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New immunosuppressive approaches: Oral administration of CD3-specific antibody to treat autoimmunity

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Abstract

One of the major goals for the immunotherapy of autoimmune diseases is the induction of regulatory T cells that mediate immunologic tolerance. Parenteral administration of anti-CD3 monoclonal antibody is an approved therapy for transplantation in humans and is effective in autoimmune diabetes. We have found that oral administration of anti-CD3 monoclonal antibody is biologically active in the gut and suppresses experimental autoimmune encephalomyelitis both prior to disease induction and at the height of disease. Oral anti-CD3 antibody acts by inducing a unique type of regulatory T cell characterized by latency-associated peptide (LAP) on its cell surface that functions *in vivo* and *in vitro* via TGF-β dependent mechanism. Orally delivered antibody would not have side effects including cytokine release syndromes, thus oral anti-CD3 antibody is clinically applicable for chronic therapy. These findings identify a novel and powerful immunologic approach that is widely applicable for the treatment of human autoimmune conditions.

Keywords

regulatory cell; TGF-β; antibody; multiple sclerosis; autoimmunity

1. Introduction

Immune tolerance is a state of immune system unresponsiveness to an antigen, and is maintained by a number of mechanisms including deletion, anergy, and active cellular regulation [1]. It is generally believed that autoimmune diseases arise in some way from in balance between autoregulatory immune system and pathogenic autoreactivity. Thus, strategies to induce immune tolerance are developing for the treatment of autoimmunity. One such approach is mucosal, oral and nasal administration of autoantigens designed to

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induce regulatory cells; that is called mucosal tolerance. Another approach is a parenteral administration of anti-CD3 antibody, which has been shown to be efficacious in animal models of autoimmunity including autoimmune diabetes [2–7] and experimental allergic encephalomyelitis [8, 9], and more recently in human trials of type 1 (autoimmune) diabetes [10–13] and psoriatic arthritis [14]. In addition, anti-CD3 monoclonal antibody given to humans intravenously has pronounced immunologic effects [15, 16] and is an approved therapy for acute transplant rejection in humans, although there are side effects and it cannot be given on a chronic basis [17–21].

We investigated immune modulation of autoimmunity by mucosal administration of autoantigens designed to induce regulatory T cells. And we found, (1) TGF-β secreting regulatory cells (Th3 cells) are generated during oral tolerance [22], (2) oral OVA induces regulatory T cells in OVA-transgenic mice [23], (3) even small peptides [24–26] and cytokines [27, 28] are biologically active when given orally, (4) in addition, it was recently reported that signalling through intravenous anti-CD3 antibody affects T cell function and induces TGF-β-dependent CD4+CD25+ regulatory T cells in NOD mice [7]. Give this background, we have taken advantage of the unique properties of the mucosal immune system and found that oral anti-CD3 induced immunologic tolerance and did not show the potential side effects such as cytokine release syndrome.

2. Therapeutic anti-CD3 antibody in NOD mice

The first demonstration that anti-CD3 antibody could affect the autoimmune diabetes was reported in the mid 1990's using non-obese diabetic (NOD) mice, which is a mouse model of type 1 diabetes [3]. 80% of NOD mice develop diabetes spontaneously, but when given intravenously or intraperitopneally anti-CD3 antibody induces disease remission within 2 to 4 weeks of treatment [3, 4]. This effect was long-term, lasting life-long of mice, and was islet antigen specific, because syngeneic islet grafts were not rejected when implanted in anti-CD3-treated mice. In addition, anti-CD3-treated mice were not immune compromised, as skin allografts were normally rejected [3]. In contrast to most other immune-interventions, parenteral administration of anti-CD3 antibody showed a unique therapeutic window of remarkable efficacy at the time of overt diabetes [4]. It was ineffective at preventing disease if applied to young prediabetic NOD mice. Similar observation was reported in a mouse model of multiple sclerosis, experimental allergic encephalomyelitis (EAE) [9]. Intravenously administered anti-CD3 antibody was highly effective in treating ongoing EAE, while no effect at all on disease prevention.

The efficacy of parenteral administration of anti-CD3 antibody in NOD mice is associated with inhibition of the pathogenic immune response, as assessed by the clearance of activated pathogenic T cells from pancreatic islets [18–21]. Anti-CD3 antibody induces depletion of T cells in the periphery, however, which is partial (40–50%) and transient. Indirect evidence suggests that recently activated effector T cells would be more sensitive to anti-CD3 antibody-mediated cell death, which supports the enhanced antibody activity at the height of autoimmune destruction. T-cell receptor (TCR) down-modulation also occurs. However, it is also transient and it only occurs during treatment phase. Thus, none of these mechanisms explain the long-term effect of anti-CD3 antibody. Within the first few days after parenteral administration of anti-CD3 antibody, a short-term Th2 polarization is observed [29]. However, this transient Th2 polarization is not essential for long-term therapeutic effect of anti-CD3 antibody, because anti-CD3-induced disease remission was observed in NOD mice deficient in IL-4 [7]. Accumulated evidence in the NOD mice suggests that the long-term effect of parenteral administration of anti-CD3 antibody is mediated by induction of regulatory T cells. In fact, anti-CD3-treated mice harbor significant numbers of diabetogenic T cells, which have sufficient competent to induce diabetes when transferred into NOD

