Immunity
Review

The Role of Retinoic Acid in Tolerance and Immunity

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DOI 10.1016/j.immuni.2011.07.002

Vitamin A elicits a broad array of immune responses through its metabolite, retinoic acid (RA). Recent evidence indicates that loss of RA leads to impaired immunity, whereas excess RA can potentially promote inflammatory disorders. In this review, we discuss recent advances showcasing the crucial contributions of RA to both immunological tolerance and the elicitation of adaptive immune responses. Further, we provide a comprehensive overview of the cell types and factors that control the production of RA and discuss how host perturbations may affect the ability of this metabolite to control tolerance and immunity or to instigate pathology.

Introduction

In the early 20th century, E.V. McCollum and Thomas Osborne independently embarked on studies to identify dietary constituents that were essential for mammalian health and survival. By using different dietary supplements, they arrived at the seminal conclusion that a single factor present in lipids was essential for growth and survival, which they coined "fat-soluble factor A" (Wolf, 1996). Studies over the years have demonstrated the pleiotropic influence of this nutrient, subsequently designated vitamin A, ranging from eyesight and organogenesis to metabolism and immunological fitness (Acin-Perez et al., 2010; Duester, 2008; Underwood, 2004; Ziouzenkova et al., 2007). Vitamin A's critical contribution to immunological health is shown by the fact that its supplementation dramatically curbs young-childhood mortality in endemic regions of malnutrition (Rahmathullah et al., 1990; Sommer, 2008; Sommer et al., 1986). The vitamin A metabolite, retinoic acid (RA), first received attention as an interventional therapy upon discovery that it could substitute for more toxic chemotherapeutic regimens to dramatically improve the prognosis of acute promyelocytic leukemia (APL), a malignancy caused by genetic translocations with the retinoic acid receptor (RAR), RARα (de Thé and Chen, 2010). Although numerous investigations of APL have highlighted the ability of RA to promote myeloid cell differentiation (Kastner et al., 2001), over the last 20 years it has become clear that this metabolite influences multiple immune cell lineages and an array of immunological functions (Cantorna et al., 1995; Chun et al., 1992). In this review, we discuss recent advances that have established RA as central to both immunological tolerance and the elicitation of adaptive immune responses. Further, we provide a comprehensive overview of the cell types and factors that control the production of RA and discuss how host perturbations may affect the ability of this metabolite to control tolerance and immunity or to instigate pathology.

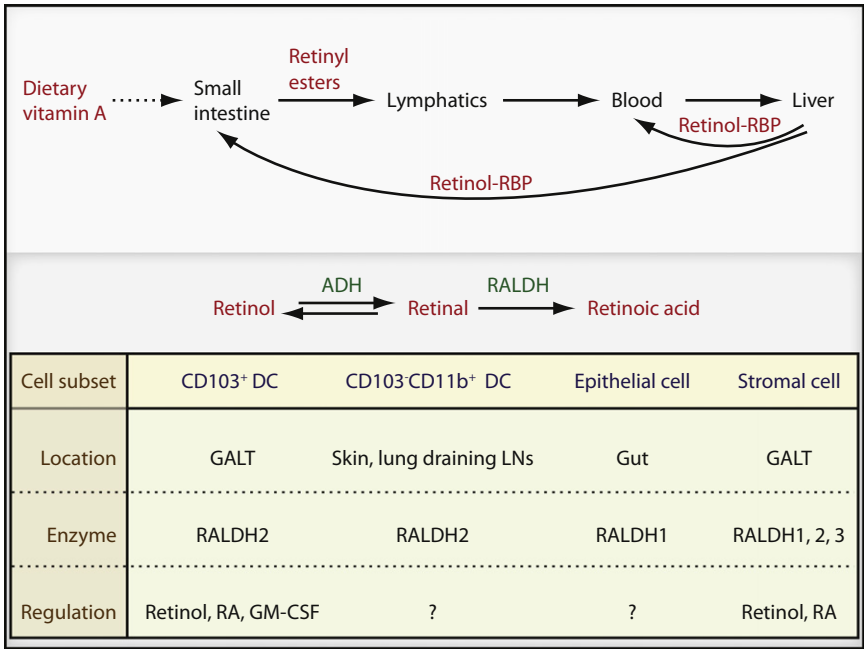
Acquisition, Storage, and Metabolism of Vitamin A

Vitamin A is a fat-soluble essential nutrient obtained through foods containing vitamin A precursors (i.e., carotenoids) or vitamin A itself in the form of retinyl esters (Figure 1; Harrison, 2005; Yeum and Russell, 2002). Subsequent to absorption and arrival into circulation, retinyl esters enter the liver, where most of the vitamin A in the body is stored (Blomhoff and Blomhoff, 2006). Liver retinyl esters are continually hydrolyzed into retinol and deployed into circulation (Wolf, 2007). Bile, which drains from the liver into the small intestinal duodenum, is also enriched in retinol (Jaensson-Gyllenback et al., 2011). Once inside a cell, widely expressed alcohol dehydrogenases (ADH) oxidize retinol into retinal, which can then bind to more selectively expressed retinal dehydrogenases (RALDH) for oxidation into retinoic acid (RA). RA can be generated in multiple isoforms; however, the all-*trans* isoform predominates in most tissues (Mic et al., 2003) and, therefore, the immunological effects of this compound will be the focus in this review. Although RA is constitutively present in serum at low amounts (Kane et al., 2008), RALDH induction is a tightly controlled process and subject to change during perturbations to homeostasis (Figure 2).

