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Would eating carrots protect your liver? A new role involving NKT cells for retinoic acid in hepatitis

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Retinoic acid (RA), which is the biologically active form of vitamin A, acts through the nuclear hormone receptor RAR (RA receptor) to induce either gene activation or repression. RA production and its effects have been linked to macrophages, dendritic cells, T and B cells, and iNKT cells in the immune system and play pro- as well as anti-inflammatory roles depending on the cell type and the immune context. In this issue of the *European Journal of Immunology*, Lee et al. [Eur. J. Immunol. 2012. 42: 1685–1694] show that RA ameliorates Con A-induced murine hepatitis by selectively downmodulating IFN-γ and IL-4 production in disease-causing NKT cells in the liver. Remarkably, this effect is restricted to this liver disease model and does not apply to αGalCer-induced murine liver injury, which is driven by other cytokines. The study identifies retinoid signaling as an important endogenous mechanism controlling immune reactions and also as a potential pharmacological target for treatment of hepatic liver injury. Furthermore, the study by Lee et al. provides additional support for the concept of metabolic regulation of immune function.

Keywords: Hepatitis · NKT cells · Retinoids · RAR

See accompanying article by Lee et al.

Presently there is an increased understanding and appreciation of the role that metabolic and lipid signaling plays in immune regulatory processes in multiple cell types (reviewed in [1]). For example, the orange pigment of carrots, beta-carotene, contributes to vitamin A levels in the body. Vitamin A is further processed into retinol, which is taken up by cells and oxidized into retinaldehyde by retinol dehydrogenase (alcohol dehydrogenase); retinaldehyde is subsequently oxidized by retinaldehyde dehydrogenase (RALDH) into retinoic acid (RA) (reviewed in [2]). RA (all-*trans* retinoic acid, RA) is one of the key biologically active compounds of vitamin A, the other (11-*cis* retinal) is involved in vision.

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RA acts as a ligand for one of the members of the nuclear hormone receptor superfamily, namely the RAR:RXR (RA receptor: retinoid X receptor) heterodimer [1]. In the absence of ligand, this receptor heterodimer binds to specific regulatory regions, termed response elements, of genes in the genome and represses their transcription. Upon ligand binding, the receptor heterodimer becomes activated and typically increases transcription [1,3]. In addition, the ligand-bound receptor can also bind to other transcription factors (e.g. NF-κB, AP1) via protein–protein interactions without directly binding to DNA, and by doing so can interfere with (i.e. repress) the transcriptional activity of these factors. This phenomenon is termed transrepression and is particularly important in the control of inflammation [1]. Therefore, the production and degradation of RA has to be very tightly regulated in order to coordinate its activating/inhibitory activities in the various cell types and tissues on which it acts. One of the functions of the RAR:RXR heterodimer is to turn on the degradation of RA by activating the

expression of a p450 enzyme CYP26 [3], thus forming a feedback loop to control RA actions.

The cellular activities of RA are widespread. It regulates cell proliferation and differentiation in many cancer cell lines, keratinocytes as well as cells of the immune system such as myeloid cells (reviewed in [1, 4]). These activities were typically identified by using exogenous, often synthetic activators or antagonists of RAR [1]. However, there is validation of these somewhat "artificial systems" since it is also well established that endogenous retinoids have immunomodulatory effects. For example, vitamin A deficiency increases childhood mortality and morbidity and increases an individual's susceptibility to infectious diseases (reviewed in [5]). In addition, there have been a large number of studies on the role of RA and/or RAR in hematopoietic differentiation and function. Of note, RAR is known to be expressed in nearly all hematopoietic lineages and to have roles in early myeloid differentiation and granulopoiesis [6, 7]. RA has a dual effect on differentiation by either inducing maturation or cell death, depending on the cellular context. It also blocks erythroid differentiation by downregulating GATA-1 [8].

Importantly, there is evidence for both pro- and anti-inflammatory activities of RA in macrophages. Regarding its anti-inflammatory actions, it has been documented that RA inhibits nitric oxide and TNF production in peritoneal macrophages [9], and that it enhances IL-10 production and reduces TNF-α and IL-12 production in LPS-stimulated macrophages [10, 11]. On the pro-inflammatory side, in rats treated with RA, the severity of TB infection is reduced, and this is accompanied by an increase in NK-cell, T-cell, and macrophage numbers in organs such as the lung and spleen, along with increased levels of TNF-α, IFN-γ, and IL-1β [12]. These data clearly show that, when assessing the role of retinoid signaling, context really matters.

