Response: Although it is widely established that type I diabetes is T cell–dependent, the nature of the target autoantigen (or autoantigens) and the precise mechanism (or mechanisms) by which the β cell is specifically destroyed are unknown. According to one scheme of diabetes pathogenesis, CD4+ T cells function as helper cells for the production of CD8+ T cells that damage β cells by a direct cytotoxic attack (1). This classical model implies that specific cytotoxic CD8+ T cells directly attack β islet cells, and it provides an explanation for the selectivity of the process of β cell destruction and for the dual requirement for CD4+ and CD8+ cells in the initiation of disease. However, more recent studies have demonstrated that islet damage may not be the result of a direct interaction between CD8+ T cells and the target β cell (2). On the basis of these findings, it has been proposed that β cell killing occurs through an "indirect pathway" from a nonspecific inflammatory response that initially involves CD4+ cells. Finally, it is possible that macrophage infiltration itself may be directly responsible for the dysfunction and death of the β cells through the release of cytokines and free oxygen radicals that may be selectively cytotoxic to β cells (3). In this latter scenario, functional T cells would still be recruited to the lesion, but the specificity of individual cells would not be essential.

The literature on NOD mice has emphasized the antigen-driven etiology of diabetes and intensive efforts have been made trying to determine whether a restricted TCR repertoire is required for the initiation of the process. TCR repertoire restriction would suggest that the presence of a single (or few) peptide epitopes of a single antigen would result in the expansion of T cells bearing specific TCR sequences that are critical to the pathogenesis of disease. Our aim in these experiments (4) was to determine whether autoimmunity would be influenced in transgenic NOD mice that were capable of expressing predominantly disease-unrelated TCR β and αβ chain genes. We reasoned (4) that if, in diabetes, pathogenic T cells recognized a very limited set of peptide epitopes, autoantigen recognition would depend on a few specific αβ TCRs and that depleting these receptors would have protected against disease.

Our transgenic studies (4) indicated that, at a minimum, the development of diabeticogenic T cells in NOD mice was the result of a redundant T cell repertoire that was not defined by particular TCR αβ combinations. Our studies suggest that the exact specificity of the TCR β chain may be unimportant and that T cells bearing a multitude of β chains, including an antigenically irrelevant transgenic β chain, might cause disease.

Although Benoist and Mathis suggest that the ratio of CD4+ to CD8+ should have been preserved in our single β chain mice and skewed in our αβ transgenic mice, we have found that the opposite occurred. The CD4/CD8 ratio was skewed toward CD8+ in our single TCR β chain mice (1.0 ± 0.11 SEM, n = 3), whereas in the double αβ TCR NOD mice the ratio was normal (2.0 ± 0.20 SEM, n = 3) and similar to that in nontransgenic controls (2.5 ± 0.17 SEM, n = 4). We know of no studies of TCR transgenic mice in which the α and β chains have been derived from T cell clones of different antigen and MHC specificities, and it is difficult to predict in which direction (CD4 or CD8), if any, T cell development would be biased. Our TCR αβ transgenic NOD mice differ from our single β chain transgenic NOD mice, which suggests (but does not prove) a functional effect of the α chain transgene.

Although we could demonstrate that the β chain transgene was expressed on the vast majority of T cells, we did not have an antibody that specifically recognized our transgenic α chain, nor was it technically possible to determine the α chain transgene surface expression or the diversity of the α chain repertoire by biochemical techniques, as we discussed (4). The few Vα monoclonal antibodies that were available were not useful because of the low (<1%) baseline expression of these Vα families in control NOD mice. We therefore quantitated the endogenous TCR repertoire in lymph node mRNA by anchored PCR. These studies revealed that 83% of the Cα positive plasmid colonies expressed the Vα3.1 transgene (Vα3.1 was not detectable in nontransgenic control NOD mice). To examine these frequencies at a cellular level, we generated a large panel of hybridomas by fusing splenic T cells from our transgenic and nontransgenic mice with the TCR (αβ)− thymoma cell line (4). These studies showed transgenic α chains frequencies (86%) similar to those in anchored PCR and demonstrated the consistent coexpression of TCR α and β transgenes within individual cells (4). Although our transcriptional data are consistent with abundant surface expression, the presence of transcripts may not guarantee cell surface expression [as our colleagues point out in regard to a recent paper (5)].

Definitive resolution of the issue of surface expression of the α chain transgene, however, awaits long-term studies to breed the RAG mutation onto the NOD background.

Our data, as we have stated (4), suggests that markedly skewing the TCR repertoire may not diminish the progression to autoimmunity in NOD mice. We did not state that "the T cells of these transgenic mice bear exclusively the transgenic TCRαβ . . ." or that we have generated a "monoclonal" mouse. Because ablating totally the expression of endogenous TCR genes by the transgenic approach is not possible, we are deriving RAG-deficient αβ TCR transgenic NOD mice. The analysis of these mice, devoid of endogenous TCR rearrangements, may enable us to further define the TCR requirements for the initiation of islet autoimmunity.

Myra A. Lipes
Research Division,
Joslin Diabetes Center,
Boston, MA 02215
Anthony Rosensweig
Vascular Research Division,
Brigham and Women's Hospital,
Boston, MA 02115
Kut-Nie Tan
Dana-Farber Cancer Institute,
Boston, MA 02115
Gary Tanigawa
Program in Cellular Biochemistry and Biophysics,
Memorial Sloan-Kettering Cancer Center,
New York, NY 10021
Jonathan G. Seidman
Department of Genetics,
Harvard Medical School,
Boston, MA 02115
George S. Eisenbarth
Barbara Davis Center for Childhood Diabetes,
Denver, CO 80262

REFERENCES

15 April 1993; accepted 25 May 1993

14 April 1993, accepted 25 May 1993
Response
Myra A. Lipes, Anthony Rosenzweig, Kut-Nie Tan, Gary Tanigawa, Jonathan G. Seidman and George S. Eisenbarth

Science 262 (5139), 1584.
DOI: 10.1126/science.262.5139.1584