Anti–CD3 Antibody Treatment: a Promising Immunotherapeutic for Type 1 Diabetes

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Abstract

Type 1 diabetes (T1D) is an autoimmune disease characterized by spontaneous pancreatic β–cells destruction. The central cause of T1D is mainly the weakening of central and peripheral tolerance. So, the induction of tolerance is a major goal for most of the immunotherapies. Investigations over the last couple of decades have shown that anti–CD3 monoclonal antibodies (mAbs) and their therapeutic variants effectively treat autoimmune disease in animal models, and have also shown promise in clinical trials. Tolerance induction by anti–CD3 mAbs is mainly related to the induction of Th2 type immune response and regulatory T cells (Tregs) development that control pathogenic autoimmune responses. Here, we review main findings of last couple of decades research, and current scenario of anti–CD3 mediated immune tolerance, and preclinical and clinical studies in which anti–CD3 mAbs have been used.

Keywords: Type 1 diabetes, OKT3, immunotherapy, mAbs, T cells

Introduction

Type 1 diabetes (T1D) is an autoimmune disease in which insulin producing pancreatic β–cells are destroyed by auto–reactive T cells, as a result of which glucose metabolism is severely affected. Insulin was the first beta–cell protein to be identified as a target of the autoimmune response in type 1 diabetic patients (Palmer et al., 1983). Consequently, people diagnosed with T1D must rely on life–long insulin administration and are susceptible to potential complications including cardiovascular disease, nephropathy, retinopathy, bone fracture and neuropathy. As per American Diabetes Association (ADA) statistics, in 2012, 29.1 million (9.3 %) Americans had diabetes, out of which 1.25 million were children and adults with T1D.

Global increased incidence in children drives attention of researchers towards the development of therapeutics. Evidences from animal models and clinical trials have demonstrated that the autoimmune reaction and the associated inflammation are the key components of the pathogenesis of T1D (Eizirik, Colli, & Ortis, 2009). Other than environmental and genetic factors, the initiation, progression and extent of the subsequent β–cell loss are due to the interplay between the innate and the adaptive immune responses. This interaction makes the strong immunological basis for the efforts to develop therapeutics to counteract the immune attack in order to prevent or delay T1D progression. These immunotherapeutics may provide ways to rely less on insulin administration (Harjutsalo, Sjoberg, & Tuomilehto, 2008).
The role of autoreactive CD4+ and CD8+ lymphocytes in T1D progression has been highly studied in the non-obese diabetic (NOD) mouse model. In the NOD mouse the disease can be transferred by adoptively transferring splenic CD4+ and CD8+ T cells into immunocompetent recipients such as NOD neonates or adult irradiated NOD mice or NOD SCID mice, and also in the human experiment in the recipients of bone marrow from donors with T1D (Lampeter, McCann, & Kolb, 1998; Wicker, Miller, & Mullen, 1986). Other than CD4+ and CD8+ T cells, Fox−P3 expressing CD4+CD25+ Treg cells, and IL4 and IL10 secreting Th2 and Tr1 cells are important in controlling disease progression in NOD mice and patients (Anderson & Bluestone, 2005; Lindley et al., 2005). Moreover, the role of dendritic cells (Phillips, Giannoukakis, & Trucco, 2009), macrophages (Martin et al., 2008) and B lymphocytes has been exploited. Chemokine CCL2 receptor (CCR2) mediated macrophage accumulation in islets accelerated the disease, which can be correlated with the number of monocytes accumulated in the islets (Martin et al., 2008). During the early stage of the disease development, islet antigen specific antibodies were also detected in the mice and human, but B cell deficient mice did not completely abrogate the occurrence of the disease, which showed that it is the T cells which are more important to initiate and progress T1D (Bjork, Velloso, Kampe, & Karlsson, 1994; Yang, Charlton, & Gautam, 1997).

Therapeutic role of anti CD3 antibody

In 1979, Kung et al. developed a monoclonal antibody (OKT3) against the ε chain of the CD3 complex which is the major signal transducing element of the T cell receptors (TCRs). The antibodies were used to prevent allograft rejection in kidney transplantation (Cosimi et al., 1981; Kung, Goldstein, Reinherz, & Schlossman, 1979). Anti–CD3 antibodies induce partial T cell depletion and interfere with T cell activation. Later in the 1990s, it was established in the non-obese diabetic (NOD) mice that self tolerance can be induced with a short-term low-dose of anti–CD3 antibodies (Chatenoud, Thervet, Primo, & Bach, 1994). However, the clinical use of OKT3 was limited because of its immunological and pharmacological side effects, and could not be extended to use in transplantation and other clinical fields such as autoimmunity.

