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INSIGHTS

License to kill: Retinoic acid programs T cells for tissue residency

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In this issue of *JEM*, Qiu et al. (2023. *J. Exp. Med.* https://doi.org/10.1084/jem.20210923) show that retinoic acid signaling during priming in the mesenteric lymph node licenses CD8⁺ T cells to develop into small intestinal tissue-resident memory cells, a finding that provides key insights into tissue-specific vaccination strategies.

CD8⁺ tissue-resident memory (T_{RM}) cells provide the first line of defense against re-infection at barrier sites (Masopust and Soerens, 2019). Understanding how these cells develop is crucial for improving T cell therapies and vaccine efficacy. Establishing a pathogen-specific T_{RM} population within tissues involves three steps: Priming of the antigen-specific T cell in the lymph node, migration to the tissue, and in situ differentiation including acclimation to the specific tissue environment mediating long-term persistence. Priming of T cells in the draining lymph node of a specific tissue enables T cell migration to the infected tissue (von Andrian and Mempel, 2003). In the case of the small intestine, this is mediated by expression of gut-homing molecules by T cells, such as CCR9 and α4β7 integrin (Iwata et al., 2004; Mora et al., 2003). As cells leave the circulation, tissue-derived signals promote the acquisition of T_{RM} characteristicsc and repress egress, e.g., TGFβ in the intestine induces expression of CD103 allowing the cells to bind to E-cadherins, thereby mediating retention in the epithelial layer (Mackay et al., 2013; Mackay et al., 2015). However, it has been unclear to which extent priming in the lymph node predisposes cells to differentiate within the tissue. In this issue of *JEM*, Qiu et al. (2023) show that priming of T cells in the mesenteric lymph node (mLN) involves more than CCR9 induction and that RA-mediated signals during mLN priming uniquely license T cells to respond to tissue factors and to differentiate into CD103⁺ T_{RM} cells.

To address the question of how priming in the lymph node affects in situ differentiation in tissues, the authors exploit two different infection routes of *Listeria monocytogenes*, intravenous and foodborne infection: intravenous infection primes T cells in the spleen and does not result in priming of T cells in mLN, whereas oral infection causes T cell priming the mLN with minimal priming in the spleen. The authors then sorted either spleen-primed T cells (after intravenous infection) or mLN-primed T cells (after foodborne infection) at day 5.5 after infection and transferred these cells into infection-matched mice. Importantly, in both cases, the recipient mice were infected orally, thus providing similar levels of inflammation and antigen in the intestine and allowing for direct comparison of how the spleen- versus mLN-primed cells differentiate in situ. In line with the previously described connection between mLN priming to gut homing, the authors found higher expression of CCR9 and α4β7 integrin in mLN-primed T cells (Hamann et al., 1994; Mora et al., 2003). Further, mLN T cells were capable of in situ differentiation and upregulated the T_{RM} markers CD69 and CD103, whereas spleen-primed T cells failed to accumulate in the lamina propria or epithelium of the small intestine or upregulate CD69 and CD103, demonstrating that priming of T cells in the mLN licensed them to respond to tissue-derived signals.

Insights from Maximilian Heeg and Ananda W. Goldrath.

To understand how priming in the mLN instructs in situ differentiation of T cells, the authors compared gene expression of spleen-primed and mLN-primed T cells by RNA sequencing. Among the differentially expressed genes were *Ccr9*, *Hic1*, *Xcl1*, *Itgae*, and *P2rx7*, all of which were elevated in mLN-primed cells and are associated with a previously described T_{RM} gene expression signature (Borges da Silva et al., 2018; Burrows et al., 2017; Crowl et al., 2022; Milner et al., 2017)—indicating that this pattern of gene expression is partially acquired prior to emigration from the lymph node. Further, induction of *Ccr9* by mLN-primed cells corroborates the importance of mLN priming in T cell migration towards

