# Immunotherapy for Type 1 Diabetes Takes Aim at Autoreactive T Cells

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We are on the cusp of treating type 1 diabetes with a new generation of highly targeted immune therapies. Current therapies—insulin, tight glucose monitoring, and treatment of complications—are inadequate to control the disease's morbidity and early mortality.[1] Patients still experience high rates of the same complications as those with type 2 diabetes: heart disease, renal failure, stroke, blindness, and amputations, among others. The severity of these complications is compounded by the earlier onset of type 1 diabetes.

High expectations have not yet been realized for a 20-year effort to transplant insulin-secreting islet cells into the pancreas. The transplanted islets eventually succumb to recurrent autoimmune attack perhaps even at accelerated rates with long-standing disease. This was vividly learned in part by the rapid recurrent autoimmune disease on genetically identical twin pancreatic islets.[2,3] Newer immune therapies, although promising, have not achieved insulin independence.[4] One important approach is to evade autoimmune attack by protecting self-peptides (ie, autoantigens), the specific autoantigens on the surface of an islet cell that trigger the autoimmune attack, which, in turn, kills the entire cell. Autoantigens thought to be dominant are insulin, pro-insulin, glutamic acid decarboxylase, (GAD), and islet-specific glucose-6-phosphatase catalytic subunit-related protein.

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Among several immune therapies tested in clinical trials, the most noteworthy is by Ludviggson and colleagues[5] who established the safety of at least one therapy of this kind, GAD. Efficacy was modest, however, and only visible by biochemical functioning of the pancreas, not a change in insulin dosing. GAD is certainly an important autoantigen, yet because its efficacy is modest, there is no consensus on which, if any, of the identified autoantigens should be protected, whether alone or in combination, and, if so, at what stage of disease, at what dose, and at what route of administration. The single-antigen approach favored by the US Food and Drug Administration for testing may not be workable for patients with advanced disease, epitope spreading, and unknown risk factors or even evolving epitope spreading.

Our immunological treatment is more direct. Its purpose is to selectively destroy the cells responsible for the attack: the autoreactive T lymphocytes, or T cells, that are cytotoxic (marked by surface glycoprotein CD8). By

destroying only autoreactive T cells while preserving other immune cells, we are adopting the time-honored strategy of exploiting the enemy's most vulnerable defect. This strategy is the culmination of years of basic research undertaken by our laboratory and others in the nonobese diabetc mouse model of type 1 diabetes to elucidate the disease's pathogenesis.[6-8] Over the last decade, autoreactive T cells have been shown to display specific functional and genetic defects in animals and in human blood samples.[9] That body of evidence has enabled us to enter and complete phase 1 clinical trials with a novel agent this year and progress within a short time period to phase 2 testing. Specifically, the agent is bacille Calmette-Guérin (BCG), a vaccine developed to treat tuberculosis in the early 20th century and now used at very high doses for bladder cancer among other applications.

BCG is ideal for type 1 diabetes based on our understanding of the mechanisms of disease. It induces secretion of the immunomodulatory cytokine tumor necrosis factor (TNF). TNF specifically kills autoreactive T cells but not normal immune cells, according to our studies and other supportive studies in an animal model of type 1 diabetes.[10-12] BCG killed autoreactive T cells, in a dose-dependent manner and at all stages of disease—not just in new-onset cases.[6,7] To understand why TNF successfully destroys autoreactive T cells requires taking a deeper look at abnormalities within autoreactive T cells and in the microenvironment surrounding them that attacks these killer T cells.

## DEFECTS IN AUTOREACTIVE T CELLS AND THEIR MICROENVIRONMENT

In healthy individuals, autoreactive T cells are destroyed during development. With autoimmune disease, the autoreactive T cells evade death during development in the thymus or bone marrow (a topic returned to later). For now, it's important to point out that once autoreactive T cells escape death and enter the circulation, they remain vulnerable to TNF-induced death by virtue of a defect in protein processing, especially if they are highly activated. Upon exposure to TNF, the defect in protein processing prevents a key protein—the transcription factor nuclear factor-kappa B (NF-kB)—from migrating from the cell body into the nucleus, where it turns on genes responsible for cell survival. Without activation of prosurvival genes by NF-kB, autoreactive T cells die upon encountering TNF in their microenvironment.[8] This problem does not apply to normal T cells or other types of cells that either constitutively express NF-kB, lack a TNF receptor, or possess other pathways for activating cell survival genes.