SCID recipient mice [4]. In addition, there is immune-cell infiltration in the periphery of the pancreatic islets similar to that observed in prediabetic mice [4]. Recently, direct evidence for the presence of regulatory T cells in anti-CD3-treated mice was reported [7]. Intravenous anti-CD3 antibody increased the proportion of CD4+CD25+ T cells in mesenteric and pancreatic lymph nodes. These CD4+CD25+ T cells induced by anti-CD3 antibody expressed forkhead box P3 (Foxp3) and were suppressive both *in vivo* and *in vitro*. When diabetogenic T cells were co-transferred with pancreatic lymph nodes cells from anti-CD3-treated mice into NOD SCID mice, recipient mice did not develop diabetes. CD4+CD25+ T cells from anti-CD3-treated NOD mice suppressed the responder cell proliferation *in vitro*, similar to conventional 'natural suppressor' regulatory T cells. CD4+CD25+ T cells from anti-CD3-treated mice produced high amount of TGF-β, and neutralizing antibody to TGF-β abrogated suppressive property of these cells both *in vivo* and *in vitro*. These data indicate that tolerance induced by intravenous anti-CD3 antibody depends on TGF-β producing CD4+CD25+ regulatory T cells, which play a central role in the long-term therapeutic effect in NOD mice.

3. Therapeutic anti-CD3 antibody in human type 1 diabets

3.1. Generation of humanized non-mitogenic anti-CD3 antibody

OKT3 is a monoclonal antibody specific for human CD3 and is mouse IgG2a subclass [30]. In 1981, first patients were treated with anti-CD3 antibody and it reversed the ongoing renal-allograft rejection [31]. After then, from 1985, anti-CD3 antibody was available for use in transplantation. However, clinical use was hampered by serious side effects linked to its mitogenecity and cytokine release potential. OKT3 is a potent mitogen that promotes T cell proliferation and cytokine production, thus leading 'flu-like' syndrome [32–38]. This mitogenic activity correlates with the capacity of Fc antibody portion to interact with Fc receptors on monocytes/macrophages. Thus, F(ab')2 fragments of anti-CD3 antibody, which lack Fc portion, are not mitogenic [3, 4, 39–41]. In addition, non-mitogenic F(ab')2 fragments of anti-CD3 antibody are immunosuppressive and fully retain their tolerogenic ability [4, 40]. Given these backgrounds, humanized FcR-non-binding anti-CD3 antibody was engineered and this form antibody was proven to be safe clinically [2, 4, 42–45]. However, FcR-non-binding anti-CD3 antibody still induces low levels of cytokine release, and is able to induce partial signaling which is responsible, at least in part, for its tolerogenic ability [4, 46, 47]

3.2. Human clinical study

There are two different forms of humanized FcR-non-binding anti-CD3 antibody, both of which are presently used in clinical trials of human type 1 diabetes. One is hOKT3γ1 Ala-Ala (Teplizumab), which is derived from OKT3 and has two mutations in its Fc portion to inhibit Fc receptor binding [44]. The other is ChAglyCD3, which is derived from the rat YTH12.5 antibody and has a single mutation leading to prevent glycosylation of its γ1 Fc portion [42]. In a Teplizumab study, Teplizumab was well tolerated and treatment group showed a beneficial effect on insulin production compared with the control group [10, 12]. This trend was confirmed until up to 2 years after the single course of treatment [12]. CD8+CD25+Foxp3+ regulatory T cells were induced *in vivo* during antibody treatment, and were considered to play a role in the effect of Teplizumab treatment [48].

Another clinical trial used ChAglyCD3 revealed that 6-day course of ChAglyCD3 treatment preserved β cell function and prevented an increase in exogenous-insulin needs for at least 18 months [13]. Humanized FcR-non-binding anti-CD3 antibodies dramatically reduced 'flu-like' symptoms, however, some degree of T cell activation is still occurred, which leads to minor side effects such as headache, fever and rash [10, 13, 49, 50].

4. Preclinical study on oral anti-CD3 antibody against autoimmunity

4.1. Effect of Oral anti-CD3 antibody on experimental autoimmune encephalomyelitis

Parenteral administration of anti-CD3 antibody is widely applied in a variety of animal models of autoimmunity other than NOD mice [9, 29, 51–53]. In experimental autoimmune encephalomyelitis (EAE), which is an animal model of multiple sclerosis, intravenous administration of non-mitogenic anti-CD3 antibody ameliorated the progression of established disease [9]. Interestingly, intravenously administered anti-CD3 antibody at the time of disease induction had no effect on disease prevention, whereas treatment at the peak of disease conferred a beneficial effect on accelerating disease remission and preventing further relapses [9]. This seems to be a unique feature of parenteral administered anti-CD3 antibody, because the similar ability was observed in NOD mice.

