Immunotherapy on Trial for New-Onset Type 1 Diabetes

Denise L. Faustman, M.D., Ph.D.

Type 1 diabetes mellitus is an autoimmune disease in which pathologic, autoreactive T cells of the immune system attack the insulin-secreting pancreatic islets of Langerhans. Cytotoxic T cells, which bear the CD8 protein on their membrane, kill islets, thereby leading to lifelong dependence on insulin for affected patients. Over time, poorly controlled blood glucose levels inevitably result in early illness and early death.[1] Invasive blood glucose monitoring, insulin administration, and medical complications of treatment can also themselves cause illness, despite decreased glycemia.

Immunosuppressive agents, one of the mainstays of approved treatments for transplantation, have been explored as therapy for patients with new-onset diabetes. Such agents ordinarily have widespread effects on immune function, leaving children and adults vulnerable to opportunistic infections and other adverse effects. The adverse effects in children sometimes outweigh the benefits, as was learned in clinical trials of the immunosuppressive drug cyclosporine for new-onset diabetes.[2-4] Cyclosporine, a calcineurin inhibitor that lacks immune specificity, succeeded in reducing insulin requirements but had serious adverse effects, particularly nephrotoxicity. Another, more recent trial used intravenous anti-CD3 monoclonal antibodies for new-onset diabetes[5,6]; although promising in that it decreased the insulin requirement for a period after disease onset, the use of anti-CD3 monoclonal antibodies is also accompanied by adverse events, although substantially fewer to date than seen with cyclosporine. Consequently, researchers have sought to identify new, more targeted, and, ideally, preventive treatments aimed at new-onset type 1 diabetes in children. But most previously tested immune therapies for new-onset disease — including oral insulin, Q fever vaccine, methotrexate, and antithymocyte globulin, among others — have shown no or minimal therapeutic benefit.[7]
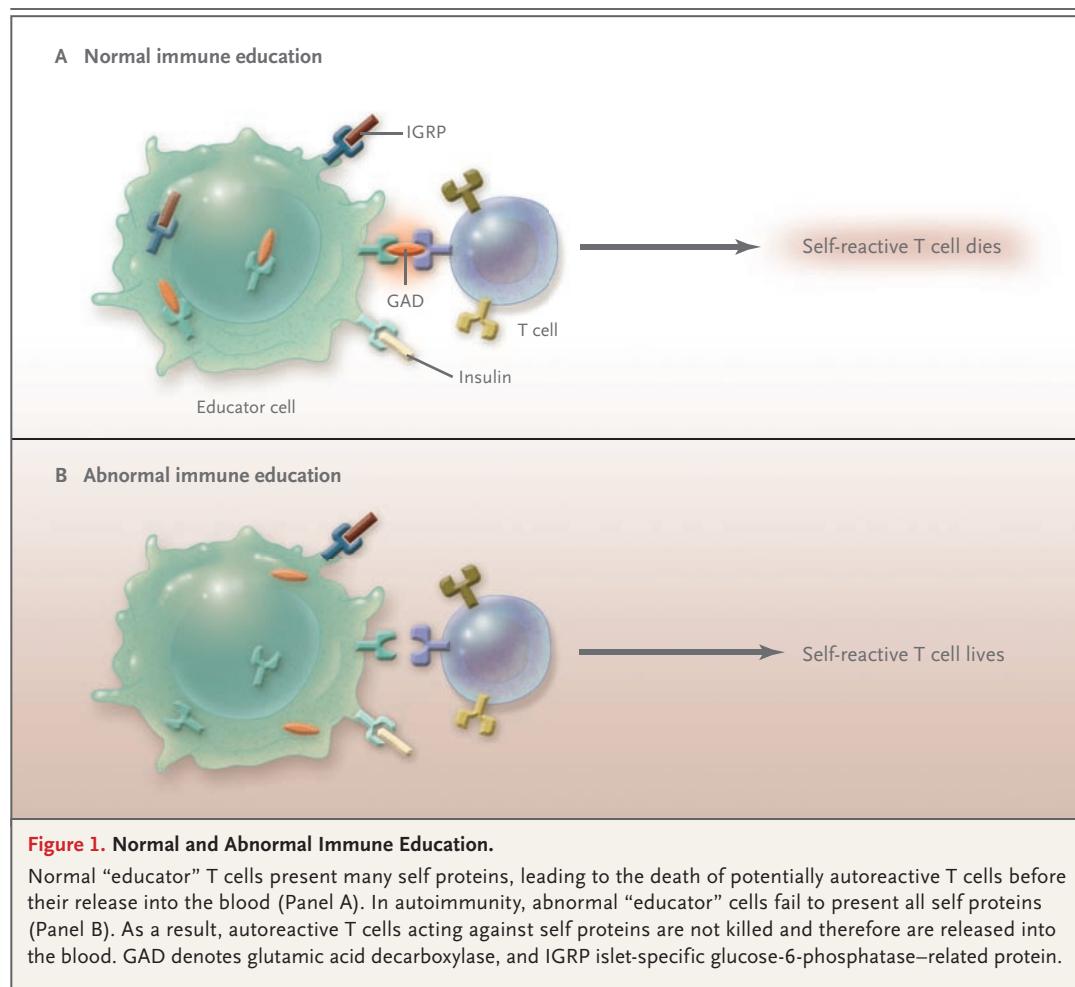
In this issue of the *Journal*, Ludvigsson et al.[8] describe a randomized, controlled clinical trial involving children with new-onset type 1 diabetes to examine the safety and efficacy of immunotherapy with the recombinant human 65-kD isoform of glutamic acid decarboxylase (GAD) in a standard vaccine formulation with alum (GAD-alum) (ClinicalTrials.gov number, NCT00435981).

