February 9th, 2016

The Editors

*Immunity*

Re: Submission of Article to Immunity

Dear Editors:

We are submitting an article entitled “Precocious Interleukin 21 expression in naïve mice defines a novel stage of T follicular helper cell development” for your consideration. The areas of investigation – the ontogenies of follicular T helper cells (TFH) and Interleukin 21 (IL21) – are expected to be of great interest to your readership and have been the topic of numerous contemporary publications in *Immunity* as well as in other high impact journals. The processes through which CD4+ T cells develop and differentiate into TFH as opposed to other effector and regulatory cell populations are major unsolved issues in immunobiology. The extent to which IL21, the signature cytokine of fully mature TFH, participates in TFH development is similarly unresolved. Essentially all studies preceding ours have relied on infection or immunization strategies in vivo or *in vitro* induction studies, typically in mature mice or humans, to help resolve these issues. This prior work was predicated on the assumption that both IL21 and TFH are end products, only elicited after strong immune stimulation by foreign antigens. Our study takes another tack by investigating the possibility that lineage decisions responsible for the development of TFH arise shortly after birth in naïve mice. We describe and take advantage of a new and highly sensitive Interleukin 21 (IL21) Venus fluorescent protein reporter knock-in mouse to identify a naturally arising population of CD4+ T cells that already express IL21 and are the earliest precursors of TFH, a populationthat we have termednatural TFH (nTFH). Remarkably, nTFH greatly outnumber alternative T-helper (TH) cell categories in neonatal mice making them the preferred differentiation state. We have extended these findings in many ways. First, we defined the cytokines and antigen-presenting cell types required for nTFH development. Second, we defined the transcriptome that is unique to nTFH through RNAseq and advanced biocomputational methods. Third, we document that nTFH are capable of differentiating to the more mature forms of TFH documented by others. Fourth, we provide evidence showing that nTFH cells develop in the thymi of naïve neonatal mice through positive selection by AIRE. Fourth, our data support a new synthesis for explaining why nTFH are the preferred type of activated TH cell to arise within weeks of birth in naïve mice, namely that they are selected to be hypersensitive to self-antigens. Finally, we show that a primary role of regulatory T cells (TREG) is to control nTFH and offer the new hypothesis that nTFH and TREG are opposing outcomes of the same ontogenetic processes.

We suggest and exclude the following reviewers.

***Suggested Reviewers:***

Warren J. Leonard M.D., National Institute of Health, Bethesda, MD - Leader in IL21 biology.

Joseph Craft M.D., Yale University, New Haven, CT - Leader in TFH biology.

Stephen Jamieson/ Kris Hogquist, University of Minnesota – Leaders in T cell development and selection.

***Excluded Reviewers (up to 3):***

Shane Crotty Ph.D., La Jolla Institute for Allergy and Immunology, La Jolla, CA - Competitor

We hope that you find our submission acceptable for publication in *Immunity*.

Sincerely yours,

Derry C. Roopenian



Herbert C. Morse III