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Vitamin A notches up CD11b^{hi} dendritic cell development

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Abstract

Vitamin A and its metabolite retinoic acid influence various aspects of immunity. Although the capacity of vitamin A to condition intestinal $CD103^{+}$ dendritic cells (DCs) to imprint tissue-specific homing programs onto activated lymphocytes is well documented, it is unclear whether vitamin A also regulates DC populations in other tissues. A study published in this issue of the *European Journal of Immunology* [Eur. J. Immunol. 2013. 43: XXXX-XXXX] now demonstrates that vitamin A exerts profound effects on the subset composition of splenic DCs. By resolving that splenic $ESAM^{hi}$ $CD11b^{hi}$ DCs are preferentially responsive to regulation by vitamin A, these novel insights not only further support the notion that ESAM expression marks two distinct lineages of splenic $CD11b^{hi}$ DCs, but also provide an important extension to our understanding of how vitamin A influences the immune system.

Dendritic cells (DCs) are rare, but widely distributed cells of hematopoietic origin that are specialized in capturing and presenting antigen to naïve T cells. Notably, DCs are comprised of multiple subsets that not only differ in phenotype and anatomical location, but, importantly, also exert distinct biological functions [1-3]. A useful strategy to divide these different subsets takes into consideration their relative ability to promote T-cell responses. In mice, surface expression of CD8α identifies DCs with a superior ability to stimulate $CD8^+$ T-cell responses, a process that often utilizes the cross-presentation pathway [4]. The appreciation that tissue-derived $CD103^+$ DCs in mice, and $BDCA3^{hi}$ DCs in humans, appear to be functionally and developmentally very closely related to $CD8^+$ DCs, but do not express CD8, has recently lead to the proposal to define this lineage of DCs by their expression of XCR1 [5, 6], a chemokine receptor that is conserved between the different DC subsets and across the species. In addition to this proposed DC lineage, DCs expressing high levels of surface CD11b appear to be functionally biased towards promoting MHC class II-restricted $CD4^+$ T-cell responses [7]. However, only a proportion of splenic $CD11b^{hi}$ DCs express CD4, and tissue-resident $CD11b^{hi}$ DCs are characterized by CD205 expression rather than CD4 [8]. Consequently, the cohort of $CD11b^{hi}$ DCs appears considerably more heterogeneous compared with the relatively well-defined $CD8^+/XCR1^+$ lineage [4, 9]. This view is supported by the diverse range of transcription factors and molecules that have been implicated in the development of $CD11b^{hi}$ DCs [10]. Interestingly, it was recently shown that differential requirement for Notch 2 receptor signaling defines two distinct lineages within the $CD11b^{hi}$ DC population [11]. The Notch 2 receptor signaling-dependent $CD11b^{hi}$ DC population is characterized by high-level expression of ESAM, an immunoglobulin superfamily molecule previously associated with neutrophil extravasation [12], and $ESAM^{hi}$ $CD11b^{hi}$ DC have been described as potent inducers of $CD4^+$ T-cell priming [11]. Conversely, $ESAM^{lo}$ $CD11b^{hi}$ DCs develop independently of Notch 2 receptor signaling and have a gene

expression signature resembling that of monocytes [11]. However, exactly how ESAM^{hi} and ESAM^{lo} CD11b^{hi} DCs diverge during development and what factors control Notch 2 receptor signaling in CD11b^{hi} DCs remains obscure.

In this issue of the *European Journal of Immunology*, Beijer et al. [13] have described an unexpected role for vitamin A in promoting the development of these newly described ESAM^{hi} CD11b^{hi} DCs within the spleen. Vitamin A, or retinol, is acquired through dietary intake and stored predominantly within the liver before release into the circulation. Upon conversion of circulating vitamin A into its active metabolite retinoic acid (RA) by retinaldehyde dehydrogenase (Raldh), RA acts as a transcriptional regulator, binding retinoic acid receptors (RAR) and retinoic X receptors (RXR) that are located in the nucleus. The binding of RA to RAR/RXR heterodimers facilitates the recruitment of co-activators and the formation of transcriptional complexes that dock onto RA response elements within the regulatory regions of target genes, which in turn initiates transcription [14]. Vitamin A has long been appreciated for its essential role in host immunity, and more recently has gained considerable attention as a major player in controlling intestinal immunity [15]. In this case, vitamin A functions in a feedback loop, whereby locally produced RA conditions intestinal CD103^{+} DCs to upregulate the enzymes involved in vitamin A metabolism. Consequently, upon migrating into the intestinal lymph nodes, CD103^{+} DCs produce RA, which in turn drives the expression of gut-specific homing receptors (CCR9 and $\alpha_4\beta_7$) by activated T and B cells [16, 17]. However, while RA is now well accepted to condition DCs within the intestine, its contribution to DC development elsewhere in the body is not yet fully resolved.

