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INSIGHTS

How decreasing T cell signaling unexpectedly results in autoimmunity

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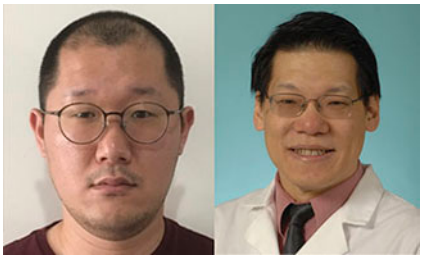
In this issue of *JEM*, Tanaka et al. (2022. *J. Exp. Med.* https://doi.org/10.1084/jem.20220386) advance our understanding of how genetic mutants that decrease T cell recognition of antigen, a critical event for immune activation to invading microbes and virus, paradoxically results in autoimmunity.

One of the defining features of adaptive immunity is the unique utilization of somatic gene rearrangement to generate a vast array of antigen receptors, allowing the individual to respond to new pathogens without necessitating evolution of the species to generate germline-encoded receptors. With clonal selection during infection for pathogen-specific receptors, the adaptive immune system represents a microcosm of evolution within each of us.

However, this great diversity inevitably results in the generation of receptors that recognize self-antigens that can lead to autoimmunity. For T cells, this is dealt with by compartmentalized development within a specialized organ, the thymus, prior to their release into the periphery where they can cause autoimmunity. In the thymus, individual T cells undergo "random" gene rearrangement to generate a genetically variable TCR, which is then tested for self-reactivity (Klein et al., 2019). T cells with a TCR that is too self-reactive are negatively selected and die, whereas an intermediate reactivity results in development into regulatory T (Treg) cells that prevent, rather than induce, inflammation (see panel A of figure; Klein et al., 2019). Consistent with the evolution metaphor, each T cell with its rearranged TCR undergoes thymic selection primarily as an individual, driven by its TCR and signaling machinery, which includes the ZAP70 molecule studied by Tanaka et al. (2022). Thus, the thymic education process integrates individual TCR signaling-dependent cell-fate decisions to generate a conventional non-Treg cell (Tconv) population for responding to pathogens that is skewed away from strong self-reactivity (see panel B of figure).

A supposition of this educational process is that the interpretation of TCR signals in the thymus should approximate that in the periphery after they exit the thymus. If TCR signaling was more sensitive in the periphery, this may increase the propensity for autoimmunity; if decreased, limit the ability to respond to pathogen. However, it has become clear that alterations in TCR signaling machinery can result in a loss of tolerance and autoimmunity (see introduction of Tanaka et al., 2022). For example, previous studies from this group have shown that a point mutation of SH2 domain of ZAP70 (skg mutant), which results in a lower affinity to CD3, leads to spontaneous autoimmune arthritis in mice (Sakaguchi et al., 2003). However, it remained unclear why this defect in TCR signaling resulted in spontaneous autoimmunity.

Initially, they quantified the development of autoimmunity in mice with ZAP70 mutations with variably lower affinities for CD3, which correlated with the ability of ZAP70 to transduce signals from the TCR. These mutants were also expressed at an approximately fivefold lower level than WT ZAP70. They observed that autoimmunity was best elicited when ZAP70 affinity was reduced eightfold (ZAP70 arthritogenic and colitogenic, ZAC mutant). Further reductions in ZAP70 affinity (15-fold from WT, skg mutant) decreased autoimmunity. Consistent with this decreased susceptibility, the induction of autoimmune arthritis in skg (Tanaka et al., 2010), but not ZAC, mice required the contribution of microbial signals. By contrast, ZAP70 mutations with much lower affinity (260-fold) were no longer able to induce autoimmunity, presumably due to an inability of Tconv cells to induce inflammation. In addition to these ZAP70 mutant studies, they confirmed that the autoimmunity was not due to a unique effect of the mutation by phenocopying the ZAC mutation with reduced WT ZAP70 expression in a tetracycline-regulated model. Thus, these data show that there is an optimum window for reduction in TCR signaling to induce autoimmunity.



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However, the mechanism by which the ZAC ZAP70 mutation induced autoimmunity remained unclear. The first clue came

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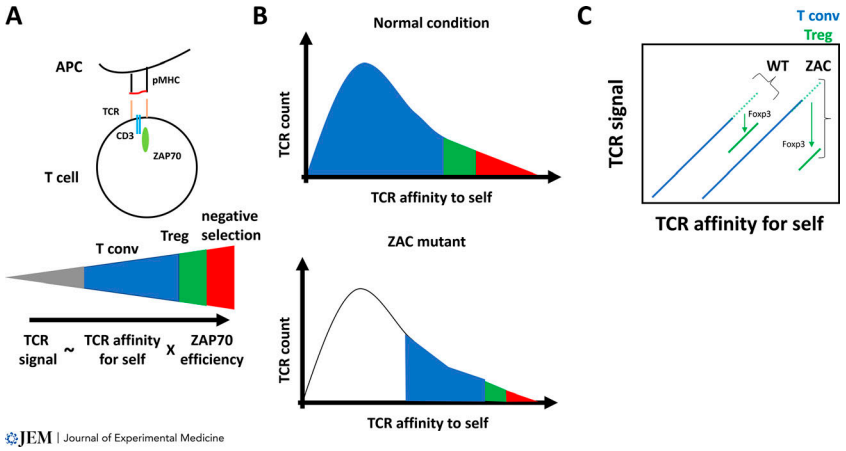

