

## IMMUNOLOGY

# Response to Comment on “Diversification of the antigen-specific T cell receptor repertoire after varicella zoster vaccination”

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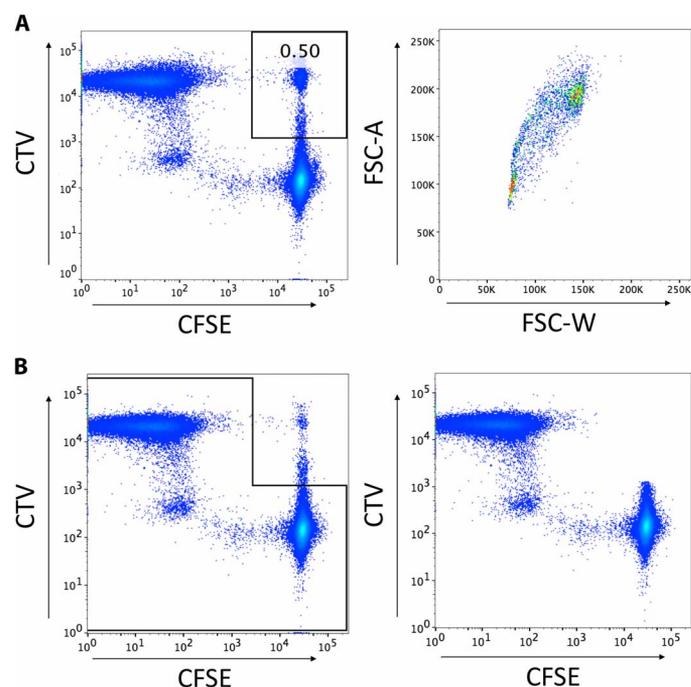
In our repertoire studies of varicella zoster virus-specific T cells, we used very stringent computational criteria to keep contamination with T cell receptor sequences from bystander-activated T cells to a minimum.

In their Technical Comment, Parizot and colleagues (1) raise two issues that, in their opinion, undermine the conclusions of our Research Article (2). We respectfully disagree. The first issue relates to the difficulties in defining antigen-specific T cells in a functional assay to perform T cell receptor repertoire studies. We are aware of these difficulties and designed our study to exclude false positives (2).

Contrary to what Parizot *et al.* (1) may have interpreted, we did not only rely in our data analysis on control stimulation with a mock lysate to exclude non-specifically activated cells, but we required the following two criteria for a T cell to be called antigen-specific in our antigen-driven cultures as clearly stated in our manuscript. First, based on the assumption that bystander T cell activation is stochastic and therefore unique for each culture, we stimulated several replicates from the same individual with antigen. Based on our previous studies on repertoire complexity, we chose a number of cells in each replicate that were large enough to include at least the majority of antigen-specific T cell receptors in a given individual (3). We then positively identified T cell receptor sequences that were found in two or more replicates. To further address the possibility that large unrelated clones have a higher chance to contaminate the analyses, that is, that contamination is not entirely stochastic, we required as a second criterion that T cell receptor sequences are enriched in the population of cells that proliferated in response to antigen. As discussed in the manuscript, we have chosen to err on missing varicella zoster virus (VZV)-reactive T cell receptor sequences and therefore underestimating diversity rather than including false positives. The experimental strategy proposed by Parizot *et al.* (1), comparing the repertoire of VZV-reactive T cells to that of T cells responsive to a control antigen to identify bystander activation, is based on the unlikely assumption that the repertoire of bystander T cells is conserved and is therefore likely to miss most bystander T cells.

The second technical issue raised by Parizot *et al.* (1) is that in co-cultures of carboxyfluorescein diacetate succinimidyl ester (CFSE)- and CellTrace Violet (CTV)-labeled T cells, a small percentage of cells have both labels, an observation to which they have apparently devoted considerable effort. In our cultures, most of these events represented doublets; upon re-gating, we agree that such cells exist at very low frequencies of around 0.1 to 0.2%, possibly lower in our autologous cultures than what they observed in an alloreactive response (Fig. 1). However, it should be noted that this observation does not affect our findings. First, the majority of these cells are non-proliferating,

whereas we were interested in single-positive cells that proliferated in response to VZV. Second, our repertoire studies were not built on this co-culture system, as we clearly state in our manuscript. To reiterate, we have isolated proliferating cells from single-color CFSE-labeled cells after stimulation with VZV and sequenced their T cell receptor. In parallel, we have purified unmanipulated naïve and memory T cells and sequenced their entire T cell receptor  $\beta$ -chain repertoires. Assignments of VZV-specific T cells to the naïve and/or memory T cell compartments were then done by re-identifying sequences. In summary, our approach has been as rigorous as possible,



**Fig. 1. Analysis of flow cytometry data.** CFSE-labeled purified naïve T cells and CTV-labeled peripheral blood mononuclear cells were cultured together in the presence of VZV lysate for 8 days. (A) The scatter plot shows gated live CD4 T cells. A small non-proliferating population of 0.5% stained high for both CFSE and CTV (left). More than 80% of these cells were identified as doublets in the forward scatter-area (FSC-A)–forward scatter–weight (FSC-W) plot (right). (B) The scatter plot shows gated live CD4 T cells after gating for single cells in the FSC-A–FSC-W plot. Single-staining CD4 T cells were gated as shown. The resulting scatter plot identifies proliferating naïve CD4 T cells (CTV-negative, CFSE-low) and proliferating total CD4 T cells (CFSE-negative, CTV-low), as was shown in Fig. 4B in (1).

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and we feel confident that our conclusions are supported by the data presented in our paper.

## REFERENCES

1. C. Parizot, H. Brisson, J. Fadlallah, D. Sterlin, G. Gorochov, Comment on "Diversification of the antigen-specific T cell receptor repertoire after varicella zoster vaccination". *Sci. Transl. Med.* **9**, eaah4501 (2017).
2. Q. Qi, M. M. Cavanagh, S. Le Saux, H. NamKoong, C. Kim, E. Turgano, Y. Liu, C. Wang, S. Mackey, G. E. Swan, C. L. Dekker, R. A. Olshen, S. D. Boyd, C. M. Weyand, L. Tian, J. J. Goronzy, Diversification of the antigen-specific T cell receptor repertoire after varicella zoster vaccination. *Sci. Transl. Med.* **8**, 332ra346 (2016).
3. Q. Qi, Y. Liu, Y. Cheng, J. Glanville, D. Zhang, J.-Y. Lee, R. A. Olshen, C. M. Weyand, S. D. Boyd, J. J. Goronzy, Diversity and clonal selection in the human T-cell repertoire. *Proc. Natl. Acad. Sci. U.S.A.* **111**, 13139–13144 (2014).

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