In order to use OKT3 for widespread purposes, humanized version of OKT3 was developed by mutating Fc regions. Bolt et al. in 1993 developed variants of OKT3 using different human heavy chains like y1, y2, y3 and y4, and found that all the variants were active and able to stimulate T cell proliferation in vitro. Also, aglycosyl version of the y1 CD3 mAbs (ChAglyCD3) (otelixizumab) were produced by site-directed mutagenesis (Asn297 to Ala) to prevent glycosylation, and consequently binding of the therapeutic antibody to Fc receptors and to complement. Aglycosyl antibodies have been tested for clinical trial in kidney transplant recipients. In another study, both the Fc variant and the activating anti–CD3 mAbs induced comparable TCR modulation and suppression of cytolytic T cell activity, in vitro. Also, in vivo administration of the Fc variant did not result in T cell activation and cytokine production (Alegre et al., 1994; Friend et al., 1999). Woodle et al. in 1999 showed that HuOKT3gamma1(Ala–Ala), a variant of OKT3 possesses the ability to reverse vigorous graft rejection in kidney and kidney–pancreas transplant recipients, and possesses minimal first dose reactions and does not show any antibody response. Also, elevations in serum IL–10, but not IL–2 levels were observed and reductions in circulating CD4+, and CD8+ T cells were observed after the first huOKT3 gamma1 (Ala–Ala) (teplizumab) dose, followed by a slow progressive return of cell counts toward pretreatment values (Woodle et al., 1999). In another study, Herold et al. used non–activating humanized monoclonal antibodies against CD3 (hOKT3gamma1(Ala–Ala)) on the loss of insulin production in patients with type 1 diabetes mellitus and showed that the treatment with the monoclonal antibody maintained or improved insulin production after one year in 9 of the 12 patients in the treatment group. Moreover, glycosylated hemoglobin levels and insulin doses were also reduced in the monoclonal antibody group without any severe side effects (Herold et al., 2002). Patients treated with ChAglyCD3 (otelixizumab) showed preserved residual beta cells mass and enhanced insulin–secreting capacity. Concomitantly, control group showed low insulin need as compared to placebo control (Keymeulen et al., 2005). Recently, a phase three clinical trial for the safety and efficacy of teplizumab showed that the patients who received the antibodies used less insulin per day, and reduced level of glycated haemoglobin A was observed (Sherry et al., 2011).

Mechanism of T cell depletion using anti CD3 Antibody:

T cells recognize antigen via specific surface receptors which are encoded by immunoglobulin (lg) like genes (Yanagi et al., 1984). These antigen receptor molecules are noncovalently associated with a molecular complex known as cluster of differentiation 3 (CD3) (Meuer et al., 1983). CD3 antibody administration induces partial T cell depletion from the circulation 30–60 minutes after the first injection (Chatenoud et al., 1982). This is caused by cytokine–release that favors increased adhesion of these cells to intercellular adhesion molecule−1 (ICAM−1) and ICAM−2 on vascular endothelium. This causes the activation of endothelial cells, leading to increased adhesiveness, lymphocyte elimination, redistribution and engulfment by reticuloendothelial cells (Buysmann et al., 1996). However, T cells depletion observed
with the use of “disabled” CD3 antibodies ruled out the possibilities of any antibody-induced depletion dependent on complement fixation or antibody dependent cell-mediated cytotoxicity (ADCC). Rather, it depends on the antibody’s fine specificity that causes redirection of T cell lysis by bridging cytototoxic T cells to other T cells, and induction of apoptosis (Wesselingh, Janssen, & Kabelitz, 1993; Wong & Colvin, 1991). More recently, Penaranda et al. showed that anti–CD3 treatment induced depletion of CD4+Foxp3– conventional T cells only, without affecting CD4+Foxp3+ Tregs cells. In contrast to previous finding by Wesselingh et al., Penaranda et al. observed that the T cell depletion induced by anti–CD3 mAb was independent of the pro-apoptotic proteins Fas, caspase–3, and Bim, and the anti–CD3 treatment can alter, and potentially stabilize, Treg function (Penaranda, Tang, & Bluestone, 2011).

Anti–CD3 treatment preferentially depletes activated T cells which are the main causes of pancreatic islet cells destruction, and regulatory T cells are spared from anti–CD3 mediated depletion (You et al., 2012). After anti–CD3 treatment the receptor complex disappears transiently on residual T cells, which are mainly CD3–TCR–CD4+ or CD3–TCR–CD8+ T cells. After the treatment stopped, these cells then rapidly restore the receptor on the cell surface by undergoing antigenic modulation of CD3/TCR (Chatenoud & Bach, 1984). In NOD mice, CD3 antibody treatment improved insulitis coinciding with a rapid return to normoglycemia. Anti–CD3 mediated effects were observed even in NOD CD8−/− mice. Moreover, co-administration of a neutralizing transforming growth factor (TGF)–beta-specific antibody abrogated CD3 antibody mediated effect on diabetes and insulitis, which suggested a critical role of TGF–β in controlling insulitis (Belghith et al., 2003). In dark agouti rat allograft model, CD3 antibodies treatment reduced T cells by 20%, and in the spleen, IL–4 and IL–5 mRNA levels were higher than control animals. This suggested that CD3 antibodies treatment is associated with increased Th2 cytokines, induction of anergy, or nonspecific activation of T cells (Plain, Chen, Merten, He, & Hall, 1999).

