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Retinoic acid takes effector T cells to the gallows: P2X7, the molecular hangman

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Effector T-cell contraction is one of the primary means by which immune homeostasis in the peripheral tissues is reestablished following an inflammatory response. In this issue of *Mucosal Immunology*, Hashimoto-Hill *et al.*[1] show that retinoic acid (RA) transcriptionally upregulates P2X7 primarily in effector T cells of the intestine sensitizing them to NAD-induced cell death (NICD); thus, demonstrating a previously unrecognized role of RA in effector T-cell contraction.

The mucosal barriers are in constant contact with myriad number of innocuous antigens derived from among others the diet and commensal microbiota. Our immune system has evolved mechanisms to tolerate these innocuous antigens while keeping the capacity to mount effective effector responses against pathogens. However, during infection, tolerance to innocuous antigens can be lost with the consequent generation and expansion of undesired inflammatory effector T (T_{EFF}) cells. Importantly, breakdown of these mechanisms of tolerance has been associated with autoimmune disorders such as food allergies and inflammatory bowel diseases. How unwanted T-cell responses are controlled has been the focus of intense research during the last years.

Tolerance to foreign antigens (peripheral tolerance) can be achieved by induction of active regulatory mechanisms, such as the generation of regulatory T cells (T_{REG}) or by depletion of antigen reactive T cells. RA, a metabolite of vitamin A, has been shown to be instrumental in inducing active regulatory mechanisms.[2] For example, RA induces a regulatory program in naive T cells in a TGF-β-dependent manner resulting in peripherally induced FoxP3$^+$ T_{REG} cells. In addition, RA is necessary to induce gut-homing molecules, such as the integrin α4β7 and the chemokine receptor CCR9, in T_{REG} cells allowing their migration to proximal small intestine, where they acquire immunosuppressive functions.[3,4] In addition, RA imprints dendritic cells with enhanced capacity to promote Foxp3$^+$ T_{REG} differentiation through the induction of RA-metabolizing enzymes (e.g., Raldh2) and likely enhancing their capacity to activate TGFβ.[5] Although the role of RA in inducing active immune regulatory mechanisms is well appreciated, the effect of RA in the elimination of antigen-reactive T cells has been underexplored.

P2X7 is a trimeric ATP-gated cation channel predominantly expressed on immune cells and is well studied for its pro-inflammatory role in promoting inflammasome formation and release of IL-1β in response to inflammatory stimuli and extracellular ATP.[6] However, recent studies have demonstrated that P2X7 have an important role in T-cell functions and survival. Particularly, P2X7 has been shown to induce apoptosis in T cells both *in vitro* and *in vivo* in response to extracellular ATP or nicotinamide adenine dinucleotide (NAD) produced at the sites of inflammation and injury.[6] Besides, RA has been shown to upregulate the expression of P2X7 on intestinal CD8$^+$ T cells and sensitizes them toward ATP- and NAD-induced cell death.[7] However, the exact mechanism of RA-mediated regulation of P2X7 is largely unknown. In this issue, Hashimoto-Hill *et al.* added another feather to the already pleiotropic cap of RA. Focusing in the resolution phase of inflammation known as "effector T cell contraction", they demonstrated that RA confine the effector CD4$^+$ T-cell pool in the intestine by inducing expression of P2X7 (**Figure 1**). They demonstrated the *in vivo* requirement of RA using mice fed with vitamin A-deficient diet (VAD) that resulted in reduced expression of P2X7 in intestinal CD4$^+$ T cells with subsequent decrease in the sensitivity toward NICD. Further, requirement of

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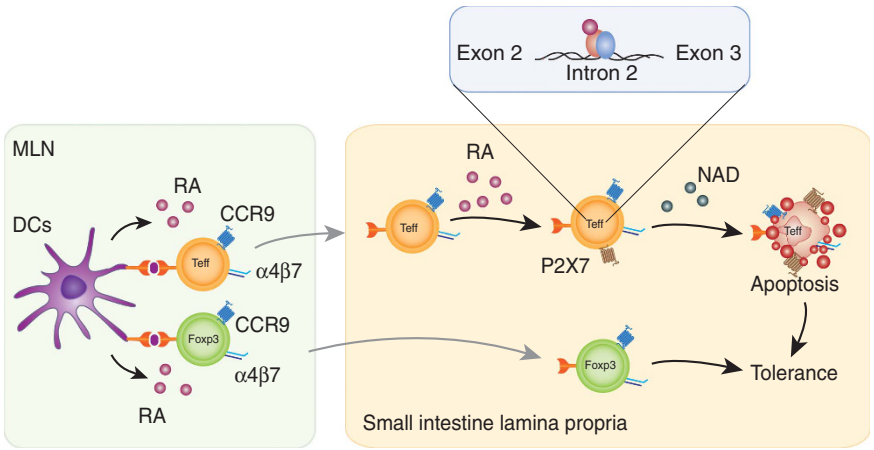

