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Reply

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To the Editor

We thank Drs. Wilde and colleagues for their interest in our study. They inquire as to whether IL-17A is produced by exon 2–deficient FoxP3 + Treg cells in patients with AAV. As mentioned in our original report, more IL-17A is produced by total T cells from AAV patients than from healthy controls, and the majority of IL-17A–producing CD4+ T cells are $CD25^{intermediate}$ $CD127^{high}$; only a small proportion of IL-17A–producing cells are $CD25^{high}CD127^{low}$ Treg cells. To address the question posed by Wilde et al, we have investigated Treg cell IL-17A production in AAV patients, using a phorbol myristate acetate (PMA)/ionomycin stimulation protocol described by Lord et al (1). We found that within the Treg cell population, both full-length and exon 2–deficient FoxP3+ cells produced IL-17A (Figure 1A). Thus, IL-17A production is not restricted to exon 2–deficient FoxP3+ cells in AAV. A caveat to this conclusion is the fact that IL-17A production was examined after PMA/ionomycin stimulation; different modes of cellular stimulation or induction with specific cytokines may yield different results. Very little is currently known about the exact function of exon 2–deficient FoxP3, but this should be explored more extensively in AAV.

As Wilde et al mention, T cell CTLA-4 and PD-1 may not function appropriately to negatively regulate T effector cell responses in AAV, and aberrant negative regulation may also contribute to Treg cell dysfunction (2,3). We did not directly test T cell CTLA-4 or PD-1 expression in our studies. However, we did examine expression of CD39 (ectonucleoside triphosphate diphosphohydrolase 1), which hydrolyzes free ATP into ADP and AMP (4,5), in AAV T cells. Circulating ATP is known to act as a “natural adjuvant” but can be removed from circulation by cells expressing CD39 (6,7). Thus, CD39 suppresses immune activation by reducing ATP levels. In humans, it is expressed by a subset of Treg cells with effector and memory-like properties (5). Interestingly, we found an increased frequency of CD39+CD4+ T cells in the peripheral blood of AAV patients compared to healthy controls (mean 11.59% versus 5.12%). Additionally, the proportion of Treg cells expressing CD39 was higher in AAV patients than in healthy controls (Figures 1B and C). These findings are reminiscent of the overexpression of the inhibitory molecules PD-1 and CTLA-4 in AAV T cells (2,3).

In conjunction with dysfunctional Treg cells, effector cell up-regulation of GITR and CD134 may alter Treg cell suppression (8). The collective data from our studies and other studies mentioned above suggest that T cell dysfunction and lack of regulation are not due to a single aberration. In AAV, and likely many other systemic autoimmune diseases, T cell dysfunction can be attributed to both the regulatory and effector compartments and may induce a positive inflammatory feedback loop. Future studies should examine the underlying

molecular cause of $CD25^{intermediate}$ T cell proliferation despite the addition of functional Treg cells.

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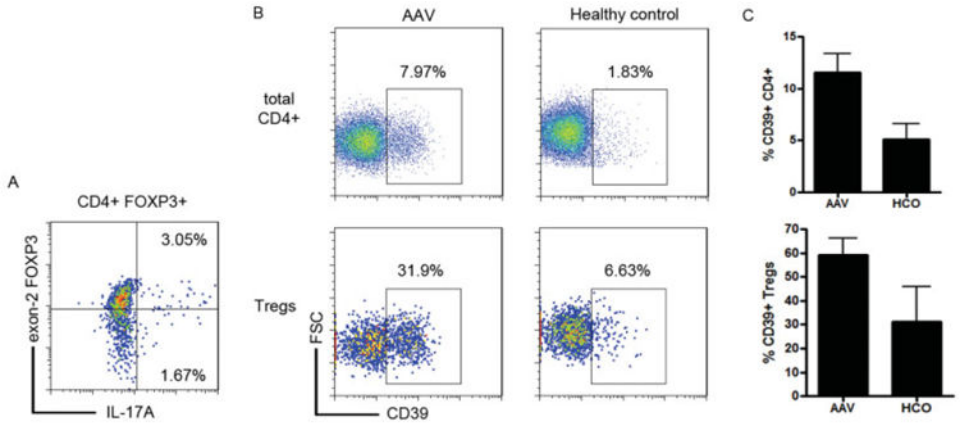

Figure 1.

Interleukin-17A (IL-17A) and CD39 expression on Treg cells from patients with antineutrophil cytoplasmic antibody–associated vasculitis (AAV). **A,** Representative flow cytometric plot of IL-17A production in FoxP3+ cells. FoxP3 antibody clone 150D, representing exon 2, is shown on the y-axis. **B,** Representative flow cytometric plots of CD39 expression on total CD4+ cells and Treg cells from patients with AAV and healthy controls (HCO). **C,** Mean ± SEM percent of total CD4+ cells and Treg cells expressing CD39 in patients with AAV and healthy controls.