Retinoic Acid Sythesis and Induction of Cellular Migration into Mucosal Sites

Prior to its function, RA binds to nuclear receptors, including retinoic acid receptors (RAR), retinoid X receptors (RXR), and, under certain circumstances, PPARβδ (Chambon, 1996; Schug et al., 2007). Notably, all-*trans* RA exclusively binds RXR via heterodimers with the RAR family, which consists of three receptors: RAR alpha (RARα), beta (RARβ), and gamma (RARγ) (Chambon, 1996).

Appropriate immune responses depend on the ability of effector and regulatory lymphocytes to home to the site of infection or injury. In this regard, dendritic cells (DCs) have been shown to foster lymphocyte migration into tissues where antigen was initially encountered (Campbell and Butcher, 2002). For instance, DCs from gastrointestinal tract and associated lymphoid tissue (GALT), but not the periphery, were shown to induce the mucosal homing markers—integrin heterodimer $\alpha_4\beta_7$ and chemokine receptor CCR9—on stimulated effector T cells (Johansson-Lindbom et al., 2003, 2005; Mora et al., 2003). The insight that mucosal DCs triggered $\alpha_4\beta_7$ and CCR9 expression through the capacity to synthesize RA was based on the seminal observation that adding RA to T cells during activation selectively induced these gut homing markers (Iwata



Cell subset	$CD103^+$ DC	$CD103^-CD11b^+$ DC	Epithelial cell	Stromal cell
Location	GALT	Skin, lung draining LNs	Gut	GALT
Enzyme	RALDH2	RALDH2	RALDH1	RALDH1, 2, 3
Regulation	Retinol, RA, GM-CSF	?	?	Retinol, RA

Figure 1. Vitamin A Metabolism and Major Cellular Sources of Retinoic Acid during Homeostasis

Dietary vitamin A is absorbed in the intestine and transported through the lymphatics into blood circulation where it enters the liver for storage. Retinol chaperoned by retinol binding protein (RBP) is constitutively deployed from the liver into circulation. It is also secreted in bile that drains into the small intestine. Upon entry into cells, retinol is reversibly oxidized into retinal via the alcohol dehydrogenase (ADH) family. Depending on the cell type (see table), retinal can undergo irreversible metabolism into retinoic acid (RA) via retinal dehydrogenases (RALDH). The table provides an overview of major cellular sources of RA during steady-state conditions. Table includes cellular location, the isoform(s) of RALDH that are expressed, and the factors known to induce RALDH expression in each cell type.

et al., 2004). In a reciprocal fashion, blockade of RAR-mediated signaling and transcription in cultures containing GALT DCs reversed induction of $\alpha_4\beta_7$ and CCR9 (Iwata et al., 2004; Svensson et al., 2008). Recently, RA was revealed to predominantly affect the α_4 subunit of $\alpha_4\beta_7$ via binding of RARα to a RAR response element within the regulatory region of the gene that encodes α_4 (DeNucci et al., 2010; Kang et al., 2011). A retinoic acid response element half-site was also recently discovered in the promoter region of CCR9, which permits RARα-RXR heterodimer binding (Ohoka et al., 2011). These data coupled with the prominent baseline expression of *Rara*, the gene encoding RARα, in $CD4^+$ T cells, pinpoint RARα as an important mediator of lymphocyte trafficking. Nevertheless, they do not exclude a role for other RARs in mediating the regulation of these markers on other defined subsets.

An assessment of vitamin A-synthesizing enzymes demonstrated that GALT DCs express mRNA for *Aldh1a2*, the gene encoding RALDH2 (Iwata et al., 2004; Schulz et al., 2009; Yokota et al., 2009). Subsequent studies have shown that basal *Aldh1a2* expression in GALT DCs is enriched in $CD103^+$ DC subsets, which induce $\alpha_4\beta_7$ and especially CCR9 far more potently than the $CD103^-$ DC compartment (Figure 1; Coombes et al., 2007; Johansson-Lindbom et al., 2005; Yokota et al., 2009). In the small intestinal LP, this population is uniquely equipped with migratory capacity and therefore accumulates in the mesenteric lymph nodes (MLN) (Bogunovic et al., 2009; Jaensson et al., 2008; Jang et al., 2006; Schulz et al., 2009). $CD103^+$ DCs within the MLN and Peyer's patches (PP) are comprised of two subsets based on expression of integrin CD11b. The $CD103^+CD11b^-$ subset is related to the $CD103^+CD11b^-$ and $CD8\alpha^+$ DC subsets outside of the GALT (Edelson et al., 2010; Ginhoux et al., 2009; Hildner et al., 2008). However, $CD103^+CD11^-$ DCs residing at other sites fail to produce appreciable RALDH, suggesting that factors within the GI tract induce RA-synthesizing capacity in this subset (Guilliams et al., 2010). In this regard, vitamin A itself is indispensible for DC production of *Aldh1a2* during homeostasis (Jaensson-Gyllenback et al., 2011; Molenaar et al., 2011; Yokota et al., 2009). This may involve Wnt-β-catenin-driven signals, because ablation of β-catenin in CD11c cells attenuates their expression of RALDH (Manicassamy et al., 2010). Microbial stimuli also appear to have an additional influence on RALDH expression; moderate decreases were observed in GALT DCs isolated from mice reared in germ-free conditions or genetically deficient in the microbial signaling adaptor MyD88 (Guilliams et al., 2010). Toll-like receptor 2 (TLR2)-stimulating ligands, in particular, were found to most potently induce RALDH expression (Manicassamy et al., 2009; Wang and Sampson, 2011).

