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INSIGHTS

A twist in the tail: Of T cell subsets and disease

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In this issue of *JEM*, the work of Joachim et al. (2023. *J. Exp. Med.* https://doi.org/10.1084/jem.20231028) on knockin mice with a specific tail mutation in *LAT* provides valuable insights about cytotoxic CD4⁺ T cells and human inflammatory diseases.

A point mutation in the cytoplasmic tail of a key T cell signaling adaptor resulted in a rodent disease model that resembles a human fibrotic inflammatory disease that has served as a prototype for a set of human diseases driven by cytotoxic CD4⁺ T cells. These diseases cannot be categorized using the widely used type 1, type 2, and type 3 immune mechanism paradigms.

In the last few decades, two extremely effective, widely used, and generally "non-specific" therapeutics have emerged that could be considered "modern-day steroids." These therapies, intravenous IgG and anti-CD20–mediated B cell depletion, were originally developed for other therapeutic indications, but are widely used today in disparate chronic inflammatory and autoimmune diseases. While the use of B cell depletion in some diseases in which autoantibodies play a crucial role is relatively easy to appreciate, beyond those disorders, there is a wide range of inflammatory diseases in which B cell depletion has proven very effective, even though the immediate drivers of these diseases are likely T cells. T and B cells infiltrate tissues in many diseases characterized by tissue inflammation. But how do B cells contribute to the induction, maintenance, or exacerbation of such diseases? Some clues have emerged from the study of a T cell signaling adaptor.

LAT (linker for activation of T cells) is a key adaptor of relevance to TCR signaling. It has a very short, three-amino-acid-long extracellular domain, and a cytoplasmic tail that is 236 amino acids long and contains nine tyrosine residues. Over two decades ago, Aguado et al. (2002) and Sommers et al. (2002) separately demonstrated that thymic T cell development was impaired in homozygous *LAT* Y136F knockin mice; these mice also exhibited peripheral lymphoproliferation and rapidly developed an autoimmune phenotype. Tyrosine 136 (tyrosine 132 in human LAT) is required for PLCγ1 recruitment and activation; downstream calcium signaling is abrogated in mutant T cells. It was later shown (Koonpaew et al., 2006) that thymic regulatory T cell (Treg) development is defective in these mice and the ratios of conventional T cells to Tregs in the periphery is very high; this may help explain the broad autoimmune phenotypes seen.

Using a single-cell transcriptomic approach, Joachim et al. (2023) serially examined immune cells in the spleens of LAT Y136F mice at different times after birth, and they also used high-dimensional flow cytometry in the spleen and in the lungs to examine T and B cell subsets that infiltrate tissues and are likely drivers of tissue inflammation. TCR levels on T cells in these mice are low and re-triggering of T cells depends heavily on the ligation of CD28. There is an interesting aspect to the cellular changes in these mice that resembles the alterations seen in a set of human inflammatory diseases. The pathological changes of inflammation and fibrosis in these diseases are best attributed to their infiltration by cytotoxic CD4⁺ T cells (CD4⁺ CTLs) and activated B cells and also, likely secondarily, CD8⁺ T cells.

CD4⁺ CTLs were initially studied in the context of viral infection and viral control. These cells were initially implicated in tissue damage, inflammation, and fibrosis in studies on the pathogenesis of an autoimmune disease called IgG4-related disease (IgG4-RD; Mattoo et al., 2016). This disease is characterized by slow-growing inflammatory fibrotic masses in a number of different organs, and by tissue infiltrates of T and B cells. Circulating IgG4 levels are elevated, as are other IgG isotypes, and in many patients, there is elevation in IgE as well. Dramatic clinical improvement is observed within a few weeks after B cell depletion (Stone et al., 2012). In this disease, the CD4⁺ effector T cells that are clonally expanded and infiltrate disease tissues were identified as CD4⁺ CTLs (Mattoo et al., 2016). The frequencies of circulating CD4⁺ CTLs and of tissue infiltrating T cells decline after B cell depletion. Inflamed tissues are also infiltrated by IgG4 expressing plasmablasts

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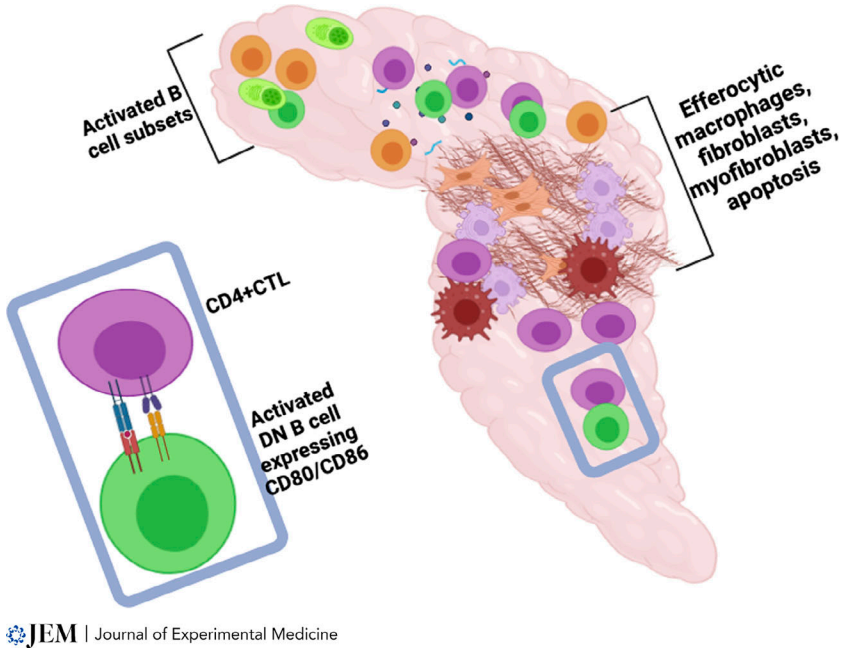