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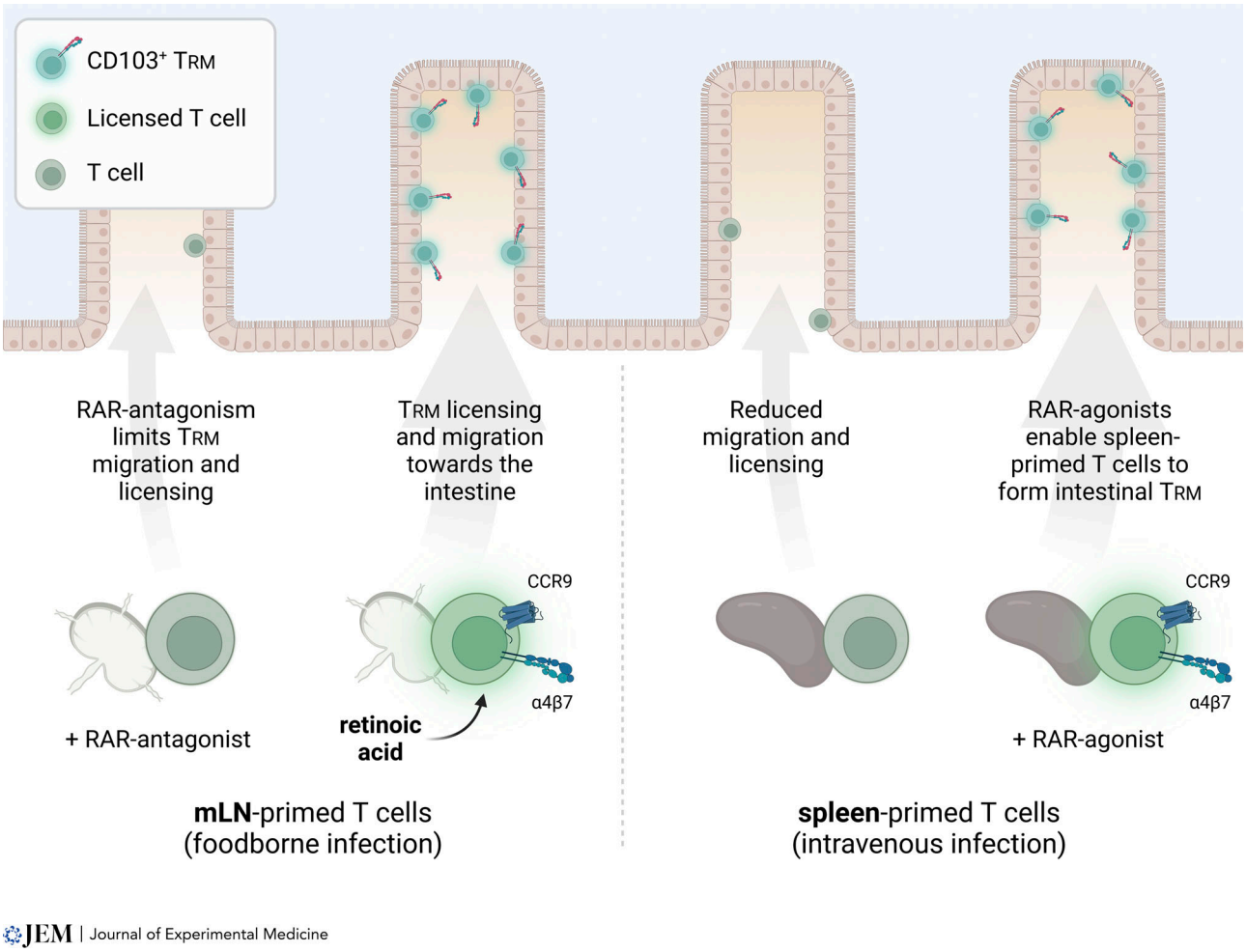

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Qiu et al. (2023) demonstrate that priming of T cells in the mLN in the presence of RA enables their migration to the intestine and licenses differentiation into T_{RM}. Spleen-primed T cells are limited in their capacity to seed the intestinal T_{RM} population. This limitation can be overcome by treatment with RAR agonists. Created with Biorender.com.

the intestinal epithelium. But what is the mechanism underlying T_{RM} licensing in the mLN? A closer examination of the most induced genes in mLN-primed versus spleen-primed T cells revealed that many of them can be induced by retinoic acid (RA), suggesting a role for this vitamin A metabolite in T_{RM} licensing. Further, RALDH2, which is involved in the conversion from vitamin A to RA, is only expressed by dendritic cells in the mLN, but not the spleen, thus corroborating the role of RA in T_{RM} licensing. To directly assess the importance of RA, Qiu et al. (2023) used pharmacological agonists and antagonists for the RA receptors (RAR). The authors used the same transfer setting of mLN-primed and spleen-primed T cells; however, this time, the mice were treated with the RAR agonist or antagonist during the first 5 d of infection. After transfer of mLN-primed T cells from mice treated with an RAR antagonist, the authors observed a substantial decrease of CCR9 and α4β7-integrin expression, but also impaired differentiation of these mLN-primed T cells into CD103⁺ T_{RM} cells. These results show that RA signaling is necessary for the priming of cells that have the potential to differentiate into CD103⁺ T_{RM} or "T_{RM} licensing." Furthermore, when transferring spleen-primed T cells from mice treated with an agonist to the RARs, the authors were able to increase expression of the gut-homing markers α4β7 and CCR9, enhance T cell numbers in the small intestine, and improve differentiation into CD103⁺ T_{RM} cells, indicating that RA is sufficient to allow for T_{RM} licensing.

