

Questioning Four Preconceived Ideas on Immunotherapy of Clinical Type 1 Diabetes: Lessons from Recent CD3 Antibody Trials

Lucienne Chatenoud and Jean-François Bach

*Université René Descartes Paris 5, INSERM U580, Hôpital Necker-Enfants Malades, Paris, France.
Address correspondence to: Jean-François Bach, e-mail: bach@necker.fr.*

Introduction

Patients presenting type 1 diabetes (T1D) are currently treated with insulin substitutive therapy that is remarkably successful. The treatment has, however, two major limitations that cannot be underestimated due to the major problems and/or discomfort they create, particularly in the numerous patients with unstable metabolic control or insufficient compliance to treatment. The first limitation is degenerative complications that remain an important concern even if their frequency has been dramatically reduced in patients with good metabolic control. The second is the worrisome daily constraints and risks (especially hypoglycemia) linked to regular insulin administration. Several alternatives to palliative insulin therapy have been proposed: pancreas or islet transplantation, β -cell regeneration and immunotherapy. The last strategy, which is in many regards the most straightforward and does not expose patients to the hazards and complications of cell therapy and transplantation, was initiated in the 1980s using cyclosporin in patients presenting new-onset T1D [1-3]. Successful remission of disease was obtained with very limited side effects. When the drug was administered at reasonable doses, neither chronic nephrotoxicity nor infections were observed [1-5].

Thus, ten years after cyclosporin treatment, patients from the French study showed perfectly normal renal function as assessed by creatinine and paraaminohypuric (PAH) acid clearance [5]. It remains true, though, that cyclosporin did not restore self-tolerance to islet autoantigens. Diabetes returned rapidly after cessation of treatment, indicating that indefinite treatment was needed to maintain the therapeutic effect, hardly an acceptable strategy in young subjects because of the long-term risk of overimmunosuppression (infections and tumors). These results justified the intensive search for alternative approaches aimed at inducing and/or restoring self-tolerance addressed by a large number of laboratories using the NOD mouse as a preclinical model [6].

Four misleading concepts emerged from these efforts: 1. tolerance induction requires the administration of β -cell antigens, the potential targets of autoimmune aggression, 2. results obtained in animal models (NOD mouse and BB rat) are not predictive of efficacy in human T1D, 3. at the time of established (even recently diagnosed) diabetes, too many β -cells are already destroyed to allow any room for effective and long-standing metabolic reconstitution, a statement which oriented investigators towards prevention trials in subjects at high risk of developing the disease, 4. to be

successful, trials should use the combination of several agents because of the unlikelihood of having a significant effect with a single agent.

The aim of this brief review, based on the results we recently obtained in a phase II randomized trial using a CD3-specific antibody [7], is to question all these four preconceived ideas.

Recovery of self-tolerance may be achieved without autoantigen administration

Induction of immunological tolerance was initially obtained in the sixties following administration of the antigen (i.e. the tolerogen). This was achieved using both soluble antigens (e.g. foreign immunoglobulins) [8, 9] and tissue antigens (using donor lymphoid cells) in transplantation [10]. It was thus a logical approach to attempt to restore self-tolerance to β -cells in NOD mice using soluble β -cell-derived autoantigens. Tolerance induction and disease prevention were obtained by several laboratories using different β -cell antigens [11-16]. The fact that tolerance extended to antigens other than the one used for its induction (through the mechanism of bystander suppression) limited the problems associated with the ill-defined molecular nature of the primary β -cell antigen(s) targeted in T1D [16]. To date, the various attempts to translate this strategy to the clinic are still inconclusive [17].

Another approach which did not require the administration of the tolerogen was investigated based on the remarkable results obtained in the field of transplantation. Treatment of rodents with anti-T cell polyclonal or monoclonal antibodies at the time of grafting allows the induction of long-term donor-specific tolerance in the absence of injection of donor alloantigens. Spectacular results were thus obtained using anti-lymphocyte serum (ALS) [18, 19] and various monoclonal antibodies or fusion proteins targeting relevant T cell receptors, including CD3, CD4 as well as CD4 and CD8 costimulation molecules [20-26]. In these transplantation models achievement of the tolerant state was confirmed by the observation of indefinite survival of second grafts from the originally tolerated donor, while third-party allografts were normally rejected.

