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Modulation of T Cell and Innate Immune Responses by Retinoic Acid

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Retinoic acid (RA) is produced by a number of cell types, including macrophages and dendritic cells, which express retinal dehydrogenases that convert vitamin A to its main biologically active metabolite, all-trans RA. All-trans RA binds to its nuclear retinoic acid receptors that are expressed in lymphoid cells and act as transcription factors to regulate cell homing and differentiation. RA production by CD103+ dendritic cells and alveolar macrophages functions with TGF-β to promote conversion of naive T cells into Foxp3+ regulatory T cells and, thereby, maintain mucosal tolerance. Furthermore, RA inhibits the differentiation of naive T cells into Th17 cells. However, Th1 and Th17 responses are constrained during vitamin A deficiency and in nuclear RA receptor α-defective mice. Furthermore, RA promotes effector T cell responses during infection or autoimmune diseases. Thus, RA plays a role in immune homeostasis in the steady-state but activates pathogenic T cells in conditions of inflammation. The Journal of Immunology, 2014, 192: 2953–2958.

Vitamin A or retinol (ROL) is essential for pre- and postnatal development, eyesight, and reproduction, and it plays a crucial role in the maintenance of the immune system (1–4). Exclusively provided through the diet, the different forms of ROL are absorbed by enterocytes and are predominantly stored in the liver (5, 6). In cells, ROL is either stored or hydrolyzed into retinal by ubiquitous alcohol dehydrogenases. A second and irreversible hydrolysis reaction allows the formation of retinoic acid (RA), the main biologically active metabolite of ROL (7). This limiting step is catalyzed by cell-specific retinaldehyde dehydrogenases (RALDH1, RALDH2, and RALDH3) and defines the range of action of RA (8).

RALDH enzymes produce two isofoms of RA: all-trans RA (atRA) and 9-cis RA (9cisRA) (9). Both atRA and 9cisRA are autocrine or paracrine ligands of the nuclear RA receptors (RARα, RARβ, and RARγ), whereas the nuclear retinoid receptors (RXRα, RXRβ, and RXRγ) only bind to 9cisRA (10, 11). Nevertheless, 9cisRA has not been detected in vivo, with the exception of the pancreas, in which it has been implicated in glucose metabolism (12). It is commonly understood that RARs act as heterodimers with RXRs and that the activity of the RXR is dependent on the activation of their ligand-inducible RAR partners (13). However, more recent studies (14–17) described intra- and extranuclear pathways driven by RARs independently of RXRs. Nevertheless, the majority of known functions of RA in immunity can be attributed to the canonical RAR/RXR pathway, driven by atRA produced by RALDH2 and acting through RARs. In this brief review, we highlight the recent key findings on the effects of vitamin A on T cells and innate immune responses.

RA in innate immunity, including its influence on APCs

A number of studies demonstrated that atRA has an important modulating role in innate immunity, with the most recent reports showing that RA has a central function in the differentiation of dendritic cells (DCs), the key APCs for activating naive T cells. Indeed, DCs express the three isotypes of RAR, especially RARα, and are able to respond directly to RA (18). It was shown that RA regulates the development and homeostasis of splenic CD11b+CD8α+CD4+Esam+DCs and the developmentally related intestinal CD11b+CD103+DCs but not other splenic or gut DC populations (19, 20). These two DC subtypes are specialized in MHC class II–restricted Ag presentation to CD4+ T cells both in vitro and in vivo and in the differentiation of IL-17–producing CD4+ T cells (Th17 cells) in vivo, respectively (21, 22). However, the mechanism by which RA regulates the fate of these DCs remains to be determined. One hypothesis proposed by several groups is that RA influences the Notch-2 pathway; Notch-2 signaling is required for the differentiation of CD11b+CD8α+CD4+Esam+ and CD11b+CD103+ DCs and is influenced by RA during development (22–25).