One of the most striking examples of tissue and cell type specific production and activity of RA has been discovered by studying intestinal dendritic cells (DCs). Iwata et al. have shown that DCs isolated from mesenteric lymph nodes and Peyer's patches of the murine intestine are able to produce RA, also showing that these cells have the necessary enzymes (alcohol dehydrogenase III and Raldh2) to convert retinol to RA [13]. The $CD103^+$ DC subset is capable of inducing robust Treg-cell development [14]. Synthetic antagonists of RAR efficiently blocked Treg-cell development [14]. Since then, additional DC subtypes located in the skin and lung have been shown to produce RA, suggesting that this activity might not be restricted to gut DCs [15]. A key development based on these findings was the dissection of the mechanism of gut-specific lymphocyte imprinting and oral tolerance and the involvement of RA. Of note with regard to gut-specific lymphocyte imprinting, Iwata et al. showed that T cells primed with RA showed preferential homing to the gut, that the expression of the α4β7 integrin and CCR9 on the T cells was essential for this homing, and that RA induced α4β7 integrin and CCR9 expression in T lymphocytes [13]. Importantly, in the $CD103^+$ DCs, blocking RAR led to the inhibition of the induction of gut homing receptors (CCR9 and α4β7) [13]. In addition, DC-derived RA has also been shown to be important for B-cell gut tropism and IgA production [16, 17]. Furthermore, in human monocyte derived DCs induction of endogenous RA production leads to increased CD1d and reduced CD1a expression and a complete rearrangement of lipid antigen-presenting capacity, favoring iNKT activation [18].

Regarding the role for RA in oral tolerance, it has been shown that inducible Treg (iTreg) cells have an important role in maintaining tolerance and that gut $CD103^+$ DC-derived RA elicits iTreg-cell development in synergy with TGF-β [14, 19, 20]. As far as the molecular mechanism is concerned, RAR has been shown to induce active histone marks on the promoter of FoxP3, a master regulator of Treg-cell development, and hence to drive FoxP3 expression [21, 22]. RA also blocks the IL-6- and TGF-β-driven induction of the pro-inflammatory IL-17-producing T (Th17) cells [23].

But here again RA has Janus's two faces, because it has been shown that RA is also required for provoking a pro-inflammatory T-cell response to mucosal vaccination and infection [24]; inhibition of RARα in T cells resulted in a cell autonomous $CD4^+$ T-cell activation defect [24]. Collectively, these data suggest that endogenously produced RA is critical in shaping T-cell responses, but its effects are complex and difficult to untangle using only pharmacological and cellular immunological methods. In vivo studies complemented with tissue-specific genetic ablation of either the receptor or key metabolic enzymes are required to gain further insight.

A new wrinkle is added to these complex roles in this issue of the *European Journal of Immunology* by Lee et al. [25], who use RA pretreatment to assess the contribution of retinoid signaling to immune-driven liver damage using two in vivo models of hepatitis. One model uses concanavalin A (Con A) to induce rapid T-cell, granulocyte, and Kupffer cell infiltration in the liver, leading to hepatocyte death and eventually the death of the animal [26]. This model is believed to depend on NKT-cell activity; NKT cells in this model produce large amounts of cytokines, such as IFN-γ, IL-4, and TNF-α, leading to hepatocyte damage [27, 28]. While animals injected with Con A all died after 6 h, mice pretreated with RA all survived for at least 24 h [24]. This remarkable difference is accompanied by reduced levels of IFN-γ and IL-4, but no change in TNF-α levels [24]. Using a pharmacological inhibitor of RA synthesis (Disulfiram), the authors also showed that the reduction of endogenous RA production could aggravate Con A-induced hepatitis. By excluding the participation of other cell types, such as Kupffer cells and Treg cells, and also by excluding changes in the activation of NKT cells per se, they pinpointed the changes in cytokine production as the cause of the in vivo phenotype. Remarkably, in the other model of NKT cell driven hepatitis, RA pretreatment was ineffective. In this model, αGalCer, the ligand of CD1d, was administered to induce hepatic tissue damage [29]. However, this model depends on FasL and TNF-α rather than IFN-γ, and while the RA-induced changes in cytokines were similar to those induced in the Con A model (i.e. reduced levels of IFN-γ and IL-4, but no change in TNF-α levels), this did not translate into a marked phenotype in α-GalCer-induced liver injury as these cytokines are not the phenotype drivers. As far as the mechanisms behind these finding are concerned, the authors propose that RA downregulates