Since *Ccr9* is induced by RA and was the most upregulated gene in mLN-primed T cells, the authors examined the importance of *Ccr9* for CD103⁺ T_{RM} differentiation. For this, the response of WT or *Ccr9*$^{-/-}$ cells to intravenous or foodborne *L. monocytogenes* infection was compared. Spleen-primed T cells do not express *Ccr9*, and thus their migration or capacity to differentiate into intestinal T_{RM} cells is not further impaired. In contrast, in foodborne infection, *Ccr9* deficiency not only resulted in impaired migration to the lamina propria and epithelium in the small intestine, but also impaired differentiation into CD103⁺ T_{RM} cells. However, a portion of *Ccr9*-deficient cells were still able to differentiate into CD103⁺ T_{RM} cells, indicating mLN-induced T cell licensing occurs through both CCR9-dependent and -independent mechanisms.

How does the lack of *Ccr9* lead to impaired T_{RM} differentiation? The co-culture of mLN-primed WT and *Ccr9*-deficient cells with TGFβ revealed that *Ccr9* expression does not affect the ability to upregulate

CD103 in response to TGFβ in vitro, which illustrates that *Ccr9* expression does not influence sensitivity to TGFβ in vitro. So how can the impaired differentiation of *Ccr9*-deficient cells into T_{RM} cells in vivo be explained? One possible and intriguing explanation lies in the well-described role of CCR9 in mediating T cell migration towards the epithelial layer. One hypothesis put forth by Qiu et al. is that bioactive TGFβ might only be available in distinct niches, and that CCR9 enhances T cell access to TGFβ signaling, thus allowing for a more efficient induction of CD103 in T cells.

Further, by combining the pharmacological modulation of RA signaling with the genetic *Ccr9*-deficient mouse model, the authors demonstrate that RA-mediated licensing occurs through both *Ccr9*-dependent and -independent mechanisms. For example, Qiu et al. (2023) show that mLN priming induces expression of *Hic1*, a ZBTB transcriptional repressor that has been shown to promote small intestinal T_{RM} differentiation (Crowl et al., 2022), and expression of *P2rx7*, an eATP receptor important for memory T cell differentiation and T_{RM} cell homeostasis (Borges da Silva et al., 2018).

Qiu et al. (2023) elegantly show that priming in the mLN not only regulates migration of T cells towards the intestine, but that specifically RA signaling during T cell priming regulates their ability to form $CD103^+$ T_{RM} in the small intestine and induces T_{RM}-associated genes. Spleen-primed T cells were inefficient at differentiating into T_{RM} cells, even if they migrated into the intestinal environment. Although RA induces CCR9 expression, and CCR9 impacts T_{RM} maturation to some extent—possibly by directing T cells into specialized microanatomical niches—RA-mediated licensing is independent of CCR9. These findings raise many important questions: for example, RA is highly abundant in the mLN but also in the intestinal epithelium—however, RA only seems to be important during priming in the mLN. Spleen-primed T cells can establish T_{RM} cells in response to a RAR agonist, but they do not respond to the abundant RA in the intestine. It is tempting to speculate that additional signals such as antigen availability or inflammation are simultaneously required to allow for T_{RM} licensing. Further, skin T_{RM} cells, which are also characterized by their expression of CD103, can develop regardless of whether T cells are primed in vivo or activated in vitro (Mackay et al., 2013; Mackay et al., 2012). In this study, however, the activated T cells were injected intradermally, therefore circumventing the necessity for T cell migration. These findings suggest that RA licensing is specific for the intestinal environment and that TGFβ-induced CD103 expression is differentially regulated between skin and intestinal T_{RM} (Hirai et al., 2019; Mani et al., 2019; Zhang and Bevan, 2013). Thus, it will be important to test if the approach of comparing priming sites could be a useful way to dissect T_{RM} precursor biology across different tissues. Combined with technological advances such as spatial transcriptomics and computational methods that assess cell–cell communication networks based on receptor–ligand pairs in a spatial context, this will allow further dissection of differentiating T_{RM} cells and the underlying molecular pathways that program T cell memory responses to specific tissues.

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