The specific defect in autoreactive T cells involves their proteasomes, which are proteolytic complexes in the cell body formed by multiple proteins. In this case, the proteasome cleaves off NF-kB from a much larger protein. Normal proteasome cleavage in T cells releases NF-kB, allowing it to migrate into the nucleus and activate prosurvival genes. Failure to cleave off from the larger protein prevents NF-kB's migration into the nucleus. Studying cultured immune cells from our mouse model, we found that autoreactive T cells, but not normal immune T cells or other cells, have no expression or reduced expression of latent membrane protein 2, a protein that forms one critical subunit responsible for proteasome functioning.[13] Another problem that lowers the levels of TNF translation is proteasomal blockage, which alters TNF mRNA instability.[14]

Low levels of TNF in the microenvironment of an autoreactive T cell are not sufficient to affect their survival, but higher levels can kill this specific subpopulation of cells. The survival of autoreactive T cells is dose-dependent, according to findings revealing that repeated doses of BCG, which induces TNF, are more effective at destroying autoreactive cells than is a single dose.[15] When TNF is administered systemically, it suppresses or precludes the onset of autoimmune disease in several animal models.[13,16-18] It is not known why TNF levels are low in autoimmunity or why at least free TNF levels are low. Research has determined that TNF levels are low in both animals and humans with autoimmune disease.[19] It is speculated that low activity may be the consequence of gene polymorphisms that reduce TNF expression or alter its production. Low TNF levels might also be a consequence of excessive expression of soluble receptors for TNF. TNF in the circulation would bind to the receptors, rendering TNF less bioavailable.[19] In other words, circulating receptors would bind to and inactivate TNF before it would have the opportunity to kill autoreactive T cells.

## DEFECTS IN T-CELL EDUCATION

Why are autoreactive T cells in the circulation in the first place? Autoreactive T cells, in healthy individuals, are not found in the circulation; they are killed during development and during life in the bone marrow and the thymus in a process known as *T-cell education*. So-called educator cells—also known as *antigen-presenting cells*—are supposed to bind self-peptides and T cells are supposed to bind this range of self-peptides with T cells exclusively reactive to each self-peptide. The T cell binding to the antigen-presenting cells kills autoreactive T cells before they reach the circulation. In individuals who have autoimmune disease, however, T-cell education goes awry. Educator cells fail to show the full complement of

self-peptides, which in the case of diabetes, are antigens specific to islet cells.[20] That failure in their education enables autoreactive T cells to evade death and to be released in the circulation. Once these autoreactive T cells enter the circulation, they become activated and cytotoxic when they encounter autoantigens on islet cells. The rogue T cells, we have discovered, still remain vulnerable to destruction by TNF as a consequence of their protein processing defect.

The attractiveness of BCG is that it induces release of TNF in the host. It has additional advantages of being inexpensive and effective at all stages of the disease, even in end-stage diabetes, according to findings in our animal model.[6,7]

## TREATMENT IMPLICATIONS

We are testing BCG in clinical trials as the first medication of its kind to treat diabetes by selectively targeting autoreactive T cells. These trials are also unique in treating individuals with type 1 diabetes who have full-fledged disease, not just an immune intervention in recent-onset type 1 diabetes. Therefore, the aim of these clinical trials is not to slow the inevitable insulin decline but rather to actually revive the pancreas and restore insulin secretion. BCG (as TNF inducer) rather than TNF itself is being used because it is known as the world's safest vaccine and has minimal systemic toxicity if used in people without AIDS or other forms of immunosuppression, either drug induced or genetic forms of immunocompromise.

BCG works in part by inducing antigen-presenting cells to secrete low levels of TNF. The primary outcome of efficacy in trials will be graded by the killing of autoreactive T cells and by the actual restoration of insulin secreting from the pancreas. Again, the trials in BCG are unique in trying to take a pancreas with no basal or induced insulin and restore insulin secretion from the organ.

Building on animal studies, we are also seeking to find a less toxic analog of TNF that is not solely a vaccine. Reasoning that TNF activates T cells via one of two receptors, TNFR1 and/or TNFR2, we studied several agonists for TNFR2. This receptor is less ubiquitously expressed than is TNFR1. One of the TNFR2 agonists we tested killed purified CD8 T cells, but not CD4 cells, in nearly 400 samples of human blood from type 1 diabetes patients.[9] The subset of CD8 cells being killed were autoreactive to insulin, one of the foremost autoantigens. The drug also displayed a dose-response pattern, and it was just as efficacious in killing autoreactive T cells as was TNF.