Results obtained in our laboratory with a CD3-specific antibody have shown that this strategy could be extended to T1D. A short-term CD3 antibody treatment (five consecutive days) in recently diagnosed diabetic NOD mice induces a permanent remission of the disease [27, 28]. The therapeutic effect is antigen-specific since mice rapidly recover full immunocompetence a few weeks following the disappearance of the

CD3 antibody from the serum [27, 28]. Underlying mechanisms involve regulatory T cells that have been shown to be TGF- β , but not IL-4 dependent [28-30].

The advantages of the antibody strategy over the autoantigen approach are essentially linked to the effectiveness of antibody treatment in mice with ongoing disease. This is not the case for autoantigen treatment, which must be administered at early disease stages, before disease onset, to be effective [6, 16]. The fact that the effect is obtained with such a short treatment is important inasmuch as it avoids the potential risks of the chemical immunosuppression by cyclosporin mentioned above. The antibody approach also has the advantage of bypassing the difficulties met in selecting the "right" antigen preparation and its proper dosage. The antibodies in question are well established pharmaceutical agents whose generalized application would not be exposed to the problems associated with the introduction of a new class of drugs.

In fact, most likely anti-T cell antibodies induce tolerance through stimulation of regulatory T cells that selectively migrate into the graft or into the organ that is the target of an autoimmune attack [29-31].

Animal models of T1D may be predictive of human application

It has become fashionable over the past few years to discredit animal models of T1D for their inability to predict clinically useful immunotherapeutic strategies. This trend was essentially based on the observation that many (too many?) agents inhibit the onset of T1D [32, 33]. Two major comments should be made, however, before applying this criticism. First, nobody knows whether very early interventions in subjects genetically predisposed to T1D would not prevent the disease using several treatments shown to be efficacious in very young NOD mice (i.e. treatments applied in 3-week-old NOD mice, 3 to 4 months before disease onset). To prove or disprove this point, one would need to treat subjects long before the onset of islet-specific antibody production, which assesses the beginning of the autoimmune aggression of the islets, something which has not been done so far. In fact, it is impossible to exclude the fact that, at such an early stage of the disease process, subtle changes in the immune system are sufficient to reset responses towards maintenance of self-tolerance. Second, the number of methods capable of stopping the progression of established disease is extremely limited, considerably less than those able to prevent disease onset. In fact, the ALS-, CD3- and CD4-specific antibodies are the only ones that have such a capacity [6, 34, 35]. This is a ma-

major fact since it relates to the clinical setting in which one would like to apply immunotherapy in T1D and, more importantly, recent data have proven that successful transfer to the clinic was obtained using one of these agents, i.e. a short CD3 antibody treatment in patients with new-onset diabetes. A Phase I open trial using the OKT3 γ 1 Ala-Ala suggested a favorable therapeutic effect [36, 37] that was definitively confirmed in a randomized double-blind multicenter phase II placebo-controlled trial that we conducted on 80 patients using the ChAglyCD3 antibody [7]. ChAglyCD3 treatment very efficiently preserved β -cell function, maintaining significantly higher levels of endogenous insulin secretion in comparison to placebos at 6, 12 and even 18 months. This also led to a significant decrease in the need for insulin [7]. Importantly, at 18 months, within the subset of patients showing a high β -cell mass (higher than the median value of the whole population) at the time of treatment, 75% of patients in the ChAglyCD3 group (versus none in the placebo group) received insulin doses ≤ 0.25 U/kg/day [7]. Short-term reactivation of Epstein Barr virus (EBV) was observed as assessed by an increase in numbers of EBV copies measured in peripheral blood mononuclear cells 10-20 days after the first injection. Within 1-3 weeks the number of EBV copies returned to normal baseline pre-treatment levels in all patients. Concomitantly, an efficient humoral and cellular immune response specific to EBV developed that was comparable in intensity and kinetics to that observed in normal subjects following infectious mononucleosis. This effective anti-EBV response is, for obvious reasons, very important in terms of safety. In addition, it suggests that, as we observed in NOD mice, the effect of ChAglyCD3 in patients is antigen-specific; that is, the antibody treatment affects the autoimmune reaction, but does not prevent immune responses to unrelated antigens such as EBV.