In addition to its impact on commitment of specific DC populations, RA influences the function of DCs. For example, atRA can modulate monocyte-derived DCs toward a mucosal-type DC, which secretes TGF-β and IL-6 and has the capacity to induce gut-homing receptors on T cells (26). atRA also enhances the migratory properties of DCs, which is crucial for their Ag-presentation function during infection. atRA induces
expression of the matrix metalloproteinases (MMP) MMP-9 and MMP-14 but not MMP-2, and it decreases the production of their inhibitors (tissue inhibitor of matrix metalloproteinase; TIMP) TIMP-1, TIMP-2, and TIMP-3, as well as the surface expression of the adhesion molecule CD11a on DCs in vitro (27, 28). MMP-9 and MMP-14 are involved in DC migration (29–31), and it was demonstrated that arRA promotes the migration of DCs in vivo toward draining lymph nodes in a murine model of cancer (27). This property of arRA is mediated through RARs, because its repression leads to a reduction in MMP-9 expression, whereas expression of CD11a on DCs and adherence of the CD11c+ DCs are both increased (28, 31). The enhancement of MMP-9 expression by arRA is likely to be mediated directly through the binding of RARs to the regulatory sequences of Mmp9 (31), although further studies are required to confirm this hypothesis.

In a proinflammatory context, arRA also has a direct influence on the Ag-presenting capacity of DCs. The positive or negative influence of arRA seems to be dependent on the proinflammatory context and/or the type of DC. During the development of experimental autoimmune encephalomyelitis, a mouse model of multiple sclerosis, arRA influences the APC function of splenic DCs by decreasing surface expression of MHC class II and the costimulatory molecules CD80 and CD86 (32). It was also shown that arRA can activate skin DCs called Langerhans cells; in the presence of the proinflammatory cytokines TNF-α and IL-1-β, arRA upregulates MHC class II and CD86 on Langerhans cells and induces binding of NF-κB to its response element on DNA (33).

In addition to its influence on DC differentiation, RA is directly produced by DCs: these cells express the RALDH enzymes, in particular RALDH2 (34). The production of RA by DCs was first described for intestinal CD103+ DCs, located mainly in the lamina propria, but also in the associated Peyer’s patches and mesenteric lymph nodes (MLNs) (34–37). Interestingly, the effect of arRA on DCs probably results from the autocrine action of RA produced by DCs themselves (34–37). Nevertheless, there are other sources of RA in the gut, such as intestinal macrophages, which express both RALDH1 and RALDH2 (38). The imprinting of intestinal CD103+ DCs to produce RA is not directly influenced by dietary vitamin A, but instead seems to rely on the stores of ROL and occurs directly in the small intestine (39). A number of studies (37, 39–41) demonstrated the importance of RA production by CD103+ DCs in the gut and its associated lymphoid tissues (GALT). However, RA is also produced in other DC populations, particularly in DCs located at the environmental interfaces, such as the skin, the lungs, and the corresponding draining lymph nodes (42, 43). At the molecular level, RA signaling in DCs is enhanced by RA itself (44, 45), as well as by the cytokines GM-CSF (35, 44), IL-4 (35, 45, 46), IL-13 (44), the MAPK p38α (45, 47), and TLR activation (18, 35, 46). Manicassamy et al. (18) showed that specific activation of the TLR2-signaling pathway induces strong expression of RALDH2 in splenic DCs and, as a consequence, SOCS3 expression, which suppresses the production of proinflammatory cytokines, including IL-23. Moreover, IL-4 enhances expression of RALDH2 and RARB at the transcriptional level, thus creating a positive-control loop on the RA pathway within inflammatory DCs (45). Interestingly, treatment of inflammatory DCs with IL-4 and arRA suppresses production of proinflammatory cytokines IL-6, IL-12p70, and TNF-α through a SOCS3-dependent mechanism (18, 45). In contrast, PGE2 appears to be a central negative regulator of RALDH expression in DCs, possibly through the induction of cAMP, a known repressor of RALDH (44). Importantly, recent studies (48, 49) showed a decrease in RALDH expression by intestinal and MLN DCs during infection with Trichuris muris and during colitis induced by T cell transfer in mice. These findings point to a crucial role for the inflammatory milieu in the regulation of RA production during inflammation. However, it is important to note that the influence of RA on the function of DCs in a proinflammatory context depends on the cytokines involved in the inflammation. For example, DePaolo et al. (50) demonstrated that IL-15 acts with RA on DCs to induce the phosphorylation of JNK, leading to the release of the proinflammatory cytokines IL-12p70 and IL-23.

Influence of RA on regulatory T cells and effectortopathenic T cells

A study by Iwata et al. (34) was one of the first to describe a role for RA in the biology of T cells; they found that RA enhances the expression of the gut-homing molecules α4β7 and CCR9 on CD4+ T cells, thus promoting preferential migration into the intestinal lamina propria and GALT. This function is supported by the intestinal RA-producing CD103+ DCs through RARα and RARB but not RARY, whereas the activation of RXR enhances the effect but appears to be dispensable (34, 51). In fact, the RAR/RARα pathway directly controls the expression of the integrin subunit α4 (52). Moreover, expression of the integrins α4β7 and α4β1 (also known as VLA-4) and implicated in the migration of T cells into tissues) is greatly decreased on the surface of T cells during a state of vitamin A deficiency (VAD), leading to defective migratory properties of these cells (52).

