Response to 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

Michael Tritt, Evridiki Sgouroudis, Eva d'Hennezel, Alexandre Albanese, and Ciriaco A. Piccirillo

We thank Thomas et al. (1) for their insightful comments regarding the functional dynamics of CD4$^+$Foxp3$^+$ regulatory T-cells (Tregs) in their model of type 1 diabetes. An impressive array of studies in the literature establishes that CD4$^+$Foxp3$^+$ Tregs play a central, master-switch role in peripheral tolerance in the NOD mouse model of spontaneous type 1 diabetes (2,3). A central question is whether the onset of spontaneous disease in NOD mice results from a decline in regulation over time or from uncontrollable activity of self-reactive T-cells. Type 1 diabetes may reflect subtle, functional deficiencies in regulatory T-cells, thus allowing the diabetogenic process to unfold. In our study (4), we attempted to determine whether temporal, quantitative, or qualitative defects in CD4$^+$Foxp3$^+$ Tregs contribute to spontaneous type 1 diabetes.

The BDC2.5 mouse model contains a highly pathogenic CD4$^+$ T-cell repertoire and represents a unique system to study CD4$^+$Foxp3$^+$ Treg-mediated control of self-reactivity. The spontaneous onset of type 1 diabetes in BDC2.5 mice, though lower than in wild-type NOD mice, may be attributed to a number of predisposing Treg-intrinsic and -extrinsic variables. We are in agreement with Thomas et al. (1) that the onset of type 1 diabetes cannot be merely attributed to quantitative fluctuations in CD4$^+$Foxp3$^+$ Tregs. Consistent with Thomas et al. (1), we also observe an age-related increase in CD4$^+$Foxp3$^+$ Tregs, particularly in secondary lymphoid tissues in BDC2.5 mice (4). This increased frequency of CD4$^+$Foxp3$^+$ Tregs in spleen and pancreatic lymph nodes is likely a response of CD4$^+$Foxp3$^+$ Tregs to the increased inflammation in these sites. However, this increase in the numbers of CD4$^+$Foxp3$^+$ Tregs in these sites is not sufficient to suppress the diabetogenic process.

Our study is in disagreement with Thomas et al. (1) as to the potency of CD4$^+$Foxp3$^+$ Treg function in young (age 3–4 weeks) and old (age 20 weeks) BDC2.5 mice. In our hands, older BDC2.5 regulatory T-cells (fluorescence-activated cell sorter [FACS] highly purified cells injected at physiological 1:10 Treg–to–effector T-cell ratios) have a reduced suppressive potential compared with that of young Tregs. Interestingly, infusion of increased numbers of older BDC2.5 Tregs into T-cell–deficient recipient mice (FACS highly purified cells injected at nonphysiological 1:2 or 1:1 Treg–to–effector T-cell ratios) reestablished disease protection, an observation consistent with those of Thomas et al. (1). This observation suggests that the cellular potency of CD4$^+$Foxp3$^+$ Tregs, while fully operative in neonatal mice, declines with age despite a stable cellular frequency of CD4$^+$Foxp3$^+$ Tregs in primary and secondary lymphoid tissues. This observation is consistent with a number of recently published studies (5–8).

In our study (4), we also observe that type 1 diabetes onset coincides with an age-dependent decline in the cycling of CD4$^+$Foxp3$^+$ Tregs, particularly within the pancreas. It is noteworthy to mention that this proliferative decline of BDC2.5 CD4$^+$Foxp3$^+$ Tregs within the pancreas occurs at a time point well before the clinical onset of type 1 diabetes but, nonetheless, at a time point when immune dysfunction (self-reactive T-cell activation and insulitis) is readily detectable in these young mice. We believe that NOD mice succumb to a temporal loss in CD4$^+$Foxp3$^+$ Treg function that coincides with a reduction in CD4$^+$Foxp3$^+$ Treg cycling in pancreatic sites; this, in turn, unleashes the diabetogenic potential of effector T-cells in older BDC2.5 mice. We suggest that a functional deficiency in CD4$^+$Foxp3$^+$ Tregs may not be visible as a sudden decline in the cellular frequency of these cells in peripheral tissues and may conceivably be related to a reduced homeostatic fitness in inflammatory sites. Evidently, these results do not exclude the possibility that time-dependent changes in Treg-extrinsic variables, like dendritic cell or effector T-cell function, may also contribute to disease onset. It remains to be seen how mouse colony/flora differences also impact these tolerogenic mechanisms.