It must be pointed out, however, that administering TNF for a one-time treatment is not likely to be limited to single dosing. In animal studies, a one-time treatment period without lifelong treatment only afforded a temporary pause in the disease prior to a reoccurence.

By targeting autoreactive T cells and now being able to measure these autoreactive T cells in blood, our goal is to treat or possibly induce permanent remission in patients with even the most intractable type 1 diabetes and at its end stages. We have translated our animal testing into an ongoing human clinical trial with an inexpensive agent that is already on the market for other purposes thus hastening the testing of this new application in clinical trials. Lastly, we hope to also prevent the onset of type 1 diabetes in its preclinical stages, a long-standing goal that may be feasible if end-stage disease can be reversed. ■

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1. Dorman JS, et al. The Pittsburgh insulin-dependent diabetes mellitus (IDDM) morbidity and mortality study. Mortality results. Diabetes. 1984;33:271–276.
2. Sutherland DE, et al. Twin-to-twin pancreas transplantation: reversal and reenactment of the pathogenesis of type I diabetes. Trans Assoc Amer Phys. 1984;97:80–87.
3. Sutherland DE, Goetz FC, Kendall DM, Najarian JS. One institution's experience with pancreas transplantation. West J Med. 1985;143:838–844.
4. Bresson D, von Herrath M. Moving towards efficient therapies in type 1 diabetes: to combine or not to combine? Autoimmun Rev. 2007;6:315–322.
5. Ludvigsson J, et al. GAD treatment and insulin secretion in recent-onset type 1 diabetes. N Engl J Med. 2008;359:1909–1920.
6. Ryu S, et al. Reversal of established autoimmune diabetes by restoration of endogenous beta cell function. J Clin Invest. 2001;108:63–72.
7. Kodama S, et al. Islet regeneration during the reversal of autoimmune diabetes in NOD mice. Science. 2003;302:1223–1227.
8. Hayashi T, Faustman D. NOD mice are defective in proteasome production and activation of NF- kappaB. Mol Cell Biol. 1999;19:8646–8659.
9. Ban L, et al. Selective death of autoreactive T cells in human diabetes by TNF or TNF receptor 2 agonism. Proc Natl Acad Sci U S A. 2008;105:13644–13649.
10. Qin HY, Chaturvedi P, Singh B. In vivo apoptosis of diabetogenic T cells in NOD mice by IFN-{gamma}/TNF-{alpha}. Int Immunol. 2004;16:1723–1732.
11. Hayashi T, Kodama S, Faustman DL. Reply to 'LMP2 expression and proteasome activity in NOD mice'. Nat Med. 2006;6:1065–1066.
12. Christen U, et al. A dual role for TNF-alpha in type 1 diabetes: islet-specific expression abrogates the ongoing autoimmune process when induced late but not early during pathogenesis. J Immunol. 2001;166:7023–7032.
13. Kodama S, Davis M, Faustman DL. Diabetes and stem cell researchers turn to the lowly spleen. Sci Aging Knowledge Environ. 2005, pe2.
14. Deleault KM, Skinner SJ, Brooks SA. Tristetraprolin regulates TNF TNF-alpha mRNA stability via a proteasome dependent mechanism involving the combined action of the ERK and p38 pathways. Mol Immunol. 2008;45:13–24.
15. Shehadeh N, et al. Repeated BCG vaccination is more effective than a single dose in preventing diabetes in non-obese diabetic (NOD) mice. Isr J Med Sci. 1997;33:711–715.
16. Satoh J, Seino H, Abo T. Recombinant human tumor necrosis factor a suppresses autoimmune diabetes in nonobese diabetic mice. J. Clin Invest. 1989;84:1345–1348.
17. Grewal I S, et al. Local expression of transgene encoded TNF alpha in islets prevents autoimmune diabetes in non-obese diabetic (NOD) mice by preventing the development of autoreactive islet specific T cells. J Exp Med. 1996;184:1963–1974.
18. Sadelain M W, et al. Prevention of diabetes in the BB rat by early immunotherapy using Freund's adjuvant. Journal of Autoimmunity. 1990;3:671–680.
19. Loetscher H, Steinmetz M, Lesslauer W. Tumor necrosis factor: receptors and inhibitors. Cancer Cells. 1991;3:221–226.
20. Faustman D, Li XP, Lin HY, Linkage of faulty major histocompatibility complex class I to autoimmune diabetes. Science. 1991;254:1756-1761.