In addition to DCs, several nonhematopoietic lineages within the gastrointestinal tract and associated lymphoid tissues (GALT), such as epithelia (expressing *Aldh1a1*, encoding RALDH1) and stromal cells (*Aldh1a1*, *Aldh1a2*, and *Aldh1a3*), share the capacity to synthesize RA (Figure 1; Edele et al., 2008; Hammerschmidt et al., 2008; Iliev et al., 2009; Iwata et al., 2004; Molenaar et al., 2011). Intestinal epithelial cells have been shown to imprint bone marrow DCs with signature characteristics of LP DCs, via provision of soluble factors, including RA (Edele et al., 2008; Iliev et al., 2009). They may also provide RA in *trans* and reinforce the expression of mucosal homing markers on lymphocytes. Stromal cells within the MLN were also found to support mucosal homing, probably through indirect effects on APCs (Figure 2; Hammerschmidt et al., 2008; Stock et al., 2011). Altogether, these findings support the observation of a prominent reduction in the number of activated T lymphocytes within the intestinal effector sites in adult mice reared on a vitamin A-deficient diet, as well as in *Rara*-deficient ($Rara^{-/-}$) mice (Hall et al., 2011; Iwata et al., 2004).

Other DC populations, particularly in the draining lymph nodes of the skin and lung, express *Aldh1a2* (Figure 1; Guilliams et al., 2010). The finding that RA signaling occurs in sites not typically associated with mucosal homing raises several interesting points of discussion. First, crosstalk with other cells in these tissues may modulate the capacity of DCs to induce mucosal homing. For example, prostaglandin E2, from the stroma of

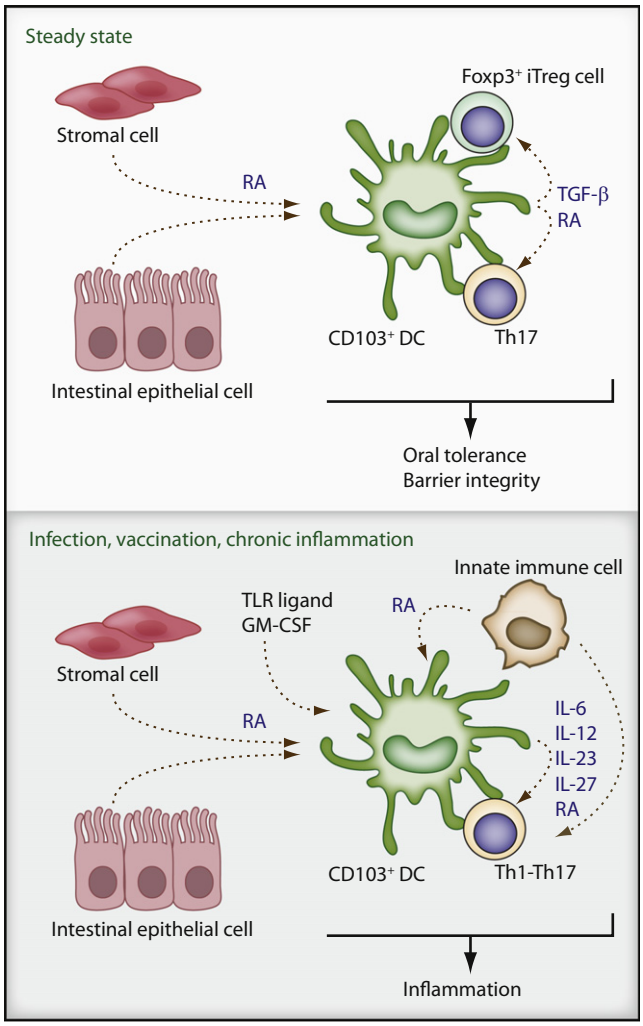


Figure 2. The Role of Retinoic Acid in the Regulation of CD4+ T Cell Homeostasis and Immunity in the GI Tract

The GALT is a retinoic acid (RA)-rich environment, containing a vast presence of RA-synthesizing cells, including resident epithelia (IECs) and stromal cells (SCs), as well as migratory $CD103^+$ DCs. RA produced by IECs may confer characteristic features to lamina propria DCs, whereas RA produced by SCs in the draining mesenteric lymph nodes may reinforce lymphocyte acquisition of mucosal homing markers and potentially other effector functions. During steady-state conditions (top), RA sustains oral tolerance and helps maintain barrier integrity. These processes are mediated in large part by the ability of RA to support the induction of $Foxp3^+$ iTreg and Th17 cells. Lamina propria $CD103^+$ DCs induce and recruit heterogeneous $CD4^+$ T cell populations during steady state, as result of their ability to respond to commensal microbial signals and produce both RA and TGF-β. During inflammation or infection (bottom), the inflammatory milieu triggers altered cytokine production by $CD103^+$ DCs, leading to RARα-dependent effector $CD4^+$ T cell activation and differentiation. Innate cell populations, including antigen-presenting cells, are recruited during inflammation and also contribute to RA production. Factors in the inflammatory milieu, including TLR-ligands and the cytokine GM-CSF, promote RALDH activity in $CD103^+$ DCs and potentially in recruited innate cells.