The observation that RA influences T cell migration was followed 3 y later by a major breakthrough on the function of RA in the maintenance of intestinal homeostasis, with the discovery that local RA produced by DCs and macrophages in the intestine and GALT promotes conversion of naïve CD4+ T cells into induced regulatory T cells (iTregs) (37, 38, 40, 53). Moreover, RA confers gut-homing properties on the regulatory T cells (Tregs) by increasing their expression of α4β7 and CCR9 (40, 54), partly through a BATF-dependent mechanism (55). In both mice and humans, RA cooperates with TGF-β to promote conversion of naïve CD4+ T cells into Foxp3+ iTregs (37, 35, 56), and this effect is mediated, at least in part, through RARα (53, 57). Interestingly, in a mouse model of Crohn’s disease, excessive stocks of vitamin A induced the conventional CCR9α4β7 Foxp3 population of Tregs, whereas a state of VAD generated CD103+CCR7Foxp3 Tregs; both Treg types helped to control intestinal inflammation (58).

The mechanism by which RA promotes iTregs is unclear, and there are a number of possibilities. RA appears to determine whether naïve CD4+ T cells differentiate into iTregs or Th17 cells, both of which require TGF-β. arRA inhibits expression of IL-6Rα on naïve T cells, which, together with TGF-β, promotes differentiation of naïve T cells into Th17 cells; IL-6 has the reciprocal effect of inhibiting the expression of RARα (41, 59, 60). Moreover arRA acts through RARα in activated CD4+ T cells to promote the expression of Foxp3, independently of the cytokine IL-2, the TGF-β–signaling molecules SMAD3
and SMAD4, and the transcription factors STAT3 and STAT5 (59, 61, 62). Furthermore, RA enhances the expression of arginine 1 in DCs in vitro, an enzyme known to promote the production of Tregs (63). In contrast, aTRA decreases T cell expression of RORγt, a transcription factor that is crucial for the differentiation of Th17 cells (60). It was reported recently that RA induces expression of the micro-RNA miR-10a in iTreg, which suppresses the negative effect of Bcl-6 and Ncor-2 on the stability of the iTreg population (64).

RA also induces iTreg conversion indirectly through inhibiting a population of CD4+CD44high memory T cells, which block the differentiation of naive T cells into Tregs, by secreting the cytokines IL-4, IL-21, and IFN-γ (59, 65, 66). In the absence of RARα-mediated modulation of CD4+CD44high memory T cells, the effect of RA on iTreg conversion is significantly decreased (59, 65). Interestingly, in the GALT, but not in lymph nodes and spleen, activation of CD44high T cells in the absence of TGF-β leads to the expression of the RA-degrading enzyme CYP26B1, which, in turn, inhibits the expression of CCR9 on T cells (67). Surprisingly, although enhancing the proportion of Tregs, both human and murine studies (68, 69) found that RA downregulates the expression of IL-10, an anti-inflammatory cytokine often produced by Treg during inflammation.

Because RA-producing DCs are found in organs at the environmental interfaces, it is important to note that in the skin a subset of DCs that, unlike RA-producing intestinal DCs, do not express CD103 but express CD11b+CD24+, express RALDH2, and produce RA (42). Moreover, these cells are able to induce Foxp3+ iTregs from naive CD4+ T cells (42). In the liver, hepatic stellate cells produce RA and are able to induce Foxp3+ Tregs in the presence of DCs and TGF-β1 (70). In the lungs, alveolar CD11c+ F4/80+ macrophages promote the induction of Foxp3+ Tregs and mediate respiratory tolerance via the production of RA and TGF-β (71). A complementary study (72) showed that lung-resident tissue macrophages coexpress TGF-β and RALDH and have regulatory functions. Furthermore, in a model of experimental allergic asthma, treatment with aTRA attenuates airways inflammation by inhibiting Th2 and Th17 responses (73).