Activated B cells expressing CD80/86 can contribute to the repeated T cell activation required for generation of disease-driving cytotoxic CD4⁺ CTLs. DN, double negative.

(Della-Torre et al., 2020), and most abundantly by double negative 3 B cells that transcriptionally resemble plasmablasts (Allard-Chamard et al., 2023). A number of other fibrotic inflammatory diseases have since been linked to clonal expansions or tissue infiltrates that resemble those seen in IgG4-RD. These include systemic sclerosis (Maehara et al., 2020), fibrosing mediastinitis, a disease that has many similarities to IgG4-RD but is linked to *Histoplasma capsulatum* (Allard-Chamard et al., 2021), the orbitopathy of Grave's disease (Wang et al., 2021), and severe COVID-19 (Kaneko et al., 2022; Allard-Chamard et al., 2023). In fibrosing mediastinitis, circulating CD4⁺ CTLs have been shown be specifically triggered by *H. capsulatum* antigens, and in severe COVID-19, CD4⁺ CTLs represent one of the most abundant SARS CoV-2 antigen-specific T cell subsets (Meckiff et al., 2020).

Extrafollicular helper T cells that express IL-4 and IL-10 are prominent in IgG4-RD (Maehara et al., 2018, Munemura et al., 2022), and these cytokines are linked IgG4 class switching. Tissue evidence does not support a type 2 immune response in IgG4-RD. T helper 2 (Th2) cells are sparse and they are also not prominent in the tissues of systemic sclerosis (Maehara et al., 2020).

Overall, the tissue phenotype of human IgG4-RD is closely mimicked by that seen in LAT Y136F knockin mice. In these mice, high IgG1 and elevated IgE levels and the presence of eosinophilic infiltrates reflect the expansion of Th cells that drive class switching. These include T follicular helper cells that express high levels of Bcl-6, GATA-3, and IL-4 and which are known to drive IgE switching (these cells likely also express IL-13, but that was not demonstrated) as well as extrafollicular helper CD4⁺ T cells that drive some switching to IgG1 and lower-affinity IgE. No Th2 cell clusters were observed, while a prominent activated CD4⁺ T cell cluster identified was made up of CD4⁺ CTLs that did not express *Gata3* or *IL4*, but did express *Eomesodermin* and *T-bet*. The inflamed lungs in these mice were infiltrated by CD4⁺ CTLs, plasma cells, and other B cells; markers for extrafollicular B cells in mice are poorly defined, so there is not yet a murine equivalent identified for human double negative B cells. So, in a broadly similar way to that seen in IgG4-related disease, the disease-driving T cells in these mice appear to be CD4⁺ CTLs, and while Th2 cells are rare, Th cells that drive class switching to "type 2–like" Ig isotypes are also prominent.

Joachim and colleagues, likely taking into account the clinical response to Rituxan in IgG4-RD, sought to understand the contribution of B cells to the inflammatory disease in these mice. While perhaps actual B cell depletion using an anti-murine CD20 monoclonal antibody may have been a preferable approach to address this question, they crossed the LAT Y16F mice into μMT mice. The marked reduction in B cells reduced but did not eliminate CD4⁺ CTLs, but they did significantly attenuate disease pathology.

There are a few interesting lessons with wide ramifications both for immunology and for the study of human disease that can be drawn from these studies.

It is known that *EOMES* expressing CD4⁺ CTLs can evolve in type 2 milieus as seen in human nasal polyps (Ma et al., 2021), so there is no formal basis to link CD4⁺ CTLs to a type 1 milieu. The serial studies from Joachim et al. (2023) clearly demonstrate that Th1 cells are not even a required intermediate for CD4⁺ CTL generation. The broader inference, therefore, is that diseases driven by CD4⁺ CTLs represent a distinct category that does not fit the type 1, type 2, or type 3 paradigms for immune responses and disease.

In both humans, as seen in IgG4-RD, and in rodents, as seen in LAT Y136F mice, a robust IgE and/or human IgG4/murine IgG1 response can evolve in the relative absence of Th2 cells. The milieus that drive Th2 cell generation can sometimes overlap with those that drive T-dependent B cell class switching, but this kind of overlap is possibly stressed more than it should be.

The expression of CD80/86 on activated B cells likely evolved to allow tissue infiltrating activated B cells to optimally trigger and re-activate tissue CD4⁺ T cells generally to facilitate pathogen elimination, but this restimulation may also contribute to CD4⁺ CTL development (see figure). It is this function of B cells that might be abrogated by B cell depletion in the context of disease.

Acknowledgments

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