GAD-alum was given to children 10 to less than 18 years of age who had recent-onset disease; daily doses of insulin were given concurrently. The authors selected GAD for targeted therapy because this autoantigen, when used alone in prehyperglycemic mice, could prevent or slow the progression to hyperglycemia.[9,10]

GAD is a naturally occurring protein found in the brain and in insulin-secreting islets of the pancreas. It is a self protein that functions as an autoantigen in patients with type 1 diabetes or stiff-person syndrome, a disabling neurologic condition.[11] In those with type 1 diabetes, self proteins can be subject to attack not only by autoreactive T cells but also by autoantibodies to GAD and a variety of other self proteins, such as the insulinoma-associated–2 autoantibody (IA-2), insulin autoantibody (IAA), and islet-specific glucose-6-phosphatase–related protein (IGRP), among others.[12]

The abnormal generation of cytotoxic T cells against these self proteins is probably due to defects in the capacity of so-called educator immune cells (e.g., dendritic cells and macrophages) to present the full repertoire of self peptides to newly forming T cells.[13] Educator cells must present thousands of self proteins to prevent the escape of any cytotoxic, autoreactive T cells into the circulation (Fig. 1). Failure to complete this self-antigen education leads to the release of autoreactive T cells and the generation of diverse autoantibodies against self proteins, often after the attacked islet tissue releases its contents. Autoimmunity may also be associated with the failure to generate regulatory T cells, whose role is to keep the immune system in balance, even where there may be rogue cytotoxic T cells that could be prevented from killing by these regulatory cells. In contrast, normal education of self proteins at lymphatic sites destroys autoreactive T cells before newly generated T cells are released from the bone marrow or thymus into the bloodstream (Fig. 1).[13]

Ludvigsson et al. present a study of 70 patients with recent-onset type 1 diabetes who were treated with two subcutaneous injections of a single self protein, GAD-alum, or of a placebo consisting of alum alone. At enrollment, the subjects, all recruited within 18 months after the diagnosis

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Figure 1. Normal and Abnormal Immune Education.

