Are T-Cell Responses to GAD65 Influential in Type 1 Diabetes?

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Autoimmune diabetes in the popular nonobese diabetic (NOD) mouse results from a T-cell–mediated destruction of the insulin-producing β-cells (1) and serves as a model for human type 1 diabetes (2,3). The mechanisms that initiate early peri-islet inflammation and the destructive components that arise later specifically targeting β-cells occur naturally in NOD mice (2,3). Two obstacles hinder our progress in unraveling the rudiments of type 1 diabetes: *1*) the identification of the antigens involved in the earliest stages of autoimmunity and *2*) the event(s) that provokes the initial autoimmunity and loss of homeostasis in the islets. The hope has been that the former would aid in sorting out the latter.

The NOD mouse provides us with a framework for understanding T-cell–mediated autoimmune diseases in general. Discovering the nature of the initial organ-infiltrating cells is a key to the prevention of inflammatory autoimmune disease. Does spontaneous insulitis, which precedes type 1 diabetes, begin by a random accumulation of lymphocytes into the pancreatic islets, or is there an essential temporal pattern to the activation and recruitment of organ-specific lymphocytes? An answer to this question seems essential to our grasping the complexities of disease susceptibility and onset. Infectious agents have long been postulated to have a role as incendiary triggers for abnormal tissue inflammation and the genesis of a tissue-specific adaptive autoimmune/immune response. Either through molecular mimicry (4,5) or cytopathic means (6), an invading microorganism could create an environment of sufficient driving force to overcome innate tolerance to self-molecules and tissues. Although tempting, the requisite "clean environment" needed to maintain a high incidence of diabetes in a NOD colony is at odds with the infectious disease concept. Alternatively, normal cellular turnover during tissue remodeling has become a favored hypothesis (7). In this scenario, tissue-associated antigens would be released from β-cells as an unintended consequence of organ development, leading to the priming and activation of islet-reactive T-cells in susceptible individuals.

Several islet antigens are able to solicit immune responses in naive NOD mice, including GAD65, one of a few β-cell proteins known to induce autoantibody; Th cells; and cytotoxic T-lymphocytes in pre-diabetic mice (8,9) and in recent-onset type 1 diabetic humans (10,11). Treatments that tolerize, deviate, or alter the anti-GAD65 response in NOD mice typically delay or prevent insulitis and diabetes (8,9). Interestingly, the immune deviation associated with GAD65-induced protection often spreads to other islet specificities as well (12), further supporting the view of a temporal pattern of immune responses in the initial stages of type 1 diabetes. Part of the charm of the molecular mimicry model is the provision of a single epitope as the initiator, which could likely manifest as a sequential pattern of autoimmunity as described in the evolution of autoimmunity in young NOD mice (8,9). However, if tissue remodeling is indeed the inciting event for β-cell autoimmunity, then we are left to explain why GAD65-specific responses preferentially expand to detectable levels early (Fig. 1) and why they can become influential to other antigenic specificities.

Previously, Jaeckel et al. (13) created NOD.GAD65.tg mice to test the premise that GAD65-specific immunity is requisite for the progression of type 1 diabetes. By placing a modified form of GAD65 under the control of the invariant chain promoter, the researchers were indeed able to suppress the response to GAD65 without altering the course of type 1 diabetes, which not only challenged the notion of GAD65 as a necessary autoantigen but to some it cast doubts on the antigen's ability to contribute to any phase of the disease. To confirm the putative tolerance, they analyzed T-cell responses following immunization with rGAD65. In this issue of *Diabetes* (14), two labs have collaborated and enhanced the examination of the spontaneous T-cell responses to β-cell antigens in NOD.GAD65.tg mice. Tian et al. (14) found the responses to GAD65 were significantly altered in all age-groups of the transgenic mice but were detectable nonetheless. Intriguingly—but consistent with previous reports from Tian et al. (12)—the blunted responses to GAD65 appear to have an age-dependent impact on other β-cell specificities. In addition, their results suggest that absolute central tolerance to GAD65 is elusive in NOD mice, even when extraordinary efforts are taken to express the antigen throughout development and in the manner expected to induce deletion of the cognate-specific T-cells. This would seem to highlight the importance of peripheral tolerance, as noted by the authors, and perhaps explain why treatment with GAD65/GAD65 peptides has been fairly effective in reducing the incidence of type 1 diabetes in NOD mice, in contrast to transgenic expression models that have produced conflicting results (13,15,16).

