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

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We failed to detect transdifferentiation of spleen cells into  $\beta$  cells following diabetes reversal in nonobese diabetic (NOD) mice, thus contradicting a key finding of a 2003 report. We respond to Faustman *et al.* by justifying the use of mouse insulin promoter–green fluorescent protein transgenic mice as an appropriate system for detecting spleen-derived  $\beta$  cells in the islets of cured NOD mice.

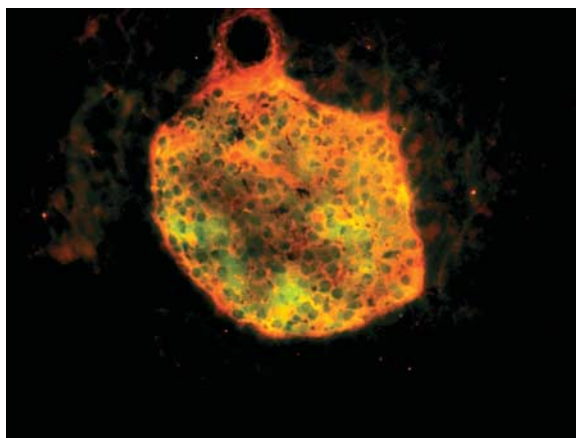
Three independent groups, including ours (1–3), reported that treatment with Freund's complete adjuvant, semi-allogeneic splenocytes, and temporary islet transplantation can reverse murine type 1 diabetes in some animals, but not by spleen cell transdifferentiation as reported by Kodama *et al.* (4). Faustman *et al.* (5) question the validity of our conclusion by suggesting that our approach of following the fate of donor spleen cells using mouse insulin promoter–green fluorescent protein transgenic (MIP-GFP) mice was technically flawed. We disagree with their assessment. The use of MIP-GFP mice to test the transdifferentiation hypothesis is not only appropriate but also more robust, more sensitive, and less prone to artifacts than their use of fluorescence in situ hybridization (FISH) for the Y chromosome to detect male donor cells.

The most important conclusion of the studies of Kodama *et al.* (4) was the putative transdifferentiation of spleen-derived cells into functional, insulin-producing  $\beta$  cells. Although Y-chromosomal FISH can potentially detect the presence of male donor cells, it cannot definitively prove that these cells are, in fact, differentiated  $\beta$  cells. In contrast, our experimental approach of using spleen cells from MIP-GFP mice had the ability to definitively demonstrate spleen-derived  $\beta$  cells in the cured NOD mice, had such cells been present.

Faustman *et al.* (5) suggest that GFP could be difficult to detect if the GFP transgene is expressed inefficiently or is silenced. This assessment is based on their previous publication containing a single low-magnification figure described as showing Y chromosome detection by FISH, but no enhanced GFP staining, in cells that should have had both (6). Castro *et al.* (7) had previously noted that

those studies did not consider the possibility that the putative Y chromosome staining could be an artifact. In Mezey *et al.* (6), the low magnification of the figures does not allow a definitive assessment, but in Kodama *et al.* (4) and Faustman *et al.* (5) the presence of signals external to the nuclei and observations of multiple signals per nucleus are consistent with the possibility that the detected signal is unrelated to the presence or absence of a nuclear Y chromosome.

The insulin promoter is the strongest promoter in the  $\beta$  cell, in which ~5% of the total cellular protein is insulin. Calibration studies have shown that as few as 100 GFP molecules



**Fig. 1.** GFP is expressed only in insulin-expressing cells in the islets from MIP-GFP mice. Pancreas sections from MIP-GFP mice were stained with antibodies to insulin (red) and antibodies to GFP (green).

per cell can be visualized (8). The inefficient expression or inadvertent silencing of GFP expression has not been reported for the MIP-GFP mouse. Stacked confocal microscopic images of isolated islets from MIP-GFP mice suggest that the steady-state levels of GFP are similar in all  $\beta$  cells and that all insulin-expressing cells coexpress GFP (Fig. 1) (9). If newly transdifferentiated  $\beta$  cells initiated the transcription of insulin, the 8.3-kb insulin promoter driving GFP would also be turned on and GFP would be produced. Conversely, the lack of GFP production from the

insulin promoter would perforce mean that the native insulin promoter was not being used and that insulin was not being produced. Such MIP-GFP spleen cells would of course be unable to reverse diabetes. Therefore, the argument that inefficient expression or silencing of GFP explains our inability to detect spleen-derived  $\beta$  cells capable of secreting insulin does not apply when the mouse insulin promoter is used to drive GFP expression.

Contrary to the suggestion of Faustman *et al.* (5), other studies have described the visualization of GFP expression in live  $\beta$  cells, without perfusion of the tissue, in this strain of MIP-GFP mice. GFP has been successfully visualized in  $\beta$  cells in MIP-GFP mice, and in MIP-GFP–derived  $\beta$  cells following transplantation into non-MIP-GFP recipients (9, 10). Chong *et al.* (1) used the same techniques to visualize GFP by fluorescence microscopy and by immunofluorescence with antibodies to GFP in positive control islets. However, no GFP was detected in the regenerated islets. Immunohistochemical approaches were also used to detect donor-derived cells expressing K<sup>b</sup> in or surrounding the islets, but, again, none were detected in the regenerated islets.

All three recent studies (1–3), using different technical approaches, were unable to demonstrate the transdifferentiation of spleen cells into  $\beta$  cells in NOD mice with restored  $\beta$  cell function. Studies are urgently needed to define the endogenous source of the  $\beta$  cells in this NOD mouse model of diabetes reversal. The therapeutic intervention of Freund's complete adjuvant, semi-allogeneic spleen cells, and temporary islet transplantation is not feasible in type 1 diabetic patients. Therefore, translating these findings into therapies that can halt autoimmunity and facilitate  $\beta$  cell regeneration in humans remains a challenge.

## References and Notes

1. A. S. Chong *et al.*, *Science* **311**, 1774 (2006).
2. J. Nishio *et al.*, *Science* **311**, 1775 (2006).
3. A. Suri *et al.*, *Science* **311**, 1778 (2006).
4. S. Kodama, W. Kuhlreiter, S. Fujimura, E. A. Dale, D. L. Faustman, *Science* **302**, 1223 (2003).
5. D. Faustman *et al.*, *Science* **314**, 1243 (2006); [www.sciencemag.org/cgi/content/full/314/5803/1243a](http://www.sciencemag.org/cgi/content/full/314/5803/1243a).
6. E. Mezey *et al.*, *Science* **299**, 1184 (2003).
7. R. F. Castro *et al.*, *Science* **297**, 1299 (2002).
8. Y. Sugiyama, I. Kawabata, K. Sobue, S. Okabe, *Nat. Methods* **2**, 677 (2005).
9. M. Hara *et al.*, *Am. J. Physiol. Endocrinol. Metab.* **284**, E177 (2003).
10. D. Yin *et al.*, *Am. J. Transplant.* **6**, 60 (2006).
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