Figure 1 Retinoic acid (RA)-mediated upregulation of P2X7 sensitizes effector T cells toward NAD-induced cell death (NICD). Retinoic acid induces gut-homing markers (i.e., α4β7 and CCR9) on effector (Teff) and regulatory (FoxP3) T cells in the mesenteric lymph nodes. Gut tropic Teff or Foxp3 T cells are equipped to migrate to the small intestinal lamina propria to exert their effector and regulatory functions, respectively. In this study Hashimoto-Hill *et al.*, demonstrate that exposure of T cells to RA in the small intestinal lamina propria leads to transcriptional upregulation of purinergic receptor P2X7 preferentially in Teff cells resulting in NICD. This is relevant during the effector T-cell contraction where depletion of antigen-reactive T cells (Teff) is a prerequisite to reestablish homeostasis following an inflammatory response. At a molecular level, RA-induced P2X7 expression is mediated by retinoic acid receptor α (RARα) binding to an intragenic enhancer in intron 2 of P2X7 (top panel).

P2X7 was demonstrated using P2rx7-deficient T cells in which NAD failed to induce apoptosis. To find out the molecular mechanism by which RA induces P2X7 expression, the authors made use of publicly available chromatin immunoprecipitation (ChIP)-Seq data to narrow down potential retinoic acid receptor α (RARα) binding promoter and enhancer regions that were further validated using ChIP and luciferase reporter assays. Employing the above-mentioned strategy, they identified a RARα binding intragenic enhancer between exon 2 and 3 that was shown to be critical in regulating levels of P2X7 in response to RA in $CD4^+$ T cells (**Figure 1**).

Although $CD4^+$ T cells within mesenteric lymph node (MLN) induce gut-homing receptors as a result of sensing RA for the first time, the biological meaning of $CD4^+$ T cells getting exposed to RA for the second time upon arriving at the small intestine lamina propria (siLP) remains unknown. With an aim to answer this question, Hashimoto-Hill *et al.* systematically characterized RA-mediated P2X7 expression in $CD4^+$ T cells with respect to cell types and differentiation stages in both intestine and lymphoid organs. An increased expression of P2X7 in the major $CD4^+$ memory/effector T-cell subsets such as Th1 and Th17 cells in the intestinal lamina propria was observed compared with lymphoid organs and was significantly abrogated in VAD mice. This points toward the role of P2X7 in effector T-cell contraction. Using $P2rx7^{-/-}$ mice and a series of *in vivo* experiments Hashimoto-Hill *et al.* demonstrated that indeed there is an increased accumulation of Th1 and Th17 effector cells in P2X7-deficient mice. This accumulation was further exacerbated when $P2rx7^{-/-}$ mice were infected with a murine enteric pathogen *Citrobacter rodentium* that shares several pathogenic features with human enteropathogenic *Escherichia coli* (EPEC) and enterohaemorrhagic *Escherichia coli* (EHEC). Complementary to this finding, *in vivo* administration of NAD resulted in shrinkage of Th1 and Th17 cells only in WT mice but not in mice deficient in P2X7. The differences observed between WT and $P2rx7^{-/-}$ mice could also be attributed to several factors such as different microbiota or altered inflammatory responses upon challenge. To rule out the influence of any of these confounding factors, the authors took one step further and elegantly demonstrated P2X7-mediated effector T-cell contraction regardless of any such environmental factors. To do so, mice subjected to sub-lethal dose of irradiation were reconstituted with WT- and $P2rx7^{-/-}$-mixed bone marrow cells (in a 1:1 ratio) allowing the comparison between WT and $P2rx7^{-/-}$ T cells under the same conditions. Nine weeks after the transfer P2X7-deficient Th1 and Th17 cells significantly outnumbered their WT counterpart in the siLP. Thus, under the same environmental conditions, WT T cells die upon challenge whereas $P2rx7^{-/-}$ T cells are resistant. Finally, the authors demonstrated the disease relevance of P2X7-mediated effector T-cell contraction using the T-cell transfer colitis model of intestinal inflammation. In agreement, *in vivo* administration of NAD into mice following T-cell transfer significantly reduced pro-inflammatory Th1 and Th17 effector T cells with concomitant amelioration of intestinal inflammation. However, whether P2X7-mediated T-cell contraction is RA dependent *in vivo* still remains to be demonstrated.

