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INSIGHTS

CD1a autoreactivity: When size does matter

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CD1a-autoreactive T cells represent a significant proportion of circulating αβ T cells in humans and appear to be enriched in the skin. How their autoreactivity is regulated remains unclear. In this issue of *JEM*, Cotton et al. (2021. *J. Exp. Med.* https://doi.org/10.1084/jem.20202699) show that CD1a molecules do not randomly survey cellular lipids but instead capture certain lipid classes that broadly interfere with the binding of autoreactive T cell antigen receptors to the target CD1a. These findings provide new potential therapeutic avenues for manipulating CD1a autoreactive T cell responses.

For most immunologists, what first comes to mind when thinking of T cells are αβ TCR–bearing cells that recognize peptide antigens presented by MHC molecules, whereby the TCR needs to specifically engage an epitope composed of the peptide antigen and the MHC molecule presenting it with sufficient affinity to trigger activation. In recent years, however, it has become more and more apparent that αβ T cells with antigenic specificities other than peptides also play important roles in immune responses (Godfrey et al., 2015). αβ T cells with such specificities primarily include CD1-restricted T cells and MR1-restricted mucosal associated invariant T cells (Godfrey et al., 2015). In humans, four types of CD1 antigen-presenting molecules exist. They have been divided into two groups based on differential cell-type expression, intracellular trafficking, and crystallographic studies showing that each CD1 molecule has a different size and architecture of their antigen-binding clefts. Group 1 CD1 molecules include three members (CD1a, CD1b, and CD1c) while group 2 CD1 has only one, CD1d. All four CD1 molecules have been shown to present self and foreign lipid antigens to T cells. In most cases where lipid antigens could be identified, the aliphatic hydrocarbon chains of the lipid antigens were found inserted into the CD1 groove, and the phosphate, sugar, or other hydrophilic head groups protruded out of the outer surface of CD1 molecules where they could be specifically contacted by TCRs, in a manner similar to the corecognition of peptide–MHC complexes.

However, in many cases, the potential antigens recognized by CD1-restricted T cells are unknown. This is particularly relevant to the spontaneous autoreactivity that T cells in the blood of healthy donors display toward CD1 molecules. Indeed, certain CD1-restricted T cell clones can be activated by exposure to CD1-expressing APCs, even without the deliberate addition of foreign lipid antigens to the cultures. In fact, it was reported that ∼7% of circulating αβ T cells show autoreactivity toward CD1-expressing APCs, with CD1a and CD1c being the most frequently recognized isotypes (de Jong et al., 2010; de Lalla et al., 2011). These results suggested that autoreactive CD1-restricted T cells might corecognize cell-endogenous lipids presented by CD1 molecules (Shamshiev et al., 1999). More recent works, however, propose a different explanation to such autoreactivity. First, it was shown that CD1a-reactive T cells clones can be activated by different CD1a-expressing cells, or even by plate-bound CD1a. Second, these responses can be augmented by the addition of small headless lipids that lack head-groups (de Jong et al., 2014). Third, several different lipids can be eluted from CD1a-lipid-TCR complexes (Birkinshaw et al., 2015). Finally, CD1a tetramers produced without any added exogenous antigen can stain large T cell pools, accounting for ∼1% of skin T cells (Cotton et al., 2021a). These results led to the "lack of interference" model, in which the major antigenic target of CD1a-reactive T cells is the CD1a molecule itself.

Insights from Laurent Gapin.

In this issue of *JEM*, the Moody and Rossjohn groups team up again to examine whether CD1a molecules on APCs capture particular classes of lipid(s) and how this might affect the autoreactivity of CD1a-restricted T cells (Cotton et al., 2021b). Using various lipidomic methods, they first

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compared the endogenous lipids bound to CD1a proteins in HEK293T cells to the pool of lipids found within these cells. While phospholipids dominated in the cell extracts, CD1a molecules preferentially captured the much less abundant sphingomyelin (SM) lipids. SM species with a combined fatty acyl and sphingosine base of 34 methylene units and one unsaturation (34:1) predominate among the cellular pool of SM lipids. Yet, CD1a eluents were found enriched for longer SM with more unsaturated alkyl chains. The most abundant species eluted from CD1a was the longer 42:2 SM, with the combined fatty acyl and sphingosine base containing 42 methylene units and two unsaturations, which was otherwise barely detectable in total cells. Although this 42:2 SM molecular formula could be easily established, its exact structure (i.e., the total number of methylene units in each the sphingosine and alkyl chains, as well as the position and stereochemistry of unsaturations) could not. Thus, in order to further assess the immunological implications that 42:2 SM might have on CD1a autoreactive T cells, the authors started using synthetic SM with known structures.