peripheral tissues, was recently shown to antagonize RALDH expression (Stock et al., 2011). Second, RA signaling may also influence migration to peripheral sites. In this regard, integrin α_4 can also form heterodimers with integrin subunit, β_1, which is rapidly upregulated in response to T cell receptor (TCR) stimulation and impedes formation of the $\alpha_4\beta_7$ heterodimer (DeNucci et al., 2010). $\alpha_4\beta_1$ binds to VCAM-1, which is present on endothelial cells and upregulated during inflammation, so RA may additionally influence migration to peripheral sites during inflammation (Figure 3; Henninger et al., 1997; Muller, 2011). Further studies are essential to understand how crosstalk between DCs and other cells can modulate RALDH activity, which may produce a better understanding of how accessory cells contribute to the regulation of immunity during both homeostasis and inflammation. Collectively these findings indicate that RA signaling occurs in tissues throughout the host and suggest that apart from mucosal homing, it plays a general role in both tolerance and inflammation.

Retinoic Acid in Plasma Cell Differentiation and Mucosal IgA

The discovery that RA was critical for the generation of immunoglobulin A (IgA)-secreting B cells offered further evidence of a multifactorial role for RA in mucosal immunity (Mora et al., 2006). A number of studies have demonstrated the potent capacity of DCs from the intestinal LP, MLN, and PP to drive naive B cell differentiation into IgA^+ B cells (Macpherson and Uhr, 2004; Mora et al., 2006; Uematsu et al., 2008), and the ability of stromal-derived cells to support IgA^+ class switching in activated B cells (Fagarasan et al., 2001; Suzuki et al., 2010). Synthesis of RA by GALT DCs is crucial for the generation of IgA^+ B cells, as shown by the fact that antagonism of RA signaling markedly reduce IgA^+ production (Mora et al., 2006; Uematsu et al., 2008). Complementing this finding, addition of RA to DC cocultures in which DCs lacked the capacity to synthesize RA restores IgA^+ production. Notably, microbial-induced cytokines, such as IL-6, are also integral cofactors in this process (Mora et al., 2006; Uematsu et al., 2008). In addition, although inhibitory at high concentrations (Mora et al., 2006), TGF-β-mediated signals also play a decisive role in IgA^+ production (Cazac and Roes, 2000). This has been verified in systems analyzing the capacity of mucosal stromal cells to foster IgA^+ B cell generation (Fagarasan et al., 2001; Suzuki et al., 2010). In a manner analogous to peripheral DCs, peripheral follicular dendritic cells are able to efficiently support IgA^+ production only when treated with RA and a MyD88-dependent microbial stimulus (Suzuki et al., 2010). This gain of function is dependent on the ability of RA signaling to induce secretion of TGF-β in peripheral follicular dendritic cells. RA signaling promotes a similar effect (i.e., induction of TGF-β production) in bone marrow-derived DCs via inhibition of suppressor of cytokine signaling 3 (SOCS3) activity (Feng et al., 2010). These findings suggest interdependency between TGF-β- and RA-propagated signals in several cell lineages.

Another significant source of IgA^+ production is B1-B cells, which contribute to mucosal integrity during homeostasis and early responses to pathogens. RA was recently shown to enforce the homeostatic maintenance of this compartment through direct regulation of the transcription factor NFATc1 (Maruya et al., 2011). Combined with the capacity of RA to generate IgA^+ B cells and facilitate their mucosal localization, vitamin A deficiency leads to a severe decrease in intestinal IgA and a marked decrease in the serum (Maruya et al., 2011; Mora et al., 2006). Altogether, these findings underscore the importance of RA in IgA responses and humoral immunity.

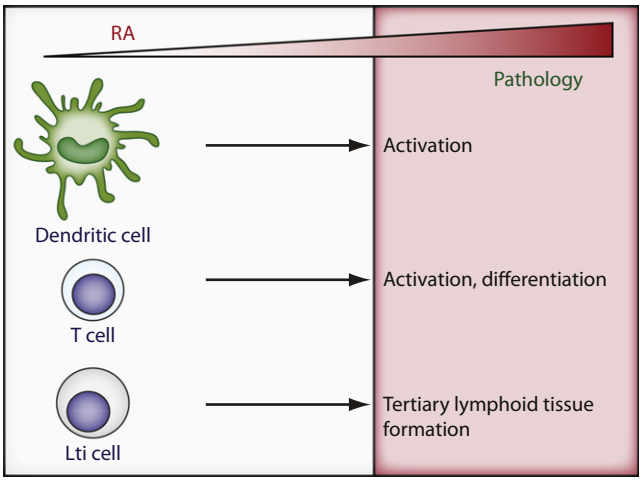


Figure 3. RA Synergizes with an Inflammatory Milieu to Promote Pathology

During inflammation, increasing RA signaling can potentiate the production of inflammatory cytokines by DCs, and in turn promote effector T cell differentiation. Direct interactions of RA with T cells may further contribute to the activation status of the cells and promote localization to inflamed tissues. RA may also engender the formation of tertiary lymphoid structures and facilitate chronic inflammation.