Normal "educator" T cells present many self proteins, leading to the death of potentially autoreactive T cells before their release into the blood (Panel A). In autoimmunity, abnormal "educator" cells fail to present all self proteins (Panel B). As a result, autoreactive T cells acting against self proteins are not killed and therefore are released into the blood. GAD denotes glutamic acid decarboxylase, and IGRP islet-specific glucose-6-phosphatase–related protein.

of diabetes, had preexisting GAD autoantibodies and residual insulin secretion, measured as stimulated C-peptide, a marker of insulin production. The primary efficacy measure chosen for the study was the fasting C-peptide level at 15 months, although the stimulated C-peptide level was also studied. C-peptide is derived from cleavage of the proinsulin molecule and is secreted along with insulin. Its presence reflects the persistence of insulin-secreting islet cells in the pancreas. The fasting C-peptide level was chosen as the primary end point because a previous study of GAD-alum in patients with latent autoimmune diabetes in adults (LADA) found that only fasting, not stimulated, C-peptide levels reflected the inevitable decline to a total lack of pancreatic insulin reserves.

The results indicate that insulin secretion, measured as the fasting C-peptide level, gradually decreased in both the treatment and placebo groups

and that the insulin requirement was unchanged by the study treatment. Treatment with GAD-alum had no significant effect on the fasting C-peptide levels at 15 months. The stimulated C-peptide level, although not the primary outcome measure, showed a lesser decline in the GAD-alum group than in the placebo group. Furthermore, GAD-alum therapy initiated 6 months or more after diagnosis did not show the protective effect on disease progression.

A number of factors affecting the immune response were also examined. GAD-alum administration persistently raised the levels of GAD autoantibody. Even 15 months after the first GAD-alum immunization, peripheral-blood mononuclear cells from treated patients continued in culture to secrete substantial amounts of inflammatory cytokines that, at least in vivo, did not cause apparent symptoms.

We do not know from this study the mecha-

nism by which GAD-alum may beneficially prolong stimulated C-peptide secretion at least for the 15-month study interval. GAD-alum immunization appears to be safe in children with new-onset type 1 diabetes, with no more adverse events in the treated group than in the placebo group. Patients treated with GAD-alum had no adverse neurologic reactions during the study period, addressing a potential concern that an induced rise in GAD autoantibodies might cause stiff-person syndrome, a neurologic disease possibly driven by anti-GAD autoantibodies in the central nervous system.[11]

The study by Ludvigsson et al. raises four important questions for the future. The first concerns the choice of monitoring methods for assessment of the remaining pancreatic function. The ultimate goal of therapeutic efficacy is to reduce or eliminate the insulin requirement. How should pancreatic insulin reserves be measured? Fasting C-peptide, stimulated C-peptide, and basal C-peptide levels are measures of different aspects of the reserves. Which of these measures is the best indicator of early therapeutic response? We do not know why some immune therapies affect only one of these three measures.

Second, it is well established that, in patients with type 1 diabetes, the immune response is driven by reactivity to not just one but many self proteins (Fig. 1). Is the future of immune therapy for new-onset diabetes likely to consist of cocktails designed to generate immune tolerance to most or all such self proteins? Third, in a breed of mouse destined to become diabetic but not yet hyperglycemic, administration of GAD succeeded in preventing the onset of type 1 diabetes.[9,10] Might GAD-alum prevent type 1 diabetes in prediabetic children more effectively than in children already diagnosed with new-onset disease? Indeed, Ludvigsson et al. observed that GAD-alum showed subtle efficacy only when administered to children within the first 6 months after diagnosis, showing no efficacy when administered after that period. This finding suggests that GAD-alum preserves residual pancreatic function but does not restore or even completely preserve pancreatic function.

Fourth, if children with new-onset diabetes are to benefit from immune therapies, should the therapies strive for pancreatic regeneration as well as pancreatic preservation? Animals with end-stage type 1 diabetes and severe hyperglycemia can, under certain conditions, have full regenera-tion, not just rescue, of the pancreas.[14] The full regenerative capacity of the human pancreas is unknown.

Finally, we should never forget that we are caring for children with a chronic disease. Before we think of efficacy, we must give first consideration to the short- and long-term safety of any immune intervention.

From the Immunobiology Laboratory, Massachusetts General Hospital and Harvard Medical School, Boston.

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