Comment on: Tritt et al. (2007) Functional Waning of Naturally Occurring CD4$^+$ Regulatory T-cells Contributes to the Onset of Autoimmune Diabetes: *Diabetes* 57:113–123, 2007

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We read with interest the recent study by Tritt et al. (1), which examined the Foxp3$^+$ regulatory T-cells (Tregs) of NOD and BDC2.5NOD mice. The authors found that Tregs from 6- to 8-week-old BDC2.5NOD mice, but not those from 3- to 4-week-old mice, were unable to suppress diabetes in an in vivo transfer model. We feel, however, that a wealth of evidence has underlined the crucial importance of these cells in BDC2.5NOD mice throughout life and that any defects in Treg function in BDC2.5NOD mice are likely to be very subtle.

First, it is surprising that defects in BDC2.5NOD Treg function are apparent so early in life. In NOD mice, which have a much higher incidence of diabetes and a less autoreactive repertoire, Treg defects were not noted until much later (2). Because of its highly autoreactive repertoire, the BDC2.5NOD mouse is heavily dependent on Foxp3$^+$ Tregs to remain tolerant. Crossing to a Foxp3^{null} background results in highly accelerated disease (3). Also, immunoregulation by interleukin-10 remains important as BDC2.5NOD mice age beyond 6 weeks (4). Our recent work has shown robust suppressive capabilities of Tregs from both young (age 4 weeks) and old (age 20 weeks) BDC2.5NOD mice (5), though we have not examined the low Treg–to–effector T-cell ratios used in the study by Tritt et al. in vivo. Furthermore, we found an age-related increase in the number and frequency of Tregs in BDC2.5NOD mice. Thus, we feel that defects in Treg function in BDC2.5NOD mice are likely to be minor given their low incidence of diabetes, the mouse's heavy dependence on the presence of Tregs, and the functionality of Tregs in even very old mice. In addition, the reported defects may be offset by the dramatic age-related rise in Treg frequency in a variety of organs.

Definitive answers on subtle changes in Treg function in the NOD strain are likely to be provided by the use of NOD mice that express green fluorescent protein under the Foxp3 promoter. This will not only allow high purity isolation of Tregs that are uncontaminated by activated CD25^{low} cells but will also include the significant number of Tregs that are Foxp3$^+$CD25$^-$.

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