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

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Faustman *et al.* present no new information to explain why three independent laboratories failed to reproduce their previous results implicating spleen cell transdifferentiation in the reversal of murine type 1 diabetes. Modulation of the immunological process in nonobese diabetic (NOD) mice has been accomplished by many laboratories using different protocols and does not represent a novel finding in their work.

The most provocative finding of Kodama *et al.* (1) was that spleen cells could reverse type 1 diabetes in NOD mice by transdifferentiating to form new β islet cells. If this was indeed true, the implications for therapies to treat diabetic patients using spleen cells would be a sensible clinical avenue to pursue. However, three independent studies have found that donor splenocytes do not contribute to islet recovery and instead implicate endogenous regeneration of β islet cells as the primary mechanism for disease reversal (2–4). Faustman *et al.* (5) present no new information to explain the differences in these results.

Despite a technically rigorous analysis using well-established controls, we found no evidence to support the replacement of spleen cells (4), either using the fluorescence in situ hybridization FISH protocol or the green fluorescent protein (GFP) manipulations reported in (1). Rather, we noticed the presence of surviving islets and functional β cells in female NOD mice that had been diabetic for 2 to 3 weeks (a time period chosen based on the report of Kodama *et al.*). The most likely explanation for this observation

is that immunomodulation during the early stages of diabetes allows for some preservation of β cell function, the extent of which depends on the degree of injury before treatment begins [see figure S6 in (4)].

Faustman *et al.* (5) cite a lack of lymphocyte infiltration as an indication of a problem with our experiments. We found that the degree of lymphocyte infiltration varied greatly, as would be expected given that the number of β cells per islet was greatly diminished. Moreover, our report mentioned the peri-islet lymphocyte infiltration observed using our second protocol involving GFP-expressing male (CByB6F1) spleen cells.

Previous studies by Faustman and colleagues reported that treatment of diabetic NOD mice with Freund's adjuvant and allogeneic spleen cells eliminated preexisting diabetogenic T cells, possibly allowing for "reeducation" of the peripheral T cell repertoire (1, 6). In our experiments, NOD mice treated with their protocol still harbored diabetogenic T cells that were present in a controlled or "quiescent" stage (4). Moreover, there was no evidence for survival of the injected allogeneic cells as reported in (1). Thus, the establishment of the chimeric state was not achieved with their protocol (3, 4), and, as would be expected, donor cells mismatched at the MHC gene loci were rejected by the host immune system.

The major finding of Kodama *et al.* (1) that donor spleen cells can give rise to new β cells, should not be confused with the reversal of diabetes in NOD mice after treatment with adjuvant and allogeneic cells. Many experiments in the past 15 years have shown the great susceptibility of the NOD mouse to interventions that prevent or reverse the disease (7). For example, an elegant report by Chatenoud *et al.* showed that treatment of newly diabetic NOD mice with monoclonal antibody to CD3 resulted in permanent reversal of the disease (8). Indeed immunomodulation, including using Freund's adjuvant, arrests an active diabetic process in the mouse (9–11). Although we remain puzzled by the differences between our results and those in (1), we are reassured by the findings of two other laboratories of the reversal of autoimmune diabetes in the absence of splenocyte-derived differentiation of new β cells (2, 3). We hope these points clarify the scientific questions raised and answered in our studies (2–4) so that the field continues to move forward.

References and Notes

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