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Response to Comment on Nishio *et al.* on Diabetes Reversal in NOD Mice

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Contrary to previous findings, we found no significant differentiation of splenocytes into pancreatic islet cells in nonobese diabetic (NOD) mice treated with an immune adjuvant and allogeneic spleen cells. We show that our single-nucleotide polymorphism assay has the requisite sensitivity to support our contention. The experiments of Faustman *et al.* lack adequate controls, and we maintain that no evidence of islet regeneration has been presented.

On the basis of *in situ* hybridization experiments, Kodama *et al.* (1) concluded that infused allogeneic splenocytes rapidly differentiated into pancreatic islet cells in diabetic mice. In contrast, we found that the pancreatic islets observed in NOD mice after the reversal of diabetes induced by the treatment protocol they described were all of NOD, rather than donor CB6 F1, origin (2). Our conclusion was based on single-nucleotide polymorphism (SNP) analysis of laser-capture microdissected islet tissue. In addition, we found that omission of CB6 splenocytes from the protocol did not affect disease reversal. Using different methods, two other groups simultaneously reported results similar to ours (3, 4).

Faustman *et al.* (5) suggest that we did not detect any chimerism reflective of donor splenocyte contribution to the recovered islets because our SNP method was not sufficiently sensitive. In support of this contention, they cite a reference mentioning the relatively low sensitivity of one SNP assay (6). However, the method described in this citation is not the one we used. More important, we have directly evaluated our assay and found it to have the requisite sensitivity. We tested our SNP assay for each of the three relevant sequences used in (2) through mixing experiments. DNA was separately extracted from the tails of a

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NOD and a B6 mouse, the two DNAs were mixed together in defined ratios in a single reaction tube, and the SNP assay, based on labeling the two strains' DNA with different fluoresceins (FAM for NOD and VIC for B6), was performed (Fig. 1). B6 DNA can be readily detected in a mixed NOD/B6 sample when it represents as little as 3% of the total DNA. This sensitivity is easily high enough to detect the macrochimerism (ranging from 29% to 79%) of CB6 F1 donor cells in the islets of successfully treated NOD mice, as reported by Kodama *et al.* (1). In addition, the fact that we obtained the same reversal of disease in the absence of the splenocyte component of the treatment protocol demonstrates that the donor splenocytes are not required.

The data presented by Faustman *et al.* for chimerism based on Y-chromosome FISH analysis remain unconvincing. Their false detection of the Y chromosome in untreated mice [table 1 in (5)] raises questions about the robustness of their detection method. In addition, inadequate controls and poor imaging quality preclude definitive identification of islet β cells and the presence/absence of staining overlay. Similarly, there has been no evidence presented to support the claimed "reeducation" of pathogenic T cells by allogeneic splenocytes (2, 7), and the expected strong response to repeated allogeneic cell infusion in this system independently demonstrated by our group (2) and Suri *et al.* (4) remains

unaddressed. Lastly, we continue to wonder why Faustman *et al.* have not performed the essential Freund's complete adjuvant alone control.

Despite the inference of Faustman *et al.* to the contrary, none of the independent groups they cited has reproduced the high rate of disease reversal they previously reported or shown a significant contribution to islet mass from allogeneic splenocyte infusion. We would also like to emphasize that none of the reports appearing so far (1–5) has provided evidence for islet regeneration, defined by the production of new β cells from immature precursors. Islet regeneration, replication, and recovery all remain possible explanations at this time.

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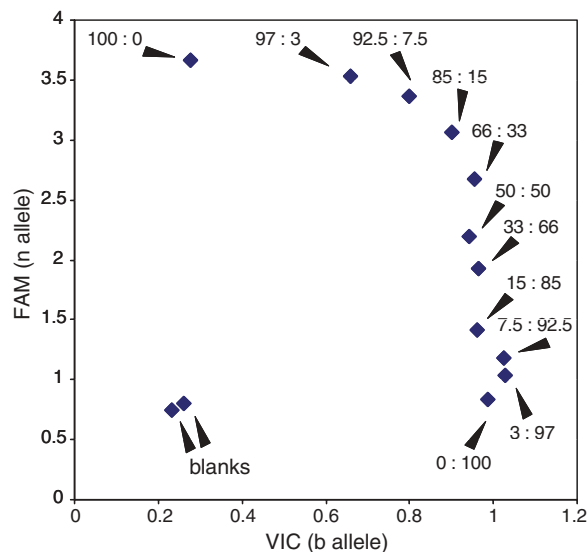


Fig. 1. Mixing experiments demonstrating the sensitivity of the SNP assay used in (2). The values shown indicate the ratio of NOD to B6 DNA mixed in one tube. Results are shown for the PCR assay specific for SNP rs4151928; the NOD versus B6 allele is detected as FAM or VIC fluorescence, respectively. Ratios of 97:3 are detectable at 600 pg of mixed DNA. Similar results were observed for the other two SNPs used in (2).