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Preservation of recall immunity in anti-CD3 treated recent onset type 1 diabetes patients.

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ABSTRACT

Background
The safety of any immune modulating agent in type 1 Diabetes Mellitus (T1DM) involves its selectivity on auto-immunity and its preservation of recall and tumour immunity.

Methods
We performed lymphocyte proliferation tests on seven newly diabetic patients treated with anti-CD3 (Otelixizumab; ChAglyCD3) and five newly diabetic patients treated with placebo, on average two years after therapy.

Results
Proliferative responses towards common viral, bacterial and yeast antigens upon *in vitro* stimulation with a range of recall antigens in anti-CD3 treated T1DM patients were highly similar to those in placebo-treated T1DM patients. Likewise, T-cell responses towards auto-antigens were equally low between the two groups, several years after diagnosis of T1DM. The proliferative response upon stimulation with the human suppressor protein p53 was invariably high in both anti-CD3 and placebo treated patients, implying preserved anti-tumour immunity in anti-CD3 treatment.

Conclusions
As long-term focus on side-effects is key, we demonstrate in this sub-cohort of recent onset T1DM patients treated with Otelixizumab that recall immunity is preserved in spite of high-dose anti-CD3 treatment, adding to the safety of anti-CD3 treatment as an immune-modulatory agent in the treatment of T1DM.

INTRODUCTION

Type 1 Diabetes Mellitus is a chronic and progressive autoimmune disease in which auto-reactive CD4+ and CD8+ T-cells attack insulin-producing beta-cells in the pancreatic islets. Targeted immune therapies have shown encouraging results in the treatment of T1DM. Preclinical studies in non obese diabetic (NOD) mice have demonstrated reversal of hyperglycaemia upon anti-CD3 treatment [1]. In clinical studies initial toxicity problems with anti-CD3 treatment, due to non-specific FcR binding, were overcome by creating humanized non-FcR binding anti-CD3 antibodies. Phase 2 clinical trials with Otelixizumab [2,3] and Teplizumab [4] have shown to preserve beta cell function for at least 18-24 months, decreasing insulin requirements and normalizing glycated haemoglobin levels in patients with recent onset T1DM.

In the successful Phase II Otelixizumab trial achieving long term preserved beta-cell function, the antibody dosage was considerably higher then that elected for the Phase III DEFEND1 study, which did not reach its primary endpoint of preserved beta-cell function. One of the reasons for dose reduction was transient Epstein Barr Virus (EBV) reactivation in the high Otelixizumab study, seen in 75% of the treated patients [2,5]. EBV copies peaked between 3 and 4 weeks after treatment with Otelixizumab and returned to pretreatement levels within a few weeks as an efficient humoral and cellular immune response specific to EBV had developed, comparable with the response observed in otherwise healthy individuals following EBV infection [2,5]. Over the 48 month follow-up period, no biological or clinical signs of EBV reactivation or EBV-related disease were observed [3]; there was no higher incidence of infections, and no lymphoma or other types of cancer. No differences in CD3+ lymphocyte counts were found between months 6 and 48.

Another safety concern in the use of immune modulating agents is how well recall immunity (the immune reaction towards pathogens patients have prior been exposed to) to other pathogens than EBV is preserved. No follow-up data on recall immunity after treatment with anti-CD3 has been available.

Previously, we reported auto- and recall immunity in patients who successfully underwent kidney-pancreas transplantation and were induced with either anti-thymocyte globulin (ATG) or Daclizumab, a monoclonal antibody against the interleukin-2 receptor (CD25) [6]. T cell autoimmunity towards islet antigens was low in both groups. Yet, immune memory responses towards bacterial, viral and tumour antigens were significantly lower in the ATG -treated group when compared to Daclizumab-treated patients, implying Daclizumab has a selective effect on auto-immunity but preserves desired recall responses.

An additional concern in the treatment of T1DM is the recurrence of autoimmunity. Monti *et al.* [7] proposed homeostatic expansion of autoreactive T-cells in T1DM patients receiving islet allografts under anti-IL-2 receptor mAb induction therapy, followed by low dose tacrolimus and rapamycin maintenance therapy. Homeostatic expansion of

autoreactive T-cells could lead to exacerbation of auto-immunity and precipitation of disease. The mechanism behind the success of anti-CD3 treatment in recent onset T1DM patients is largely unknown. Short-lived antigenic modulation, apoptosis and anergy are found immediately after treatment [9]. There is compelling evidence that the lasting effect is achieved through the induction of regulatory CD4+ and CD8+ T cells [8,9]. In vitro studies on prediabetic glutamic acid decarboxylase (GAD) 65-antigen specific autoreactive T-cells revealed that both Daclizumab and Otelixizumab operate in a non-depleting fashion, by proliferation inhibition and antigen modulation, hence not by creating a niche for autoreactive T-cells [10].

MATERIALS AND METHODS

We performed lymphocyte proliferation tests on average two years after therapy on seven newly diabetic patients treated with anti-CD3] (Otelixizumab; ChAglyCD3) and five newly diabetic patients treated with placebo, as previously described [2,6]. These patients represent a sub-cohort of the European phase II placebo-controlled trial, in which a cumulative dose of 48 mg Otelixizumab was administered intravenously over 6 days [2].