Despite its role in promoting the differentiation of Tregs required to maintain mucosal homeostasis, recent studies established a central role for RA in the activation of effector CD4+ T cells during inflammation (Fig. 1). Thus, in a proinflammatory context, the RA-signaling pathway in CD4+ T cells is enhanced at the site of inflammation (74). CD4+ T cell effector function and migration to the site of inflammation were inhibited by conditional ablation of RA signaling in T cells (74). Hall et al. (75) showed that the RA–RARα axis is essential for the production of the proinflammatory cytokines IFN-γ and IL-17A by Th1 and Th17 cells in response to infection. Moreover, in a proinflammatory context involving cytokines, such as IL-15, RA acts through DCs to decrease conversion of naive T cells into Tregs and to enhance Th1 cell polarization (50). RA also has direct effects on CD8+ T cells; Allie et al. (76) found that the absence of functional RARα resulted in a lack of effector CD8+ T cells, whereas the population of central memory CD8+ T cells appeared to be increased. Importantly, both splenic and MLN DCs are able to enhance the RA pathway in CD8+ T cells (77), and both MLN and intestinal CD103+ DCs induced the RARα-dependent expression of the gut-homing molecules on activated CD8+ T cells (77–79).

Impact of RA on γδ T cells, NK cells, and innate lymphoid cells

In addition to its effect on T cells and DCs, RA modulates innate lymphoid cells (ILCs) and γδ T cells at the interface of...
innate and adaptive immunity. Consistent with the effect of CD4+ T cells, we found that RA inhibits IL-17A production by γδT cells but, interestingly, enhances the production of IL-22 by γδ T cells and group 3 ILCs stimulated with IL-1β and IL-23 (80). Furthermore, in vivo administration of RA promotes recovery from intestinal inflammation induced by dextran sulfate sodium or following infection with Citrobacter rodentium, and this is associated with enhanced production of IL-22 and the antimicrobial peptides REG3β and REG3γ (80). Consistent with the protective effects of aTRA, Chenery et al. (81) showed that mice lacking the RA-degrading enzyme CYP26B1 are protected against T cell–induced intestinal inflammation. In a model of autoimmune disease, experimental autoimmune uveitis, administration of aTRA is associated with a decrease in expression of the activation markers CD25 and CD69 on pathogenic γδ T cells. Furthermore, because aTRA reduces IL-17A production by γδ T cells (80) and because IL-17A and IL-21 produced by γδT cells can activate Th17 cells (82), RA may decrease the ability of γδ T cells to activate autoreactive Th17 cells (83). This provides further evidence that RA can have direct and indirect effects on CD4+ effector T cells. Studies in humans (84) showed that the aTRA–RARα pathway induces expression of the gut-homing integrin α4β7 and its ligand addressin (MAdCAM-1) on circulating γδ2+ γδ T cells, while decreasing their production of the skin-homing receptor cutaneous lymphocyte Ag.

There is also evidence that RA can influence the function of NK cells. RA enhances expression of the lipid Ag-presenting molecules CD1d on DCs, thereby promoting activation of NKT cells (85). Moreover, the number of circulating NK and NKT cells in humans is positively regulated by the level of the ROL stores (69). In tumor cells, RA also increases the expression of the RA early inducible genes RAE-1 and MICA/B, which activate NK cells through binding to NKG2D (86, 87).

Conclusions

Although the benefits of dietary vitamin A supplementation in controlling child mortality in developing countries is still controversial (88), there is evidence both from human and mouse studies that VAD is associated with defective immune responses to infection or following vaccination (75, 89). These studies led to the conclusion that vitamin A or its metabolite RA plays a role in driving adaptive immunity. Ironically, the early studies (37, 41) on the immunomodulatory functions of RA demonstrated a role, with TGF-β, in promoting the conversion of naive T cells to iTregs. RA produced by gut-resident CD103+ DCs helps to maintain intestinal tolerance by promoting expansion of Tregs in vitro and inhibiting Th17 cells (90). Furthermore, we demonstrated that treatment of mice with an RARα inhibitor can enhance the protective efficacy of a DC vaccine against B16 tumors, suppressing the induction of Tregs and promoting Th1 responses (90). Therefore, modulating RA signaling by dietary supplementation with vitamin A, treatment with agonists, or inhibitors of RAR has potential for enhancing natural and vaccine-induced immunity against infections or cancer, as well as in the treatment of immunemediated diseases in humans.

Disclosures

K.H.G.M. is a cofounder and shareholder in Opsona Therapeutics and Tri-Mod Therapeutics, university spin-out companies from Trinity College involved in the development of immunotherapeutics.

References


