Control of Autoimmune Diabetes

We have several questions about Yoon et al.’s (1) report. The degree to which anti-glutamic acid decarboxylase (GAD) was identified by immunohistochemistry and Western blotting of islets from standard (transgene negative) animals was remarkably high, especially in view of the accompanying figure 1 [in (1)] involving insulin staining. Most studies suggest that GAD is a minute quantity in mouse islet cells (2). The diabetes frequency and immune response data from antisense animals may be questionable because it is unclear whether Yoon et al. performed genetic analyses to ensure that disease resistance alleles of donor strain origin were not co-transferred with the transgene to the NOD background or whether the transgenes were not inserted into regions vital to the formation of the disease (3). We are concerned that the observed diabetes resistance may result from factors other than the GAD antisense transgene. Yoon et al.’s data show that T cells from these mice did not transfer disease to NOD-scid recipients. If the transgene was only expressed in pancreatic β cells, it is unclear how this could alter T cell selection and block the development of diabetogenic effectors. While the target antigen may have been absent in pancreatic β cells, GAD autoreactive T cells should still have been generated in the transgenic mice that were capable of initiating diabetes development upon transfer into NOD-scid recipients. Potential metabolic changes afforded by the introduction of antisense GAD into β cells and their effect on immunogenicity at a local and/or systemic level were not fully considered by Yoon et al. Altering the metabolic physiology of NOD mice can modify the frequency of disease and degree of insulitis (4).

Finally, why islet cells from the GAD antisense animals were not destroyed when transplanted into overtly diabetic NOD recipients remains unclear. Through antigenic spreading, recipient animals should have contained populations of lymphocytes reactive to non-GAD β cell antigens capable of inducing type 1 disease (5). Caution should be exercised in assigning the therapeutic and pathogenic significance of this work until these issues are adequately addressed.

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tastain Elite ABC kit, which became a sensitive method for the detection of minute amounts of GAD antigen in cells. A different protocol was used for insulin staining of serial sections because an amplification procedure was not required. The GAD and insulin antibody reaction was optimized for each antigen and differed with respect to incubation times for the reaction with specific primary antibodies and for color development. In short, the GAD staining shown in figure 1 [of (1)] cannot be compared directly with the insulin staining. Our method for detection of GAD protein by Western blot analysis was also adapted to detect minute quantities of GAD in islets. We applied a highly sensitive biotin-streptavidin-peroxidase method with a chemiluminescence system to amplify the signal to detect GAD protein. Band intensity was amplified by adjustment of the exposure time to x-ray film.

We were also aware of the possible cotransfer of diabetes resistance alleles originating from the donor strain along with the transgene. We therefore established six lines of antisense GAD transgenic NOD mice, two of which showed protection from diabetes and from diabetogenic effector cells. These two lines showed no detectable GAD in β cells, whereas those lines with some degree of expression of GAD in β cells developed diabetes, even though the antisense transgene was incorporated into the genomic DNA. These findings suggest that our observations are a consequence of β cell GAD suppression rather than transfer of diabetes resistance alleles to NOD mice. Further support comes from studies using cyclophosphamide (CY) (2). When diabetes-protected antisense GAD transgenic (H-AS-GAD) NOD mice were treated with CY, two out of five became diabetic, presumably by activation of T cells reactive to other (non-GAD) β cell antigens that are present but not sufficient to cause disease. However, we cannot absolutely exclude the possibility of the transfer of a diabetes resistance gene.

Atkinson et al. questioned why T cells from antisense GAD transgenic mice did not transfer diabetes to NOD-scid recipients. We cannot answer this question with certainty. We suggest that if islet-reactive T cells are present in the nondoniabetic antisense GAD transgenic NOD mice, then the number of these cells is insufficient to cause disease. Our published data support this view, as splenic T cells from antisense GAD transgenic mice did not proliferate against GAD and show reduced responses to other antigens. These findings are consistent with data suggesting that GAD is not expressed in the pancreatic α, δ, and pancreatic polypeptide cells of mice and rats (3) and is expressed in pancreatic β cells and in the brain, it being an immunologically privileged site. Thus, we assumed that in the absence of GAD expression in the β cells in H-AS-GAD-NOD mice, β cell–specific GAD-reactive T cells either do not develop or remain naïve.

With regard to the protection of GAD-suppressed islets from destruction by effector T cells present in acutely diabetic NOD mice, we, too, are surprised at this observation and agree that a conclusion that GAD is the sole antigen initiating the disease process should be made with caution. There are several possible explanations that we did not adequately address in our report. First, other T cells directed at non-GAD islet cell antigens may not have been sufficient (in the absence of GAD-reactive T cells) to induce recurrence of diabetes, although they did destroy a portion of the β cell mass. Approximately 20% of the GAD-suppressed islets showed insulitis. Although there are a variety of T cells recognizing other (non-GAD) autoantigens and diabetes can be produced by many of these different β cell–reactive T cell lines and clones, the T cells in spontaneously diabetic NOD mice are different from amplified T cells grown in vitro, thus precluding direct comparison. Second, the suppression of GAD in β cells may have rendered them more resistant to destruction by T cells and/or macrophages. Normal β cells expressing GAD show expression of tissue transglutaminase (tTG), which is known to promote apoptosis of cells. Our preliminary studies indicate that our GAD-suppressed β cells also show suppression of tTG. Thus, the inhibition of tTG in GAD-suppressed β cells may have rendered the β cells resistant to death by means of apoptotic mechanisms. A third possibility is that a diabetes resistance gene from the strain of origin may have been transmitted to NOD mice along with the transgene, making islets from the antisense GAD transgenic mice more resistant to effector cells from NOD mice. We think this is less likely. Finally, it is possible that the suppression of GAD in β cells induce some alteration in intra-islet physiology and that this has an effect on the capability of the β cell to trigger autoimmunity. We agree with Tian and Kaufman that GABA deficiency in the islet might have reduced β cell metabolism causing “β cell rest” or that the lack of GABA in the islet might have influenced effector/regulatory T cell interactions locally within the islet. Both the physiologic role of GABA in the islet and the effect of intra-islet GABA on islet autoimmunity remain unknown.

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