Finally, a similar case could be made for most of the other differences frequently highlighted between NOD mice and T1D patients [33]. The genetic predisposing and protective factors and the role of the microbial environmental factors on disease incidence are highly comparable [38-40]. Only minor differences exist, which are predictable inasmuch as human T1D is in essence genetically heterogeneous, whereas NOD mice are in fact multiple copies of a single individual.

Most β -cells are not destroyed at the time of diabetes onset

It was a remarkable step forward to prove that autoimmune aggression to β -cell starts long before the

onset of clinical disease, as evidenced by the presence of islet-specific autoantibodies several years before disease onset. A debate persists, however, on the proportion of residual β -cells when the disease is first diagnosed. Morphometric studies are interesting, but not fully informative, the more so since they use insulin as the main β -cell marker. This poses the question of its reliability when insulin production is decreased (as we know it is) due to the pro-inflammatory activity of the islet infiltrate [41, 42]. Additionally, histological data on human T1D are very limited due to the risks associated with pancreas biopsies. The fact that T cell-targeted treatment both in NOD mice (in the absence of concomitant insulin therapy) [27, 34, 43] and in man [1, 3, 7] induces a rapid remission of clinical disease strongly indicates that T cells do not only act by destroying β -cells but that part of their action involves a reversible, probably cytokine-mediated inflammatory inhibition of β -cell function. This is a critical point conceptually, in keeping with the recovery of β -cell function by islets from recently diagnosed diabetic NOD mice once the mononuclear cell islet infiltrate is cleared, following *in vitro* culture [41]. It also provides a straightforward rationale for immunointervention in overt recent-onset diabetes with the practical advantages of this clinical condition over that of prediabetes in terms of the number of patients needed and the time frame to conduct high-powered controlled studies (easier enrollment of patients without the costly screening for autoantibody positive subjects, application only to patients without previous family T1D history, shorter duration for evaluating the treatment effect). This does not mean that prevention approaches should not be considered. We would indeed encourage major efforts in enrolling patients as soon as diabetes can be detected (several weeks are often wasted before starting immunotherapy). One may hope that the recently reported results with CD3 antibodies will emphasize the urgency of the treatment. In fact, it proved to be much more efficient in patients with good residual β -cell function. One could think of including prediabetic patients defined as 'high risk patients' already presenting infraclinical metabolic dysfunction (as detected after glucose load). We must be more cautious, at least for the time being, concerning prediabetic subjects without detectable alterations of β -cell function. These subjects may already have an immunologically progressing disease but their identification is still complex, laborious and uncertain. Once the efficacy of a given treatment is well-established in overt disease, with a satisfactory risk/benefit ratio, it will be easier to consider treating such subjects.

Importance of combination

Coming back to the case of organ transplantation, it is true that the combination of several immunosuppressive drugs represented a major clinical breakthrough. One should realize, however, that in this case, the drugs used were all essentially acting as non-antigen-specific immunosuppressants. Combining several drugs allows a decrease in the dosage of individual compounds, thus decreasing their toxicity. The problem is different in T1D, where one does not use chemical immunosuppression. In fact, the CD3 antibodies used in NOD mice and man are fully efficacious on their own [7, 27]. Synergy with β -cell antigens has been reported in the mouse, but then one has to use an infra-optimal schedule of CD3-specific antibody treatment, whose advantage over optimal schedules still remains to be proven. In fact, in our opinion the problem is more that of the long-term outcome of responders to CD3 antibody therapy. If in the end the

disease returns, it might be wise to administer soluble β -cell antigens in combination with other forms of immunotherapy. This is an interesting approach, perhaps more attractive than repeated CD3 antibody administration, which could be successful but only if considering sufficiently long intervals and only in patients who are not sensitized to the antibody.

Conclusions

The remarkable efficacy of CD3 antibody therapy in recent-onset T1D is very encouraging. One may hope that results can be improved by recruiting patients at an earlier disease stage. One will also have to evaluate the risks associated with the side effects presently observed, essentially the short-term EBV replication. In any event, these results reiterate the hopes invested in immunotherapy of T1D with the possible goal of a cure for the disease and/or its prevention.

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