Clearly, a robust T-cell response to GAD65 is not required for type 1 diabetes in NOD mice (13,17). Yet, does this disqualify the antigen as a contributor to type 1 diabetes or as a viable target in immunotherapy? What remains is a need to advance our understanding of the mechanisms that create a connection between GAD65

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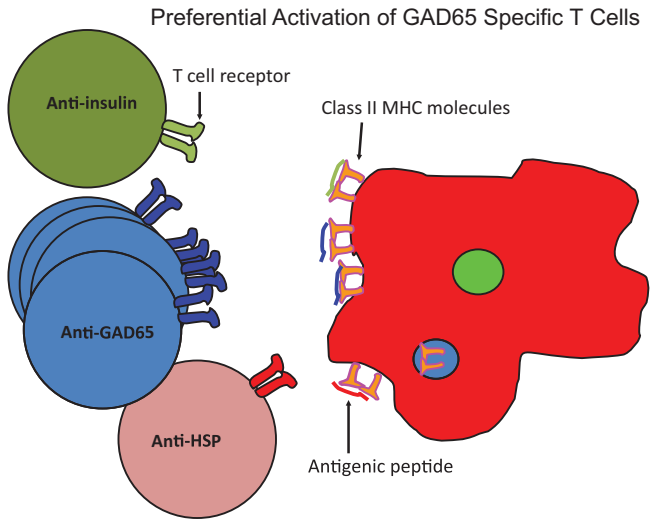

FIG. 1. Following the release of β-cell antigens, the frequency of GAD65-reactive T-cells expands in the pancreatic nodes of young NOD mice, reaching detectable levels ahead of clonotypes specific for other islet antigens. Inadequate tolerance, due in part to limited expression in the periphery and/or enhanced antigen presentation, may help drive the preferential expansion of clones that recognize dominant GAD65 epitopes.

immunity and the activation/expansion (17) and, most important, the regulation (12) of other autoreactive T-cells. The earliest events in islet inflammation lead to the propagation of diabetogenic T-cells; thus, prevention necessitates knowledge of the steps that occur prior to the recruitment of these effectors.

The notions of tissue remodeling and sequential expansion of antigen-specific T-cell repertoires may seem incompatible. However, if we cease to assume that all antigens are "created equal" and take into consideration the powerful influence of antigenic vigor, we can reconcile that the simultaneous release of antigen is not synonymous to simultaneous priming. Perhaps GAD65 is one of the more immunogenic islet antigens because GAD65 peptides and plasmids readily recruit adaptive immune responses. Human GAD65 is less soluble than its isomeric counterpart GAD67 and predominantly bound to vesicular membranes (sequestered) in the cell (18,19), features that could enhance GAD65's capture by antigen-presenting cells. GAD65's influence in type 1 diabetes may relate more to its ability to overcome tolerance, activate cognate-specific T-cells, and dictate the milieu of the islets rather than to its direct diabetogenic potential (Fig. 1).