Retinoic Acid in Extrathymic Treg Cell Induction and Oral Tolerance

Foxp3 regulatory T (Treg) cells maintain both peripheral and mucosal homeostasis throughout the lifespan of the host (Josefowicz and Rudensky, 2009; Kim et al., 2007). Treg cells typically develop during thymic selection processes; however, they also develop extrathymically in response to chronic antigen stimulation or exposure to environmental and food antigen at mucosal sites (Curotto de Lafaille and Lafaille, 2009). Development of inducible Treg (iTreg) cells, but not thymic-derived Treg cells, requires transcription factor binding to the intronic enhancer element (enhancer-1) of the foxp3 locus, also known as conserved noncoding sequence 1 (CNS1). iTreg cell development is also dependent on several soluble mediators, including TGF-β, IL-2, and, as recent data demonstrate, RA (Hall et al., 2011; Knoechel et al., 2005; Kretschmer et al., 2005; Mucida et al., 2005; Tone et al., 2008; Zheng et al., 2010).

The insight that RA served as a cofactor in the generation of iTreg cells stemmed from in vitro findings that relative to splenic DCs, MLN and LP DCs potently induced iTreg cell differentiation in the presence of TGF-β (Coombes et al., 2007; Mucida et al., 2007; Sun et al., 2007). Separation of MLN DCs and LP DCs based on CD103 expression revealed that the $CD103^+$ subsets were specifically able to yield iTreg cells in the absence of exogenous factors, implying that RA signaling potentially contributed to this process (Coombes et al., 2007; Sun et al., 2007). Indeed, a pan-RAR antagonist strongly inhibited iTreg cell generation (Coombes et al., 2007; Mucida et al., 2007; Sun et al., 2007).

Blockade of TGF-β in cocultures with $CD103^+$ GALT DCs also diminished iTreg cell generation. The coordinate ability of GALT DCs to produce both RA and TGF-β may involve conditioning signals from RA during developmental maturation (Figure 2; Feng et al., 2010). Assessment of DCs in a retinoic acid response reporter mouse revealed abundant RAR binding activity in GALT $CD103^+$ DCs (Jaensson-Gyllenback et al., 2011). The level of RAR binding activity in DCs from several tissues correlated with both the concentration of retinol and the percentage of $CD103^+$ DCs expressing RALDH2 in those tissues, which suggests that DC conditioning by RA may occur in situ. Furthermore, GALT $CD103^+$ DCs highly expressed mRNA for the gene encoding RARα, implicating this receptor as a dominant mediator of such conditioning signals (Jaensson-Gyllenback et al., 2011).

The addition of RA to cocultures with splenic DCs and TGF-β also dramatically enhanced iTreg cell induction (Benson et al., 2007; Mucida et al., 2007, 2009; Sun et al., 2007). This effect is dependent on T cell expression of RARα (Hill et al., 2008), which is upregulated upon stimulation in the presence of TGF-β (Schambach et al., 2007). Remarkably, exogenous RA can sustain iTreg cell generation in conditions that typically oppose it, such as the presence of certain inflammatory cytokines (IL-6, IL-21) and high costimulatory environments (Benson et al., 2007; Mucida et al., 2007; Xiao et al., 2008). Stimulation of $CD4^+$ T cells in the presence of TGF-β induces IL-6R expression, which is reversed with the addition of RA (Figure 2; Hill et al., 2008; Xiao et al., 2008). Because RA did not appear to enhance the inherently unstable phenotype of iTreg cells (Floess et al., 2007; Hill et al., 2008), RA-mediated repression of IL-6R could potentially explain why iTreg cells generated in the presence of RA were reportedly more stable in vivo after adoptive transfer (Benson et al., 2007). Corroborating in vitro findings, the generation of iTreg cells in response to antigen feeding is abrogated in animals deficient in vitamin A and, therefore, lacking RA (Hall et al., 2011).

Oral tolerance—the active suppression of inflammatory responses to food and other orally ingested antigens—is critically dependent on the generation of iTreg cells (Curotto de Lafaille et al., 2008; Weiner et al., 2011). Recent evidence indicates that in addition to supporting iTreg cell differentiation, RA-mediated trafficking of T cells is required for a sustained expansion of iTreg cell numbers in the gut (Hadis et al., 2011). This expansion in numbers is propagated through IL-10-mediated interactions with resident $CD103^-$ antigen-presenting cells (APCs). In previous studies, similar interactions were shown to contribute to both the induction and maintenance of Treg cells (Denning et al., 2007; Murai et al., 2009). Altogether, these findings indicate that RA signaling is a keystone in the development of oral tolerance.

In the absence of RA, other mechanisms may compensate for the loss of these tolerogenic processes. For instance, elevated frequencies of $Foxp3^-IL\text{-}10^+$ Tr1 cells have been reported in vitamin A-deficient mice and could be generated in vitro in the presence of RAR antagonists and microbial-induced DC production of IL-6 and IL-21 (Maynard et al., 2009). Despite the importance of RA for generation of iTreg cells, neither the frequency nor absolute numbers of Treg cells are reduced during vitamin A deficiency (Hall et al., 2011). Thymic Treg cell differentiation is also intact in *Rara*$^{-/-}$ animals (Hill et al., 2008). Although these findings suggest that there are distinct requirements for inducible versus thymic-derived Treg cells, it still remains unclear whether intrathymic Treg cell differentiation and regulatory function is impacted in the absence of RA.

