COMMENT ON LIU ET AL.

Immune and Metabolic Effects of Antigen-Specific Immunotherapy Using Multiple β-Cell Peptides in Type 1 Diabetes. Diabetes 2022;71:722–732

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The article by Liu et al. (1), on immune and metabolic effects of antigen-specific immunotherapy via multiple β-cell–specific peptides in type 1 diabetes, uses a popular method to estimate the number of regulatory T cells (Tregs) in peripheral blood that has been shown to have defects (2,3). Essentially, by considering as Tregs all $CD3^+CD4^+CD25^{high}CD127^{low/-}$ cells, one unavoidably includes many non-Tregs in this population. The "high" and "low/−" intensities of CD25 and CD127, respectively, are subjective because of a continuum of intensity values in these parameters (e.g., see Supplementary Fig. 1 of Liu et al. [1]). A more accurate estimation of effector/active Tregs and naive/resting Tregs can be achieved via the use of the CD3/CD4/CD25/CD45RA-RO panel, where the former are $CD3^+CD4^+CD25^{high}CD45RA^-$ (fraction II) and the latter are $CD3^+CD4^+CD25^{int}CD45RA^+$ (fraction I), inherently avoiding such subjectiveness (2). The $CD127^{low/-}$ and $FoxP3^+$ populations of $CD3^+CD4^+CD25^{high}$ cells are not congruent (see Supplementary Fig. 1 of Liu et al. [1]). In fact, there is a 13.5% increase in the $FoxP3^+$ cells compared with the $CD127^{low/-}$ ones (7.70% vs. 6.78%). This is not surprising, as the method of Miyara et al. (2) for Treg delineation identified a $FoxP3^+$ population that lacks regulatory activity ($CD3^+CD4^+CD25^{int}CD45RA^-$; fraction III). By differentially coloring the six fractions in a CD25/CD127 dot plot, we were able to show that demarcation of Tregs via the $CD3^+CD4^+CD25^{high}CD127^{low/-}$ criterion unavoidably excludes a sizeable portion of naive Tregs but includes many cells from fraction III (non-Tregs) as well as cells from the uncharacterized fraction of $CD3^+CD4^+CD25^{int/low}CD45RA^-$ cells (gray dots in Fig. 3*B* of Petsiou et al. [3]).

Detailed proteomic/transcriptomic investigation of the properties of five of the six fractions has uncovered the several adaptive mechanisms in cellular signaling by which fractions I and II maintain their Treg identity, while $CD4^+$ T cells from other fractions are activated in the canonical manner via select stimuli (4). Besides identifying and characterizing the properties of at least six $CD4^+$ T-cell fractions, this method holds promise for identifying the fraction(s) bearing (self or foreign) antigen-specific T cells and enumerating their respective specificities via pMHCII tetramer staining (5). This is an important piece of information when dealing with peptide-specific immunotherapy of type 1 diabetes, as Liu et al. do. The monoclonal antibody panel required for this manner of Treg delineation is already part of the large panel that the authors used for immunophenotyping (see Supplementary Table 2 of Liu et al. [1]). Thus, the data are there, they just need to be analyzed in this suggested manner. Last, there is the outstanding issue of the presence of $FoxP3^+$ in the $CD4^+CD25^{low}$ T-cell population (otherwise uncharacterized) in the peripheral blood of patients with type 1 diabetes (and from several autoimmune diseases), as cited in Petsiou et al. (3). This, in parallel to the diminution of Tregs from the $CD4^+CD25^{high}$ cell population, makes the method of Miyara et al. (2) the method of choice for delineation of $CD4^+$ T cell fractions:

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two Treg populations as well as variably activated and naive ones. The convenient separation of the CD25 intensity of expression into four scales (negative, low, intermediate, and high) to delineate different $CD4^+$ T-cell fractions should be taken advantage of.

Duality of Interest. No potential conflicts of interest relevant to this article were reported.

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