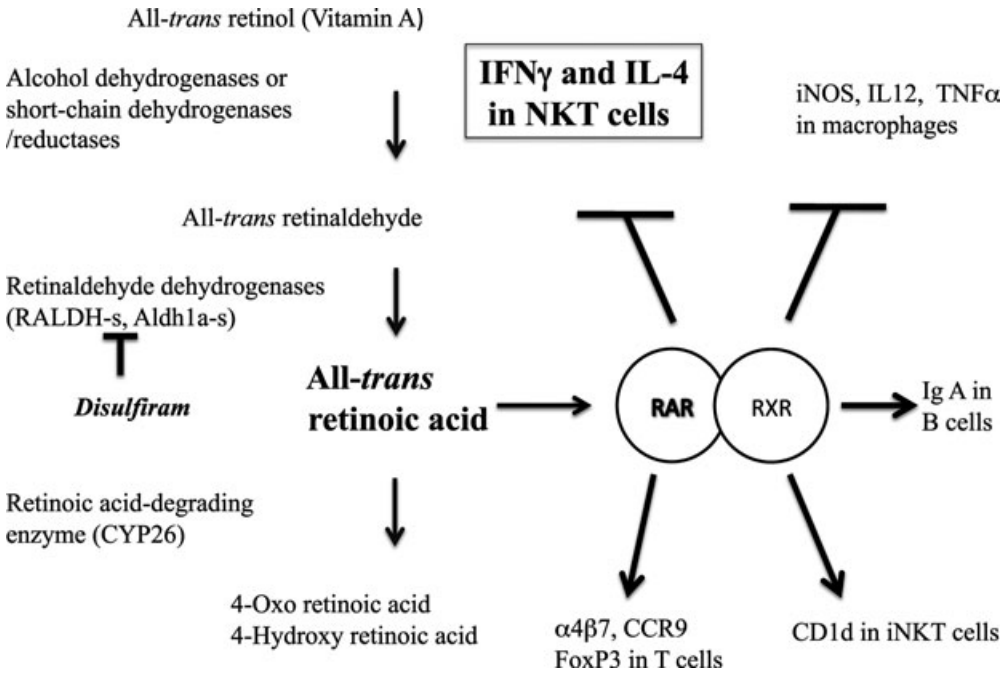

Figure 1. RA selectively regulates cytokine production in NKT cells.
The left side of the figure shows the metabolic steps leading to all *trans*-RA production and degradation. The enzymatic step inhibited by the synthetic enzyme inhibitor, Disulfiram, a compound inhibiting acetaldehyde dehydrogenase and retinaldehyde dehydrogenase as well is indicated. All *trans*-RA directly binds to and activates the RAR:RXR heterodimeric receptor, which activates (arrow) or represses (blunt arrow) the indicated genes expression in the various indicated cell types. The list is partial and is intended only to illustrate the variety of effects RA has on various immune cells. The new activity identified by Lee et al. in this issue of the *European Journal of Immunology* [25] is marked by a box.

IFN-γ and IL-4 production by a MAPK-dependent mechanism, while the NFAT-dependent TNF-α induction would be unaltered, hence explaining the differential effect on cytokine production (Fig. 1).

These new data are important as they strongly implicate RA and, critically, its endogenous production, in the control of NKT-cell cytokine production and, by doing so, provide new pharmacological targets for controlling hepatic inflammation in vivo. These findings also provide support for the concept that lipid signaling, metabolism, and diet are important in the immune regulation of T-cell subpopulations. Finally, they also highlight the need for further research into the mechanistic details of the pathophysiological and gene regulatory events triggered by RA that, hopefully, will lead to further therapeutic targets for the regulation of inflammation.

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Abbreviation: **RA**: retinoic acid

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