In vivo requirements notwithstanding, RA is insufficient to induce Foxp3 in the absence of TGF-β (Benson et al., 2007;

Mucida et al., 2007; Sun et al., 2007). These findings imply that responsiveness to TGF-β is a prerequisite for RA to access Foxp3 differentiation programs. In this regard, blockade of TGF-βRI kinase activity, which inhibits TGF-β-induced Smad2 and Smad3 phosphorylation (Sorrentino et al., 2008), diminished TGF-β-mediated iTreg cell generation and abrogated the additive effect of RA (Xu et al., 2010). Several preceding studies noted that RA enhances the total expression of Smad3 in activated $CD4^+$ T cells; however, TGF-β is required to trigger Smad3 activation (i.e., phosphorylation) (Nolting et al., 2009; Xiao et al., 2008). Furthermore, TGF-β was observed to prevent the intracellular degradation of RA (Takeuchi et al., 2011), prompting the possibility that RA and TGF-β cooperatively promote enhanced Smad3 activity. Based on the importance of Smad3 activation in CNS1-mediated Foxp3 expression (Tone et al., 2008), the role of RA in the regulation of CNS-1 was examined, which led to the identification of a potential RAR binding site (Xu et al., 2010). Accordingly, RA was shown to dramatically enhance TGF-β-induced chromatin accessibility and Smad3 binding in CNS1. The strong association between RA and the regulation of CNS1 not only provides a tentative molecular mechanism for the enhancement of iTreg cell generation, but further suggests that RA synthesis pathways may be manipulated during infection and inflammation to shift the balance between Treg and effector T cells and in turn influence immunopathology.

Influence of Retinoic Acid on Effector $CD4^+$ T Cell Differentiation and Function

The role of RA in TGF-β-dependent responses has been further evaluated in Th17 cell differentiation. Th17 cells, which produce IL-17A (IL-17), IL-17F, IL-21, and IL-22, promote control of bacteria and fungal infections at mucosal sites (Littman and Rudensky, 2010). They are induced in response to TGF-β, combinations of the Stat3 signaling cytokines—IL-6, IL-21, and IL-23—and IL-1 (Korn et al., 2009). Although linked to the pathogenesis of autoimmune responses (Iwakura et al., 2011), Th17 cells provide an important layer of protection at mucosal interfaces and are typically detected during steady state in these regions (Ivanov et al., 2006, 2008). Several recent studies revealed that Th17 cells are virtually ablated in the GALT of mice reared on a vitamin A-deficient diet during steady state (Cha et al., 2010; Wang et al., 2010). In concert with the loss of Th17 cells, the ability of APCs to produce IL-6, which promotes Th17 cell polarization, is reduced in vitamin A-deficient mice (Hall et al., 2011). Despite the likelihood that additional factors are complicit in the diminished number of Th17 cells during vitamin A deficiency, these data suggest that RA is critical for the in vivo differentiation and/or survival of Th17 cells. In support of this, in conjunction with microbial stimulation, a low dose of RA (~1 nM) in splenic DC cocultures was found to potentiate Th17 cell generation (Uematsu et al., 2008). These findings suggest that a synergy between RA- and microbial-driven signals promote Th17 cell differentiation in vivo (Figure 2). Importantly, the ability of RA to support Th17 cell differentiation probably results from combined actions on DCs and T cells, because addition of RA to Th17 cell-polarizing conditions in APC-less cultures did not enhance Th17 cell differentiation (Wang et al., 2010).

In parallel with the initial discovery that RA enhanced iTreg cell differentiation, RA was observed to suppress Th17 cell generation (Elias et al., 2008; Kang et al., 2007; Mucida et al., 2007). RA was shown to inhibit IL-6R and IL-23R upregulation induced by TGF-β and IL-6, respectively (Xiao et al., 2008; Zhou et al., 2007). Accordingly, RA supplementation in vitamin A-replete settings could suppress Th17 cell responses and IL-23-driven immunopathology during *Listeria monocytogenes* infection and autoimmune experimental encephalitis (Mucida et al., 2007; Xiao et al., 2008). Thus, RA is required for the in vivo promotion of Th17 cell differentiation, and it also may directly contribute to Th17 cell regulation.

In addition to Th17 cells, RA can exert direct regulatory effects on other effector T cell populations (Hill et al., 2008). For instance, RA was shown to inhibit IFN-γ production from $CD8^+$ T cells and Th1 cells (Cantorna et al., 1995, 1996; Stephensen et al., 2002). A previous study argued that such regulation might have contributed to impaired Th2 cell responses to the parasitic infection *Trichinella spiralis* during vitamin A deficiency (Carman et al., 1992). RA was also shown to relieve the inhibitory influence of effector T cells in the generation iTreg cells by directly suppressing their production of IL-4 and IL-21, which was previously demonstrated to potently antagonize iTreg cell generation (Hill et al., 2008; Korn et al., 2007; Nurieva et al., 2007; Wei et al., 2007). Further investigation into the temporal regulation of retinoid receptor expression during helper T cell polarization should clarify the molecular mechanisms by which RA controls cytokine production in various effector T cell subsets. Although the direct effects of RA on effector T cells should continue to be explored in more pathological contexts, these findings support a model in which RA-rich microenvironments can limit cytokine production by terminally differentiated effector T cells and, thus, T cell-mediated tissue pathology.