In short, peripheral blood mononuclear cells (PBMC) were isolated from freshly drawn heparinized blood. 150.000 PBMC were cultured in tissue-coated, round bottomed 96-well plates (Costar, Cambridge, MA, USA) in Iscove's modified Dulbecco's medium with 2 mmol/l glutamine (GIBCO, Paisley, Scotland, UK) supplemented with 10% human type AB pool serum in the presence of antigen, recombinant IL-2 or medium alone in 150 ul at 37°C, 5% CO2. After 5 days, RPMI-1640 (Dutch Modification; GIBCO) containing 0.5 uCi [^{3}H]-thymidine per well was added, and incubation was continued for 16 hours. Cultures were harvested on glass-fibre filters and [^{3}H]-thymidine incorporation was measured by liquid scintillation counting. The stimuli used were insulin (Sigma), recombinant human proinsulin and islet autoantigen IA-$2_{602\text{-}979}$, GAD65 (Diamyd Medicals, Stockholm, Sweden), tumour suppressor protein p53, recombinant IL-2, tetanus toxoid (1.5 Limes flocculationes/ml or 12.0 international units/ml) (National Institute of Public Health and Environmental Protection, the Netherlands), Candida, purified protein derivate (PPD) (tuberculin) and *Haemophilus influenzae* matrix protein M1. The results are expressed as stimulation indexes (SI); CPM in the presence of stimulus divided by CPM with medium alone.

RESULTS

Upon *in vitro* stimulation with different recall antigens, anti-CD3 treated T1DM patients had proliferative responses towards common viral, bacterial and yeast antigens that were highly comparable to those in placebo-treated T1DM patients (Fig.1). In addition, several years after diagnose, T-cell responses towards auto-antigens did not differ significantly between these two groups.

The proliferative response upon stimulation with the human suppressor protein p53 was invariably high in both the anti-CD3 and the placebo treated group. These responses underline preserved anti-tumour immunity in anti-CD3 treatment. This observation is in line with the 48 month clinical follow-up where no lymphoma or other types of cancer were observed [3].

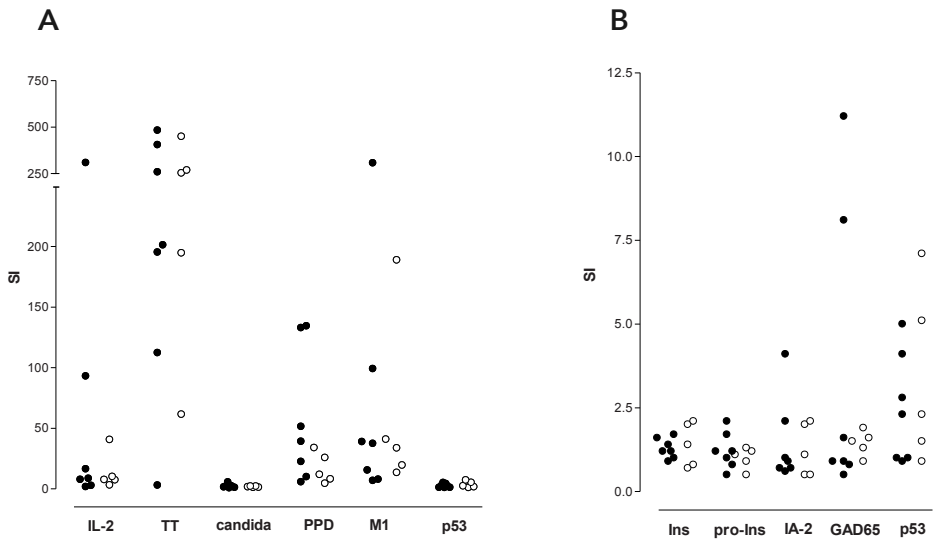

Figure 1 | Memory responses towards recall antigens (A) and auto-antigens (B).
Open circles for placebo (n=5), closed circles for anti-CD3 treatment (n=7). Lymphocyte proliferation tests, expressed as stimulation indices of median responses in triplicate experiments. Average background for placebo treated and anti-CD3 treated patients was 333±80 and 450±194 cpm, respectively.

DISCUSSION

Over the last decade the use of anti-CD3 in the treatment of T1DM has moved from bench to bedside. Two separate phase II trials with anti-CD3 mono-therapy have shown prolonged preservation of beta cell function with decreased insulin requirements. While we continue to focus on long-term side-effects, we demonstrate in this sub-cohort of recent onset T1DM patients treated with Otelixizumab that in spite of high-dose anti-CD3 treatment recall immunity is preserved. This follow-up data adds to the safety of anti-CD3 treatment as an immune-modulatory agent in the treatment of T1DM.

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DUALITY OF INTEREST

Drs. Keymeulen and Mathieu participated in a GSK funded phase 1 study with subcutaneous Otelixizumab and a phase 3 study with intravenous Otelixizumab (Defend 2). Dr. Roep was member of the scientific advisory board of TolerX from October 2010 till April 2011. Dr. Waldmann is co-founder of TolerX.

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