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REFERENCES

1. Delovitch TL, Singh B. The nonobese diabetic mouse as a model of autoimmune diabetes: immune dysregulation gets the NOD. Immunity 1997;7:727–738
2. Cahill GF Jr, McDevitt HO. Insulin-dependent diabetes mellitus: the initial lesion. N Engl J Med 1981;304:1454–1465
3. Cahill GF Jr. Diabetes mellitus: an overview. Curr Concepts Nutr 1981;10:145–151
4. Honeyman MC, Coulson BS, Stone NL, Gellert SA, Goldwater PN, Steele CE, Couper JJ, Tait BD, Colman PG, Harrison LC. Association between rotavirus infection and pancreatic islet autoimmunity in children at risk of developing type 1 diabetes. Diabetes 2000;49:1319–1324
5. Atkinson MA, Bowman MA, Campbell L, Darrow BL, Kaufman DL, Maclaren NK. Cellular immunity to a determinant common to glutamate decarboxylase and coxsackie virus in insulin-dependent diabetes. J Clin Invest 1994;94:2125–2129
6. Horwitz MS, Bradley LM, Harbertson J, Krahl T, Lee J, Sarvetnick N. Diabetes induced by Coxsackie virus: initiation by bystander damage and not molecular mimicry. Nat Med 1998;4:781–785
7. Turley S, Poirot L, Hattori M, Benoist C, Mathis D. Physiological beta cell death triggers priming of self-reactive T cells by dendritic cells in a type-1 diabetes model. J Exp Med 2003;198:1527–1537
8. Tisch R, Yang XD, Singer SM, Liblau RS, Fugger L, McDevitt HO. Immune response to glutamic acid decarboxylase correlates with insulitis in non-obese diabetic mice. Nature 1993;366:72–75
9. Kaufman DL, Clare-Salzler M, Tian J, Forsthuber T, Ting GS, Robinson P, Atkinson MA, Sercarz EE, Tobin AJ, Lehmann PV. Spontaneous loss of T-cell tolerance to glutamic acid decarboxylase in murine insulin-dependent diabetes. Nature 1993;366:69–72
10. Nepom GT. Conversations with GAD. J Autoimmun 2003;20:195–198
11. Mallone R, Kochik SA, Laughlin EM, Gersuk VH, Reijonen H, Kwok WW, Nepom GT. Differential recognition and activation thresholds in human autoreactive GAD-specific T-cells. Diabetes 2004;53:971–977
12. Tian J, Clare-Salzler M, Herschenfeld A, Middleton B, Newman D, Mueller R, Arita S, Evans C, Atkinson MA, Mullen Y, Sarvetnick N, Tobin AJ, Lehmann PV, Kaufman DL. Modulating autoimmune responses to GAD inhibits disease progression and prolongs islet graft survival in diabetes-prone mice. Nat Med 1996;2:1348–1353
13. Jaeckel E, Klein L, Martin-Orozco N, von Boehmer H. Normal incidence of diabetes in NOD mice tolerant to glutamic acid decarboxylase. J Exp Med 2003;197:1635–1644
14. Tian J, Dang H, von Boehmer H, Jaeckel E, Kaufman DL. Transgenically induced GAD tolerance curtails the development of early β-cell autoreactivities but causes the subsequent development of supernormal autoreactivities to other β-cell antigens. Diabetes 2009;58:2843–2850
15. Yoon JW, Yoon CS, Lim HW, Huang QQ, Kang Y, Pyun KH, Hirasawa K, Sherwin RS, Jun HS. Control of autoimmune diabetes in NOD mice by GAD expression or suppression in beta cells. Science 1999;284:1183–1187
16. Geng L, Solimena M, Flavell RA, Sherwin RS, Hayday AC. Widespread expression of an autoantigen-GAD65 transgene does not tolerize non-obese diabetic mice and can exacerbate disease. Proc Natl Acad Sci U S A 1998;95:10055–10060
17. Kanazawa Y, Shimada A, Oikawa Y, Okubo Y, Tada A, Imai T, Miyazaki J, Itoh H. Induction of anti-whole GAD65 reactivity in vivo results in disease suppression in type 1 diabetes. J Autoimmun 2009;32:104–109
18. Christgau S, Schierbeck H, Aanstoot HJ, Aagaard L, Begley K, Kofod H, Hejnaes K, Baekkeskov S. Pancreatic beta cells express two autoantigenic forms of glutamic acid decarboxylase, a 65-kDa hydrophilic form and a 64-kDa amphiphilic form which can be both membrane-bound and soluble. J Biol Chem 1991;266:21257–21264
19. Kaufman DL, Houser CR, Tobin AJ. Two forms of the gamma-aminobutyric acid synthetic enzyme glutamate decarboxylase have distinct intraneuronal distributions and cofactor interactions. J Neurochem 1991;56:720–723