Given this association with intestinal immunity, Beijer et al. [13] set out to examine whether vitamin A influences the splenic DC composition and made the intriguing discovery that,

relative to splenic $CD8^+$ DCs ($CD11b^{lo}CD4^-CD8^{hi}$), splenic $CD4^+$ DCs ($CD11b^{hi}CD4^{hi}CD8^-$) and splenic DN DCs ($CD11b^{hi}CD4^-CD8^-$) have elevated expression of a number of RA target genes (*MMP9, gp91hox* and *TG2).* It was also observed that $CD4^+$ DCs and DN DCs express gene signatures indicative of preferential RA metabolism and utilization. To determine whether these RA responsive elements in $CD4^+$ DCs and DN DCs reflect developmental or functional dependencies on vitamin A, the authors fed new-born mice (day 7.5-10 of gestation) a vitamin A-deficient diet and analyzed the relative proportion of the three DC subsets in the spleen after at least 9 weeks of diet. Strikingly, while the relative proportion of $CD8^+$ DCs remained unaffected by the absence of RA, there was a significant reduction in the proportion of both $CD4^+$ DCs and DN DCs. Collectively; this suggests that in contrast to $CD8^+$ DCs, $CD11b^{hi}$ DCs are subject to RA signaling and that these signaling events are necessary for their differentiation within the spleen. To further probe the activity of RA in shaping the differentiation of splenic DCs, Beijer et al. [13] performed the reverse experiment, placing mice on a RA-rich diet before examining the relative proportion of the three DC subsets in the spleen. Here, excessive RA resulted in a shift towards DN DCs. Specifically, the frequency of $CD11b^{hi}$ DN DCs increased dramatically in the spleen, while the proportion of $CD8^+$ DCs and, unexpectedly, $CD4^+$ DCs was significantly suppressed in mice fed the vitamin A-rich diet. The lack of an increase in $CD4^+$ DCs in response to RA overexposure and subtle, but significant differences in the expression patterns of some of the nuclear RA receptors (RXRα, RARα, RXRβ) between $CD4^+$ DCs and DN DCs are likely related to heterogeneity within the $CD11b^{hi}$ DC population. Indeed, when Beijer et al. [13] segregated $CD11b^{hi}$ DCs on the basis of ESAM expression, which has recently been shown to resolve two distinct subsets within the $CD11b^{hi}$ DC population [11], they noted that RA specifically affected $ESAM^{hi}$ $CD11b^{hi}$ DCs with this subset being selectively reduced in the absence of RA and increased upon overexposure to RA. The utilization of the well-established FMS-like

tyrosine kinase ligand (Flt3L) culture system, which allows the revisiting of key steps of splenic DC development in vitro [18], further substantiated these in vivo findings. Specifically, it was found that addition of RA during Flt3L-driven DC development skewed the culture drastically towards the SIRPα^{hi} CD11b^{hi} DC subtype, although it was not examined whether ESAM was also expressed under these conditions. Whether the concomitant reduction in the culture equivalents of the CD8^{hi}/XCR1^{hi} lineage (SIRPα^{lo} CD11b^{lo}), and the reduced proportion of CD8^{+} DCs in mice kept on a vitamin A-rich diet indicates that RA also actively represses the development of CD8^{hi}/XCR1^{hi} DCs is an exciting possibility that remains to be tested.

