cells, while the remaining T effector cells and Treg cells undergo immunomodulation. The second phase is long lasting and occurs after completion of treatment with restoration of tolerance to pancreatic β-cells and induction of a population of Tregs (53, 55, 56). This induction of tolerance requires activation of the Treg population and may be blocked by the T-cell inhibitor ciclosporin. The anti-CD3 mAb induced Tregs that were identified as $CD4^+CD25^+$ cells that mediated inhibition through a TGF-β-dependent mechanism (53, 55). These properties of tolerance induction in mice meant that there was no need for recurrent administration of anti-CD3 mAbs to achieve cure of diabetes in NOD mice (54).

The first-generation anti-CD3 mAb OKT3 was developed to treat solid organ rejection in humans (57, 58). OKT3 was clinically effective in treating acute rejection; however, its administration was associated with a severe cytokine release syndrome due to binding of the Fc portion of the Ig to Fc receptors and cross-linking of the T-cell receptor, leading to T-cell activation (59, 60). To overcome this problem and minimize the mitogenic potential, humanized CD3-specfic Fc-mutated mAbs have been produced to decrease the binding to Fc receptors. Both hOKT3g1 Ala-Ala (teplizumab, which has two alanine-for-leucine substitutions at positions 234 and 235) and chAglyCD3 (otelixizumab, which has an alanine substitution at position 297 of the Fc portion of the Ig molecule) have been tested (52).

Translation of these findings was achieved with the first reported human trial of teplizumab in patients with recent-onset diabetes. This clinical trial demonstrated that a single course of treatment led to improvement of C-peptide response for up to 2 years after diagnosis. Treated patients also had sufficiently improved metabolic control, with lower HbA1c and reduced insulin use (7, 61). Another clinical trial with otelixizumab found that at 18 months after treatment patients had maintained insulin secretion and had lower insulin requirements. The greatest treatment effects were seen in patients who had the highest endogenous insulin production at the time of entry into the trial (8). Subsequent follow-up has shown significant improvement in insulin use for 4 years after diagnosis and there is evidence for preservation of endogenous insulin production for up to 5 years after treatment (62, 63). Computer modeling analysis of the treatment of type 1 diabetes patients with a course of teplizumab suggests that patients may have an improved life expectancy as compared to conventional treatment (64).

Side effects have included mild cytokine release in a small proportion of subjects with the initial doses of the drug. Importantly, there is no evidence for sustained immune suppression. In the study of otelixizumab, Keymeulen et al. reported transient symptomatic reactivation of Epstein–Barr virus (EBV) in a high proportion of subjects (8). Within 7-21 days of treatment the number of copies of EBV had returned to pretreatment levels due to the development of cellular and humoral responses to EBV (65). The effective immune response to EBV in humans after treatment with otelixizumab demonstrates that reconstitution of immune responses to foreign antigens had occurred (66). A similar experience of symptomatic reactivation of EBV was not reported in studies of teplizumab, although the dosing of the two drugs may have been different and viral loads were not monitored. At later time points, there has not been evidence for loss of immune function in terms of titers to vaccines or other clinical parameters. The antibodies are as effective in metabolic terms as ciclosporin, without the same risk of renal toxicity and global persistent immune suppression (67-69).

In contrast to the preclinical mouse data, the improvement in metabolic and C-peptide responses in most patients was not permanent in human clinical trials (53). A recent small clinical trial of teplizumab in six drug-treated subjects found that higher doses increased side effects but not efficacy of the drug. The results of this trial concur with a recent study in the NOD mouse that found that low-dose regimens of an anti-CD3 mAb may be effective in inducing remission of diabetes (70). Comparisons between patients treated with a non-FcR-binding anti-CD3 mAb and placebo showed that there was a rapid reduction in T cells at the initiation of anti-CD3 treatment followed by a rapid recovery of T cells following treatment (62). This reduction and reappearance in T cells appears not to be mediated by anti-CD3 depletion of T cells, but rather margination of cells out of the peripheral circulation (71). This suggests that pathogenetic T cells are not depleted, but rather leave the circulation –preclinical and clinical studies support the notion that when T cells return, they are suppressed by a newly activated and expanded Treg population (72). With time, the progression of autoimmunity recurs with loss of Treg-mediated tolerance and dominance of pathogenetic T effector cells (73). The role of regulatory T cells following anti-CD3 mAb therapy in humans remains largely unresolved. We have identified adaptive $CD8^+$ Tregs that require exposure to TNF for their induction and inhibit antigen-specific $CD4^+$ T cells through a contact-dependent mechanism (74). One of the limitations of the clinical mechanistic studies involves the restriction to cells in the peripheral blood, whereas the induced Tregs described in mice were found in pancreatic lymph nodes (75).

FUTURE STRATEGIES

In recent years, the number of immunomodulating agents available and clinical trials performed has grown exponentially. The non-FcR-binding anti-CD3 mAbs teplizumab and otelixizumab have both demonstrated the ability to preserve endogenous insulin production when administered to patients with new-onset disease. Questions about the use of these drugs in diabetes still remain and will be addressed by ongoing clinical trials. These include whether the product will be effective in patients with a longer duration of diabetes and whether treatment will prevent the onset of disease in high-risk individuals. In addition, the evidence to date from trials of patients with recent-onset diabetes indicates that single-agent immunotherapy is unlikely to produce a sustained remission from autoimmunity with adequate β-cell mass to ensure insulin independence. Combinations of immunotherapy including anti-CD3 mAbs appear to be synergistic

and might also suggest an approach to sustain the effects of anti-CD3 mAbs on insulin production. For example the combination of intranasal proinsulin or the GLP-1 receptor agonist exendin-4 and anti-CD3 therapy has proved effective in preclinical models (76).

Finally, the long-term effects of immunomodulatory therapies, even if successful, on clinical outcomes remain to be proven. Data from the Diabetes Control and Complication Trial and other studies suggest that retention of C-peptide responses is associated with reduced secondary end-organ complications and a reduced rate of severe hypoglycemia. These clinical benefits are the ultimate measure of the products' success, but would require extended follow-up of a large number of patients. These goals will be realized only after the use of these non-insulin-based therapies is introduced.

DISCLOSURES

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