Currently, our ability to isolate CD4$^+$Foxp3$^+$ Tregs in the NOD model is limited to the isolation of CD4$^+$CD25$^+$ T-cells, which likely constitute a phenotypic and functional heterogenous population including a variety of effector and regulatory subsets. Thus, a more discriminate characterization of CD25$^-$ and CD25^{low} T-cells relative to CD25$^+$ Tregs is imperative. The next step to examine the functional dynamics of Tregs will be to rigorously track and characterize CD4$^+$Foxp3$^+$ Treg development, function, and homeostasis in type 1 diabetes by means of NOD.Foxp3–green fluorescent protein reporter mice. These experiments are well underway.

From the Department of Microbiology and Immunology and McGill Center for the Study of Host Resistance, McGill University, Montreal, Quebec, Canada, H3A 2B4.

Address correspondence and reprint requests to Dr. Ciriaco A. Piccirillo, Department of Microbiology and Immunology, McGill University, 3775 University St., Room 510, Lyman Duff Medical Building, Montreal, QC, Canada, H3A 2B4. E-mail: ciro.piccirillo@mcgill.ca.

Received for publication 5 December 2007 and accepted in revised form 6 December 2007.

DOI: 10.2337/db07-1710

REFERENCES

1. Thomas DC, Mellanby RJ, Cooke A: Comment on: Tritt et al. (2007) Functional waning of naturally occurring $CD4^+$ Tregs contributes to the onset of autoimmune diabetes (Letter). *Diabetes* 57:e6, 2008. DOI: 10.2237/db07-1572
2. Anderson MS, BluestoneJA: The NOD mouse: a model of immune dysregulation. *Annu Rev Immunol* 23:447–485, 2005
3. Chatenoud L, Bach JF: Regulatory T cells in the control of autoimmune diabetes: the case of the NOD mouse. *Int Rev Immunol* 24:247–267, 2005
4. Tritt M, Sgouroudis E, d'Hennezel E, Albanese A, Piccirillo CA: Functional waning of naturally occurring $CD4^+$ regulatory T cells contributes to the onset of autoimmune diabetes. *Diabetes* 57:113–123, 2007
5. Gregg RK, Jain R, Schoenleber SJ, Divekar R, Bell JJ, Lee HH, Yu P, Zaghouani H: A sudden decline in active membrane-bound TGF-beta impairs both T regulatory cell function and protection against autoimmune diabetes. *J Immunol* 173:7308–7316, 2004
6. Pop SM, Wong CP, Culton DA, Clarke SH, and Tisch R: Single cell analysis shows decreasing FoxP3 and TGFbeta1 coexpressing $CD4^+CD25^+$ regulatory T cells during autoimmune diabetes. *J Exp Med* 201:1333–1346, 2005
7. You S, Belghith M, Cobbold S, Alyanakian MA, Gouarin C, Barriot S, Garcia C, Waldmann H, Bach JF, and Chatenoud L: Autoimmune diabetes onset results from qualitative rather than quantitative age-dependent changes in pathogenic T-cells. *Diabetes* 54:1415–1422, 2005
8. Gregori S, Giarratana N, Smiroldo S, Adorini L: Dynamics of pathogenic and suppressor T cells in autoimmune diabetes development. *J Immunol* 171:4040–4047, 2003