Herold et al. showed that non–FcR binding anti–CD3 MAb delivers different activation signal as compared with FcR binding anti–CD3 MAb, former activates T cells in such a way that T cells result in disproportionate production of interleukin–10 (IL–10) relative to interferon–gamma (IFN–gamma) in vitro, and detectable levels of IL–10, IL–5, but rarely IFN–gamma or IL–2 in the serum after treatment. Also, the MAb induces a population of CD4+IL–10–CCR4– cells in vivo (Herold & Taylor, 2003). In a trial of a modified anti–CD3 mAb [hOKT3gamma(Ala–Ala)] in patients with type 1 diabetes, an increase in the number of peripheral blood CD8+ cells was observed. Anti–CD3 treatment caused activation of CD8+ T cells and induced CD8’CD25+ regulatory T cells. Also Foxp3 was induced on CD8+ T cells in patients during mAb treatment (Biskirksa, Colgan, Luban, Bluestone, & Herold, 2005). Moreover, a novel combination treatment with anti–CD3–ε–specific antibody and i.n. proinsulin peptide can reverse recent–onset diabetes in murine diabetes models. The combination therapy strongly induced expansion of CD25+Foxp3+ and insulin–specific Tregs producing IL–10, TGF–beta, and IL–4 (Bresson et al., 2006). Ishikawa et al. showed that even oral administration of anti–CD3 antibodies treatment protected incidence of insulitis in TGF–β dependent manner. Oral administration of anti–CD3 mAb at doses of 50 and 250 μg/feeding suppressed the incidence of diabetes in streptozotocin induce diabetic mouse model with the best effects seen at the 50 μg/dose (Ishikawa et al., 2007).

Side effects of CD3–specific antibodies:

The main drawback in early OKT3 antibody treatment was occurrence of an anti–globulin response to the xenogeneic protein, because it promoted the rapid clearance and neutralization of OKT3. Immunological response to OKT3 is mainly specific for isotypic (OKT3 is a mouse IgG2a) and idiotypic determinants (Chatenoud, Baudrihaye, et al., 1986). Idiotype antibodies are mainly neutralizing antibodies as they compete with target antigen for binding to OKT3 (Baudrihaye, Chatenoud, Kreis, Goldstein, & Bach, 1984). Moreover, the occurrence of OKT3–specific IgE antibodies has also been described which is associated with a potential risk of anaphylaxis (Abramowicz, Crusiaux, & Goldman, 1992). Anti–globulin response associated with anti–CD3 treatment is oligoclonal and recruits only a limited specificity B cells clones which restricts the neutralization potential of anti–CD3 antibodies (Chatenoud, Jonker, Villerain, Goldstein, & Bach, 1986). Moreover, the use of OKT3 in association with conventional immunosuppressants helped to decrease the anti–globulin response. Adding corticosteroids, cyclosporin and azathioprine decreased the frequency of OKT3 specific antibody response (Hricik, Mayes, & Schulak, 1990).

Second important concern associated with anti–CD3 treatment is mitogenic potential of therapeutic antibodies as antibody administration induced T cells proliferation and cytokine production in vitro (Van Wauwe, De Mey, & Goossens, 1980). Mitogenic potential is mainly due to the interaction of the antibody’s Fc portion with FcRs that are present on phagocytes and NK cells. Also, in vivo administration of antibodies led to the large scale production of cytokines within the initial hours after the first injection (Chatenoud et al., 1989). This short term cytokine response - which is a combination of TNF, IL–6 and interferon–γ (IFN–γ) - leads to a ‘flu-like’ syndrome, which is characterized by fever, chills,
Conclusion

Type 1 diabetes is predominantly autoimmune in nature, which is initiated with the immune attack by mainly T cells to β-cells, which are involved in insulin secretion. The susceptibility factors include genetic predisposition, dietary, environmental, and viral infections. Somehow these factors collectively trigger the injury to β-cells. This initial β-cells injury provides the fertile ground to other immune cells like macrophage and T cells. Then the process of priming, presentation and cross presentation of β-cell specific peptides in MHC I and MHC II restricted manner to CD8 and CD4 T cells exacerbate the disease. Moreover, many of the bystander cells are not left unaffected. This complex immune interplay generates specific cytotoxic T cells which directly or indirectly mediate killing of β-cells mainly via cytokine release. Moreover, this complex immune interplay creates an imbalance between the autoreactive T cells and regulatory T cells. Because of its complex immune nature, the immune manipulation has been the best way to deal with the disease for a long time. This can be achieved in two ways; selectively eliminating autoreactive T cells, or, selectively increasing Treg cell function. Anti–CD3 approach mainly focuses on decreasing islet antigen specific T cells activation. Researchers have developed several variations of anti–CD3 in order to make it more effective, and of course, to reduce the side effects associated with the therapy. On the other hand, targeted delivery of antigens/peptides to dendritic cells via DEC–205 receptor–a leptin like receptor expressed mainly on dendritic cells–is also found to be effective to induce tolerance in NOD mouse model (Mukhopadhyaya et al., 2008). Moreover, in future the use of targeted delivery of auto antigens or antigenic peptides along with anti–CD3 would be a good approach to control the exacerbation of the disease, or at least to preserve remaining β–cells mass.

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References


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