In contrast to parenteral administration of anti-CD3 antibody, we found that oral administration of antibody was effective at both preventing and treating established disease in EAE [54]. The Fc portion of antibody was not required for this suppression, as orally delivered F(ab')2 fragments of anti-CD3 antibody suppressed EAE as well. In addition, the effect of oral anti-CD3 antibody was not antigen specific and thus, is useful in a wide range of autoimmune conditions. Furthermore, because oral anti-CD3 antibody did not appear in the bloodstream, there was no mitogenic effect and no evidence of cytokine release syndrome such as wasted appearance or ruffled fur in treated mice. In 1988, a trial in patients with multiple sclerosis showed that OKT3 suppressed relapse, however, it was not pursued because of its side effects [55]. Taken together, these results suggest that oral delivery of anti-CD3 antibody is a safe and novel therapeutic approach for autoimmunity.

4.2. Mechanisms of oral anti-CD3 antibody

Orally administered anti-CD3 antibody appeared in the villous epithelium within 30 min and increased 1 hr and 3 hr after feeding, whereas intravenously administered anti-CD3 antibody appeared in the serous lining the gut [54]. In addition, there was a fundamental immunological difference between oral and intravenous administration of anti-CD3 antibody. Oral anti-CD3 antibody did not induce any TCR down-modulation, depletion of T cells nor T cell division, even at high dose. However, oral anti-CD3 antibody delivered a weak signal to T cells in the gut that preferentially induced unique subset of regulatory T cells. These are CD4+CD25- regulatory T cells that express latency-associated peptide (LAP) on their surface, and their regulatory function depends on transforming growth factor-β (TGF-β) both *in vivo* and *in vitro*. In fact, oral anti-CD3 antibody enhanced the expression of CD69 activation marker on CD4+CD25-LAP+ T cells in the gut and TGF-β production from these T cells. TGF-β is secreted as a homodimer noncovalently bound to LAP. Extracellular dissociation of TGF-β from LAP releases biologically active TGF-β, thus, LAP contributes to the prevention of uncontrolled activation of TGF-β signaling. One of unique features of LAP+ T cells is the positivity for thrombospondin [56]. Thrombospondin can convert biologically inactive TGF-β to the active form; therefore, thrombospondin is also thought to play an important role in LAP+ regulatory cell function.

These regulatory T cells migrate from the gut to lymph nodes draining tissues that are the target of autoimmune inflammation, where they exert their regulatory effects. Oral anti-CD3 antibody was effective before disease onset in EAE, because it directly induced regulatory T cells that prevent the induction of pathogenic effector cells. In the EAE model, it was also effective at the peak of disease, presumably by boosting the regulatory T-cell response that occurs during recovery of EAE [57].

Notably, suppressive effect of oral anti-CD3 antibody on EAE was observed at low dose, but not at high dose. This dose response correlated with the induction of regulatory cells in

the gut. A similar dose effect is described in oral tolerance in which lower doses of protein autoantigens are most efficient in the induction of TGF-β-dependent regulatory T cell [58, 59]. Although the exact relationship between CD4+CD25-LAP+ regulatory T cells and the Th3 regulatory cells that appear after oral administration of protein autoantigens remains to be determined, it is possible to postulate that LAP+ T cells are precursors of Th3 cells [60] and either differentiate into Th3 cells upon secretion of TGF-β or induce regulatory T cells by the secretion of TGF-β. Once Th3 cells are stimulated, they may lose LAP on their surface.

4.3. Oral anti-CD3 antibody in the model of other autoimmunity

We also found that oral administration of non-mitogenic anti-CD3 antibody was effective in prevention of autoimmune diabetes in AKR mice in which the low dose streptozocin induced autoimmunity against the β-cells of the islets [61]. Analogous to what was observed in the mouse model of EAE, this suppression was mediated by induction of CD4+CD25-LAP+ regulatory T cells that suppressed *in vitro* and *in vivo* in a TGF-β dependent fashion. In addition, we found that oral and nasal administration of anti-CD3 antibody worked in a mode of lupus (unpublished data).

5. Conclusion

Oral administration of anti-CD3 antibody has been shown to induce immune tolerance and to be effective treatment for animal models of autoimmunity. The immunological effects of oral anti-CD3 antibody are not antigen specific, thus, which could be useful in a wide range of autoimmunity. In addition, oral administration of anti-CD3 antibody is clinically applicable for chronic use and would not be expected to have side effects including cytokine release syndromes and anti-globulin response. Human clinical trials also demonstrated that orally delivered bovine antibodies were effective in preventing rotavirus, enterogenic *Escherichia coli*, shigella infection, and necrotizing enterocolitis [62]. Taken together, our results indicate that oral administration of antibody directed at other structures on immune cells may be a new avenue for the immunotherapy. Based on our animal data, we have initiated dosing studies of oral administration of anti-CD3 antibody in normal human subjects to test for toxicity and immunologic effects.

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