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INSIGHTS

Blood-thirsty: S1PR5 and T_{RM}

Victoria M. Hallisey and Susan R. Schwab

In this elegant study, Evrard et al. (2021. *J. Exp. Med.* https://doi.org/10.1084/jem.20210116) find that sphingosine 1-phosphate receptor 5 (S1PR5) powerfully impairs tissue-resident memory T cell (T_{RM}) formation, and that tissue-derived TGF-β limits *S1pr5* expression by infiltrating T cells.

To eliminate an invading pathogen, activated T cells migrate into infected tissues. Some of these T cells stay on after the resolution of inflammation to serve as frontline defense against future infection. These long-lived cells are referred to as tissue-resident memory T cells (T_{RM} cells). Here, Evrard et al. (2021) demonstrate that downregulation of the homing receptor sphingosine 1-phosphate receptor 5 (S1PR5) by T cells in infected tissues is essential to retain these cells in the tissue, and hence to establish a robust army of CD8$^+$ T_{RM} cells.

Evrard et al. became interested in the role of S1PR5 in T_{RM} because *S1pr5* downregulation has been observed in T_{RM} cells in multiple settings, because S1PR5 directs exit of natural killer (NK) cells from lymphoid organs into circulation, and because effector CD8$^+$ T cells must lose the related receptor S1P receptor 1 (S1PR1) to park in tissues and become T_{RM} cells (Jenne et al., 2009; Walzer et al., 2007; Mackay et al., 2015; Skon et al., 2013). The best characterized role of S1P in the immune system is to act as a "circulation marker" (Baeyens and Schwab, 2020). The concentration of S1P is, in most cases, relatively low in tissues and high in blood and lymph. Leukocytes follow this S1P gradient out of tissues, and they sense the gradient using varying combinations of S1P receptors 1–5.

Evrard et al. began by examining expression of *S1pr5* by CD8$^+$ T_{RM} cells established in the skin after infection with HSV, in the lung after influenza infection, and in the small intestine after lymphocytic choriomeningitis virus infection. They found consistent strong loss of *S1pr5* mRNA in T_{RM} cells compared with circulating memory cells. *S1pr5* levels were low in naive CD8$^+$ T cells, induced upon T cell activation in the spleen in response to skin HSV infection, and extinguished as the cells settled into the skin. As in NK cells and in other models of CD8$^+$ T cell activation, the transcription factor T-bet was required to induce *S1pr5*, and a key intermediate step was T-bet's induction of the transcription factor *Zeb2* (Jenne et al., 2009; Dominguez et al., 2015).

Evrard et al. then asked whether the loss of *S1pr5* was important for T_{RM} cell establishment. They found that *S1pr5* limited T cell extravasation from blood into skin, as well as from blood into lymph nodes and splenic white pulp, by adoptively transferring activated CD8$^+$ T cells overexpressing *S1pr5* into blood and tracking their arrival in tissues. Furthermore, they found that *S1pr5* promoted T cell exit from the skin, by adoptively transferring activated CD8$^+$ T cells overexpressing *S1pr5* into the dermis and tracking their numbers over time. Conversely, *S1pr5*$^{-/-}$ T cells preferentially accumulated in skin compared with control T cells after HSV infection. *S1pr5*$^{-/-}$ type 1 innate lymphoid cells, which have strong T-bet expression, similarly accumulated in the small intestine and salivary glands compared with control cells.

Finally, Evrard et al. asked how T cell infiltration of skin led to loss of *S1pr5*.

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Tissue-derived cytokines including TGF-β have been shown to shape T_{RM} cell formation, and to down-modulate T-bet and *Zeb2* (Guan et al., 2018). Indeed, in an adoptive transfer model, very few TGF-β receptor 2-KO CD8$^+$ T cells were found in the skin after HSV infection, and those few cells had elevated expression of *Zeb2* and *S1pr5*.

A long-standing question has been why effector T cells might express S1PR5 in addition to S1PR1. Both receptors bind S1P with high affinity and couple to Gαi, although S1PR5 has been reported to couple to Gα12 as well (Malek et al., 2001; Mandala et al., 2002). One important difference between the receptors lies in their interaction with the early activation marker CD69. S1PR1 physically binds CD69, and the two are internalized together (Shiow et al., 2006). This traps cells in place just after activation. It's easy to imagine the utility of this for naive T cells, which might need to stay in the lymph node to survey for antigen after

Skirball Institute of Biomolecular Medicine, New York University Grossman School of Medicine, New York, NY.

Susan R. Schwab: susan.schwab@med.nyu.edu.

sensing inflammatory cytokines, or to stay in the lymph node to be activated just after receiving a signal through the T cell receptor. By contrast, S1PR5 does not detectably interact with CD69, and might enable activated cells to travel among tissues (Jenne et al., 2009).

This paper highlights a second important difference between the receptors: their sensitivity to ligand-induced internalization. In cultured cells expressing FLAG-tagged S1PR5, S1PR5 remains on the cell surface after incubation with S1P at a high concentration that results in full internalization of FLAG-tagged S1PR1. The same is true for incubation with pFTY720, a drug that targets both S1PR1 and S1PR5 (Jenne et al., 2009). In this paper, Evrard et al. (2021) found that S1PR5 limited T cell extravasation from blood to tissues, and one appealing hypothesis to explain this is that S1PR5 might remain sensitive to blood S1P and therefore keep T cells in circulation. In support of this hypothesis, internalization of S1PR1 by blood S1P is important for its function in trafficking. When S1PR1 cannot be internalized, T cells in blood continue to be attracted to blood S1P and are slow to enter lymph nodes (Arnon et al., 2011). Limited desensitization of S1PR5 might also explain why FTY720 has little effect on S1PR5-mediated migration (Walzer et al., 2007). Treatment with FTY720 (or treatment with the S1P lyase inhibitor deoxypyridoxine) might not ablate the ligand gradient. Any remaining gradient could be followed by S1PR5, but not by S1PR1 because it is fully internalized.

A major contribution of this paper is to identify a third important difference between the receptors: their transcriptional control. Evrard et al. elegantly show that while both *S1pr1* and *S1pr5* are downregulated during the establishment of resident memory, with TGF-β signaling a critical driving force, the pathways to loss of *S1pr1* and *S1pr5* differ. KLF2 plays a key role in regulating *S1pr1* expression, with little effect on *S1pr5*; conversely, ZEB2 is key for *S1pr5* but not *S1pr1*. It will be fascinating to explore further how these receptors are regulated over the course of different types of immune response in different tissues. One particularly interesting question is how expression of these receptors might be altered as a subset of activated T_{RM} cells returns to circulation upon reinfection (Fonseca et al., 2020, Behr et al., 2020).

Therapeutically, it may be important to release T_{RM} cells to treat chronic inflammation, or to release exhausted cells before cancer immunotherapy. It will be informative to address whether reactivation of S1P signaling in T_{RM} cells is sufficient to accomplish this. Conversely, in the context of vaccination, it may be important to trap effector T cells in specific tissues to generate T_{RM} cells. Manipulating $CD8^+$ T cell tissue egress via *S1pr1* or *S1pr5* regulation might offer a strategy to induce tissue-specific T_{RM} cells.

Overall, this paper sheds fundamentally important new light on the role of S1PR5 in establishment of T_{RM} cells and raises provocative questions about how manipulation of S1PR5 and tissue egress might be a novel strategy for treatment of disease.

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