Retinoic Acid-Retinoic Acid Receptor Signaling in $CD4^+$ T Cell Activation

The recent descriptions of RA in immunoregulation have in some ways overshadowed the importance of this metabolite in generating functional immunity. Indeed, several studies have noted potent adjuvant effects of RA during infection (Dawson et al., 2009; Yamada et al., 2007). One aspect in the amplification of these responses may involve the vital role of RA in T cell activation. For instance, in serum-free cultures, RA dramatically enhances TCR-mediated $CD4^+$ T cell proliferation in an IL-2-dependent manner (Engedal et al., 2006). The NFAT family of transcription factors regulates an array of functions in multiple cell types; in T cells these include production of IL-2 and the full acquisition of effector properties (Macian, 2005; Peng et al., 2001). mRNA expression of multiple NFAT isoforms, which is reduced in B1-B cells during vitamin A deficiency, rebounds to normal amounts after treatment with RA. NFAT proteins are also markedly reduced in T cells during vitamin A deficiency and it is possible that RA regulates NFAT transcription and/or stability in these cells, as well (Maruya et al., 2011). Interestingly, NFATc2 was recently shown to cooperate with RARα-RXR heterodimers to induce CCR9, whereas NFATc1 inhibited CCR9 induction (Ohoka et al., 2011). These findings suggest that RA establishes an intimate link between T cell activation, effector function, and homing properties. It will be important to determine whether

T cell expression and localization of NFAT proteins are differentially regulated in distinct anatomical sites and how their modulation converges with RA availability in these sites to influence expression of homing markers.

Naive CD4$^+$ T cells basally express the genes encoding RARα (*Rara*) and RARγ (*Rarg*) (Hall et al., 2011; Ohoka et al., 2011). Although previous data suggest that RARγ is dispensable for CD4$^+$ T cell activation (Dzhagalov et al., 2007), recent data reveal that RARα is important for this process (Hall et al., 2011). Specifically, Ca^{2+} mobilization in response to TCR-CD3 engagement is impaired in CD4$^+$ T cells from *Rara*$^{-/-}$ mice or normal cells exposed to a pan-RAR antagonist (Hall et al., 2011). Ca^{2+} mobilization results in NFAT translocation into the nucleus, so these data suggest that both early and sustained T cell activation are impaired when RA signaling is deficient (Feske, 2007). In this regard, activation of the mTOR kinase pathways, which play important roles in directing helper T cell responses, are also reduced in *Rara*$^{-/-}$ T cells (Delgoffe et al., 2009, 2011; Zhang et al., 2011). The reductions in these signaling pathways may, in part, contribute to the immunodeficient state observed in animals devoid of RA-RARα signaling, which will be discussed below.

Precisely how RA-RARα signals mediate early T cell activation events is unclear. RARα is potent transcriptional regulator of gene networks and known to constitutively bind to DNA. Such binding may exert a tonic influence on the DNA binding capacity of other proteins involved in the regulation of T cell activation. For instance, RA-RARα regulates DNA binding of the AP-1 transcription factor, c-Jun, which is involved in responses to stress- and TCR-mediated signals (Schüle et al., 1991). In addition to its well-appreciated nuclear activity, extranuclear functions of RARα have also been described (Rochette-Egly and Germain, 2009). In this regard, RARα was observed to regulate expression of the phosphatase MKP-1, which is an important mediator of effector T cell differentiation (Lee et al., 1999; Zhang et al., 2009). Furthermore, RARα may associate with a membrane and/or cytosolic signaling scaffold important in T cell activation (Rochette-Egly and Germain, 2009). Recent findings indicate that RXR expression is barely detectable in naive T cells, suggesting that the effects of RARα on T cell activation may proceed independently of RXR heterodimerization. Conversely, RAR-RXR heterodimerization is required for the acquisition of mucosal homing markers (Ohoka et al., 2011). Although further research into the mechanism by which RA-RARα regulates T cell signaling is needed, one can construct a model in which RA regulates adaptive T cell responses through dichotomous roles: on the one hand promoting the initiation of effector T cell differentiation and, on the other hand, restraining inflammatory T cell responses in tissues.

Retinoic Acid in Infection and Immunity

Recent human data highlight the correlation between vitamin A status and T cell function (Ahmad et al., 2009). Although vitamin A has gained widespread acceptance as a clinical health intervention, skepticism of its efficacy has lingered because of inconsistency in outcomes of various vitamin A supplementation programs and a subpar understanding of the mechanisms it employs to combat infectious disease (Sommer, 2008; Wintergerst et al., 2007). The latter, in and of itself, poses a significant challenge to developing efficacious supplementation programs. Studies utilizing various animal models of vitamin A or retinoid receptor deficiency have begun to close this knowledge gap, revealing an integral role for RA in vitamin A-dependent immunity. Impaired and/or dysregulated T cell responses have been observed in various models of infection and vaccination strategies during vitamin A and/or retinoid receptor deficiency (Carman et al., 1992; Dzhagalov et al., 2007; Hall et al., 2011; Stephensen et al., 2004). During infection with *Toxoplasma gondii*, an intracellular replicating pathogen controlled by IFN-γ (Suzuki et al., 1988), the acute Th1 cell response and parasite clearance are substantially impaired in vitamin A-deficient mice (Hall et al., 2011). Similarly, vaccination with an *E. coli*-derived heat-labile enterotoxin mucosal adjuvant, LT(R129G), which simultaneously elicits Th1 and Th17 cells (Hall et al., 2008), yields diminished Th1 and Th17 cell responses in these animals. Short-term treatment with RA immediately prior to and during challenge completely rescued CD4$^+$ T cell responses both to acute *T. gondii* infection and vaccination in vitamin A-deficient mice (Hall et al., 2011). These findings demonstrated an essential role for RA in the development of Th1 and Th17 cell responses.