CD1a tetramers loaded with endogenous lipids (CD1a-endo) that are derived from the cellular expression system used to produce CD1 molecules readily stain a fraction of skin T cells (Cotton et al., 2021a) as well as CD1a-autoreactive clones (de Jong et al., 2014). Surprisingly, 42:2 SM treatment of CD1a-endo interfered with this staining. Similarly, increasing concentrations of synthetic 42:2 SM to plate-bound CD1a proteins co-cultured with one of the CD1a-autoreactive T cell clones inhibited IFNγ production. Altogether, these results suggested that 42:2 SM might act as an inhibitor and block the potential binding of the TCRs expressed by CD1a autoreactive T cells. Further refined analysis using synthetic lipids of varying lengths and saturation confirmed these findings, showing that increased C6-8 increment chain length and a second unsaturation conferred increased inhibitory effects. To examine how these different SM might affect the overall architecture of the CD1a-lipid complex, the authors solved the crystal structures of CD1a bound to 36:2 SM, 42:1 SM and 42:2 SM. In each case, clear unbiased electron density in the cleft of CD1a could be observed, leading to the unambiguous assignment of the lipid position in the groove. Both 42:1 SM and 42:2 SM bound CD1a in a manner similar to each other, with 16–17% of the ligand protruding and being solvent exposed on the outer surface of CD1a. By contrast, the shorter acyl-chain in 36:2 SM allowed for a deep seating within the CD1a-binding groove, positioning the phosphate head group ∼7 Å deeper into CD1a and maintaining the integrity of the CD1a A′ roof. In this lack of interference scenario, this would allow for autoreactive TCRs to dock, thereby triggering a response. Thus, the size (and unsaturation) of the acyl chain of SM lipids loaded into CD1a matters in controlling the reactivity of CD1a autoreactive T cells. Under steady-state conditions, "blocker" lipids are preferentially loaded into CD1a and are interfering with TCR recognition. The authors propose that a balance between "permissive" versus blocker lipids loaded into the CD1a molecules might be controlling this autoreactivity. In humans, CD1a is found expressed on thymocytes, myeloid dendritic cells, and Langerhans cells (Meunier et al., 1996). In the periphery, CD1a is found predominantly in the skin, where epidermal LCs express CD1a at extremely high cell surface density. In disease conditions such as psoriasis and contact dermatitis (Cheung et al., 2016; Kim et al., 2016; Nicolai et al., 2020), small lipids are thought to displace these natural dominant negative blockers, thereby awakening the underlying autoreactivity of CD1a-restricted T cells. As such, these new findings should help in the design of exogenous lipids, which, based on their chain length and unsaturation, could be used for modulating the response of CD1a autoreactive T cells. Interestingly, major fatty acid (FA) species in cells include long-chain FAs (LCFAs) with carbon chain lengths of 12–20 and very-long chain FAs (VLCFAs) with carbon chain lengths ≥ 24. Both are generated by distinct fatty acyl elongases (ELOVL) that have tissue-specific expression (Kihara, 2012; Sassa and Kihara, 2014; Tanno et al., 2021). The authors determined that inhibitory 42:2 SM could be formally defined as VLCFA-SMs, while shorter permissive SM (34:1 SM) are LCFA-SMs, and that the ratio of these species varied by cell type, with higher 42/34 ratio in skin-derived samples. One could therefore imagine manipulating the activity of CD1a autoreactive T cells by changing the ratio of permissive/blocker self-lipids through the modulation of expression and/or activity of these ELOVLs. As such, it would be interesting to assess the activation status of CD1a autoreactive T cells in patients with mutations in *VLCFA*-related genes (Kihara, 2012) and/or upon manipulation of ELOVL expression. For example, IFNγ induces the down-regulation of ELOVL1 and ELOVL4 in cultured keratinocytes (Kanoh et al., 2019), while LCFAs tend to accumulate in the tumor microenvironment (Manzo et al., 2020). Although the role of CD1a autoreactive T cells in skin cancers remains largely unexplored, these new findings have the potential to provide novel therapeutic approaches for the manipulation of CD1a autoreactive T cells in a broad range of conditions.

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