Altered thymic selection and impaired TCR signal in Treg cells are responsible for induction of autoimmunity caused by reduced TCR signal. (A) TCR signal strength, which is a key factor in thymic selection, is determined by TCR affinity for self-antigen and the signal cascade transduced by TCR-proximal molecules such as ZAP70. TCR-dependent cell-fate decisions include negative selection and Treg cell development. (B) TCR affinity to self drives selection of mature $CD4^+CD8^-$ thymocytes into Tconv (blue), Treg (green), or death (negative selection, red). Deficiency in TCR signaling machinery due to ZAP70 mutation skews towards compensatory usage of a more self-reactive TCR repertoire. (C) Foxp3-mediated desensitization of TCR signaling renders Treg cells more susceptible to loss of TCR-dependent function due to low-affinity ZAP70 mutation. APC, antigen-presenting cell.

from the observation that there was a substantial reduction of mature thymocytes. Maturation via positive selection requires TCR signaling, which we simplify here as equaling the product of TCR affinity for self and output of molecules such as ZAP70 (see panel A of figure). A shift toward TCRs with higher self-reactivity in ZAC was supported by studies that control the TCR affinity for self using superantigens or defined TCRs encountering its cognate antigen in the thymus, which showed that the cell fates shifted to outcomes consistent with lower TCR signals in ZAC, such that TCRs that normally delete instead induce Treg cells, and so forth (see panel B of figure). Although additional experimental validation in developing thymic Treg cells is required, the observation that peripheral ZAC Tconv and Treg TCRs are less diverse and more oligoclonal is consistent with the shift toward the use of a much smaller TCR pool with greater self-reactivity in the thymus of ZAC mice. By reducing the number of T cells, a.k.a. lymphopenia, this would generate an environment known to facilitate the development of autoimmunity.

A second clue came from studies of autoreactivity using T cell transfers into lymphopenic mice. Here, they observed that both thymic and splenic CD4 T cells with reduced ZAP70 expression could induce autoimmunity. Importantly, the disease was dependent on the level of ZAP70 expression in the donor mice, and not after transfer, arguing that thymic negative selection and Treg development was impaired, resulting in a Tconv population with enhanced self-reactivity and autoimmune potential.

A final clue came from examination of TCR signaling following in vitro TCR stimulation. Consistent with in vivo observations, TCR signal induction as reported by Nur77 induction was similar between ZAC Tconv and WT Tconv and Treg cells. However, ZAC Treg cells showed a marked reduction in Nur77 induction. As TCR signaling is crucial not only for Treg differentiation in the thymus but also for suppressive function (Li and Rudensky, 2016), this suggested that ZAC Treg cells were much more compromised in terms of TCR signaling compared with their Tconv counterparts (see panel C of figure)—a mismatch permissive for the development of autoimmunity.

A defect in ZAC Treg cells was supported by the rescue of autoimmunity by WT Treg cells transferred into ZAC mice. Further investigation of the ZAC Treg signaling defect confirmed that ZAP70 expression in Treg cells was reduced via transcriptional repression by Foxp3 binding to the *Zap70* promoter (Ohkura et al., 2012). Moreover, the authors also found other genes, such as *Cd45*, *Ptpn22*, *Slp76*, and *Cblb* to be similarly regulated by Foxp3. Interestingly, these molecules together with CD5 have been thought to be involved with desensitization process of TCR signal on Tconv cells to avoid activation of potentially harmful self-reactive T cells (Cho and Sprent, 2018). These findings suggest that TCR signaling in Treg cells is more strictly regulated than that on Tconv cells, which may exist to compensate for the higher self-reactivity of Treg cells and limit Treg expansion in the periphery. Consistent with this hypothesis, experimental upregulation of ZAP70 induced robust proliferation of Treg cells after in vitro stimulation, which was not seen with normal levels of ZAP70. Together, these data suggest that this negative feedback loop results in an exaggerated effect of ZAP70 mutations on TCR signaling in Treg cells relative to Tconv cells, resulting in ZAC Treg cells with greatly reduced function.

These findings regarding TCR signaling are reminiscent of the findings two to three decades ago that were, at that time, paradoxical, as they revealed that many of the molecules thought to be important for effector T cells such as IL-2 (Sadlack et al., 1993) and CD28 (Salomon et al., 2000; among others) were eventually discovered to be more important for Treg cell development, survival, and/or function. A differential effect on Tconv vs. Treg cells may also underly the observations that cyclosporine, a potent immunosuppressive that inhibits TCR-driven NFAT signals, can at low dose unexpectedly lead to worsening of autoimmunity in experimental models and occasionally in human patients (Flores et al., 2019), analogous to the findings reported here. Thus, a better understanding of the differences in the TCR signaling machinery driving effector and Treg cell function may lead to the development of new therapies that modulate the immune system for cancer and autoimmunity.

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