Although the current study clearly demonstrates that RA-mediated expression of P2X7 in effector T cells has an important role in effector T-cell contraction, the same remains to be addressed for $FoxP3^+$ T_{REG} cells. Previous studies have demonstrated that $FoxP3^+$ spleenic T_{REG} that express P2X7 are more susceptible to ATP- or NAD-induced apoptosis compared with non-T_{REG}.[8] In the present

study the authors partly addressed this issue by comparing the expression levels of P2X7 in T_{REG} and non-T_{REG} according to their activation status and location (lymphoid organs or intestine). They observed that in the spleen and MLN even naive-like T_{REG} express P2X7 at medium level compared with the very low expression by the non-T_{REG} naive $CD4^+$ T cells. On the contrary, in the small intestine non-T_{REG} effector T cells express much higher level of P2X7 compared with the T_{REG}. One possible explanation is that as T_{REG} already express P2X7 at the naive stage, may depend less on RA for P2X7 expression during differentiation and maturation. However, how sensitive are intestinal T_{REG} to NAD-induced apoptosis remains to be further investigated.

This study by Hashimoto-Hill *et al.* evokes several interesting questions. For example, Esplugues *et al.*,[9] demonstrated that pro-inflammatory Th17 cells generated elsewhere can be redirected to the intestine in a CCR6-CCL20-dependent manner and controlled by flushing out part of the Th17 cells into the intestinal lumen. This suggests that intestine can act as an "immunologic sink" where excess of pathogenic cells can be dumped to maintain the overall immune homeostasis. In the same light, based on the findings of Hashimoto-Hill *et al.*, it is tempting to speculate an additional regulatory check point imposed by RA on T_{EFF} cells ensuring unwarranted expansion of the antigen-specific T cells that needs to be contracted following inflammation. This is achieved by RA-mediated upregulation of P2X7 preferentially on the effector T-cell populations in the mucosal sites. However, if P2X7 is capable of inducing different fates in different cell types, what determines its specific role in specific cell types. For example, in contrast to previous studies Hashimoto-Hill *et al.* demonstrated that memory or effector type $CD4^+$ T cells expresses higher levels of P2X7 compared with their naive counterparts both in the intestine and the lymphoid tissues resulting in increased susceptibility toward NICD. Although increased expression of P2X7 on effector T cells seems plausible with respect to T_{EFF} cell contraction, how the memory T cells and in particular tissue-resident memory T cells (T_{RM}), which survive the T-cell contraction phase, escape NICD.

Another interesting observation is differential expression of P2X7 on $CD4^+$ T cells based on their tissue location, which was higher in the intestine compared to MLN. As $CD4^+$ T cells are exposed to RA in both the environments, what accounts for such differential expression? This could be explained by a two-step sensing of RA by $CD4^+$ T cells. First, in MLN RA induces expression of gut-homing markers on $CD4^+$ T cells. Second, after their arrival in the intestinal lamina propria re-exposure to RA might lead to several fold upregulation of P2X7 compared to their MLN counterparts. Thereby, ensuring that the T_{EFF} are capable of mounting an effective effector response before being cleared by NICD (upon encountering ATP and NAD at inflammatory sites). However, this hypothesis needs to be experimentally tested.

Nevertheless, by showing that RA takes T cells to the gallows leading to their death by directly upregulating expression of P2X7, a molecular hangman, Hashimoto-Hill *et al.*[1] highlights a previously unappreciated role of RA in effector T-cell contraction.

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DISCLOSURE

The authors declared no conflict of interest.

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