Taken together Beijer et al. [13] demonstrate that RA signaling is necessary for the development of the ESAM^{hi} CD11b^{hi} DC subset within the spleen. These insights bring into focus a number of questions. For example, at what developmental stage are DCs subjected to RA conditioning? In this regard, it is interesting to note that hematopoietic stem cells in the bone marrow are among the few leukocytes that produce RA [19]. Analogous to the exposure of CD103^{+} DCs to RA in the intestine rather than in lymphoid organs [15], it is possible that pre-DCs would be exposed to RA in the bone marrow prior to their migration towards the spleen (Fig. 1A). Given that the proportion of circulating pre-DCs was unaffected in the absence of vitamin A, such a conditioning effect of RA would have to be regulated at a qualitative level rather than simply by altering the precursor product relationship numerically. Nevertheless, whether these events take place at the pre-DC level in the bone marrow or occur in the spleen requires further investigation. Another consideration arising from these findings relates to the potential mechanism by which RA signaling promotes the development of ESAM^{hi} CD11b^{hi} DCs. Given that ESAM^{hi} CD11b^{hi} DC differentiation is dependent upon Notch 2 receptor signaling, an interesting explanation maybe linked to RA regulating the

expression of Notch 2 receptor and/or its ligands (Fig. 1B). Indeed, it has recently been demonstrated that Raldh2-deficient mice, which are unable to convert vitamin A to RA, have diminished Notch signaling in developing neural tissues [20]. Thus, it is possible that RA signaling directly drives the expression of Notch 2 receptor and/or the ligands, and in this fashion, is necessary for the development of ESAM^{hi} CD11b^{hi} DCs. It will be interesting to examine whether the RA-dependent DC subsets express differential levels of Notch 2 receptor relative to other DC subsets. Similarly, future studies should investigate whether the Notch 2 receptor and/or its ligands contain RA-responsive elements within their promoter regions and are thereby directly regulated by RA. An alternative, although not mutually exclusive, mechanistic explanation may be that RA conditions pre-DCs to localize to distinct areas within the spleen that favour the differentiation of ESAM^{hi} CD11b^{hi} DCs (Fig. 1B). Since RA is well known to regulate homing receptor expression and migratory patterns of cells [21], it is conceivable that RA-conditioned DCs may preferentially migrate to regions of the spleen that are rich in Notch 2 receptor ligands, such as the marginal zone [22]. Stimulation of the Notch 2 receptor pathway could then promote ESAM^{hi} DC differentiation locally. It is interesting to contemplate this issue in light of the very recent finding that the chemokine receptor EBI2 (GPR183) and its ligand 7α,25-dihydroxycholesterol are critical for the positioning of CD4-expressing CD11b^{hi} DCs in the spleen [23]. Finally, as the observations by Beijer et al. were focussed on the spleen, it will be important to examine whether CD11b^{hi} DCs in the lymph nodes or tissues, such as dermal DCs or interstitial DCs, differentiate with comparable requirements for vitamin A and RA.

While the mode-of-action remains to be further defined, the findings of Beijer et al. [13] presented within this issue of the *European Journal of Immunology* clearly highlight a previously unappreciated role for RA signaling in regulating the diversity of splenic DCs.

Thus, vitamin A appears to play an ever-growing role in DC development, acting in both the intestinal and splenic compartment.

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Conflict of interest

The authors declare no financial or commercial conflict of interest.

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Figure 1. Retinoic acid (RA) drives ESAM^{hi} CD11b^{hi} DC differentiation.

(A) Pre-DCs seeding the spleen give rise to heterogeneous populations of DCs that can be roughly divided into the XCR1^{+}/CD8^{+} DCs and CD11b^{hi} DCs. In this issue of the *European Journal of Immunology*, Beijer et al. [13] describe that vitamin A-derived RA promotes the differentiation of a subset of CD11b^{hi} DCs that is characterized by the expression of ESAM. These findings not only uncover a novel link between vitamin A and splenic DC differentiation, but also raise some interesting questions. For example, is the bias in the ability of RA to drive the differentiation of ESAM^{hi} CD11b^{hi} DC accompanied by active suppression of the XCR1^{+}/CD8^{+} DC lineage? (B) Moreover, with RA linked to regulating the responsiveness of some cells to Notch ligands [20], and Notch 2 receptor expression required for the differentiation of ESAM^{hi} CD11b^{hi} DCs [11], is Notch signaling in DCs subject to regulation by RA? Finally, given the existence of Notch ligand gradients within the spleen [22], and the recent appreciation that the local splenic microenvironment influences CD11b^{hi} DC development [23], does RA affect splenic DC differentiation by impacting on their local distribution in the spleen?