RA signaling appears to control the fate of T cell immunity largely through RARα and RARγ. The CD4$^+$ T cell response to vaccination with LT(R129G) was strongly diminished in *Rara*$^{-/-}$ mice (Hall et al., 2011). CD4$^+$ T cell activation was reduced in the absence of RARα, but additional functional impairments may have also factored into this outcome. Preliminary evidence suggests that RARα regulates not just the proper maturation of DCs in the GALT (Jaensson-Gyllenback et al., 2011) but also their ability to drive inflammatory responses in certain pathologic settings, which will be discussed below (Depaolo et al., 2011). Future studies employing lineage-targeting strategies will be integral for discerning the contributions of RARα in individual cell types to immunological outcomes. Although the role of RARα in CD8$^+$ T cell function remains to be explored, RARγ, which is dispensable for CD4$^+$ T cell and humoral responses, was shown to be required for full effector differentiation of CD8$^+$ T cells in response to infection with *Listeria monocytogenes* (Dzhagalov et al., 2007).

A direct role for RARγ in CD8$^+$ T cell activation has not yet been addressed; however, this receptor controls optimal macrophage production of inflammatory cytokines in response to microbial stimuli (Dzhagalov et al., 2007). A role for RA signaling in macrophages is consistent with another study, which showed that RA enhanced macrophage activation in response to in vitro infection with *Mycobacterium tuberculosis* (Yamada et al., 2007). Altogether, these data illustrate the ability of RA to regulate a network of innate and adaptive immune cell functions, which through nonredundant receptor signaling pathways power functional immune responses. More research is required to elucidate how RARβ signals fit into the control of vitamin A-dependent immunity. In contrast to the other RARs, RARβ expression appears to largely depend on RA itself and, therefore, may play a prominent role in the regulation of mucosal immune responses (Molenaar et al., 2011; Suzuki et al., 2010). RARβ was also found to regulate the recruitment of lymphoid tissue-inducer cells during embryogenesis via stromal cell induction of CXCL13 (van de Pavert et al., 2009). Thus, it will be worthwhile to examine the role of RA in the formation of tertiary lymphoid structures during chronic inflammation.

Retinoic Acid in Inflammation

Chronic inflammatory syndromes arise as a consequence of genetic polymorphisms in concert with accumulating environmental exposure to toxins, pathogens, and diet (Yazdanbakhsh et al., 2002). Western diets are becoming increasingly associated with a higher prevalence of inflammatory disorders, including allergies and inflammatory bowel diseases (Garrett et al., 2010; Wang and Sampson, 2011). Several studies have suggested that in vivo iTreg cell generation can prevent and/or mitigate these manifestations (Curotto de Lafaille and Lafaille, 2009; Lacy-Hulbert et al., 2007; Travis et al., 2007). In this regard, treatments that promote RA metabolism may constitute an effective strategy to restore the regulatory balance during chronic inflammation. For example, treatment with the TLR2 agonist zymosan was shown to induce RALDH production by nonmucosal DCs and ameliorated pathology in a model of autoimmunity (Figure 2; Manicassamy et al., 2009). Nevertheless, in previous studies, mice fed a diet high in vitamin A exhibited more vigorous responses against grafts and tumors, suggesting that elevated retinoid levels were potentially detrimental in certain inflammatory contexts (Figure 3; Malkovský et al., 1983a, 1983b). In this regard, a recent study demonstrated that RA-driven signals in an inflammatory environment fostered reactivity to dietary glutens in a mouse model of celiac disease (Figure 3; Depaolo et al., 2011; Jabri and Sollid, 2009). In particular, RA was demonstrated to synergize with an IL-15-rich milieu and potentiate production of IL-12 and IL-23 by mucosal DCs, diminishing their capacity to promote iTreg cells and leading to exacerbated responses to Gliadin (Depaolo et al., 2011). These data reflect several reports describing a possible association between pharmacological retinoid treatment and spontaneous development of inflammatory bowel disease and point to elevated activity of vitamin A metabolic pathways as potential instigators of chronic inflammation (Figure 3; Crockett et al., 2010; Reddy et al., 2006).

Concluding Remarks

Recent insights into the role of RA in the promotion and regulation of multiple immunological pathways draw new attention to the sweeping influence of vitamin A in immunity. Though long on the radar of health experts trying to combat immunodeficiencies in developing nations, vitamin A is often overlooked in developed regions where access to this nutrient is plentiful. New data reveal that multiple factors influence the generation of RA, including vitamin A itself, fatty acids, TLR ligands, and GM-CSF, which promote RA synthesis and prostaglandin E2, which inhibits RA synthesis (Manicassamy et al., 2009; Stock et al., 2011; Szatmari et al., 2006; Yokota et al., 2009). Greater understanding of how these factors play into RA synthesis during homeostasis and inflammation will be essential for assessing their efficacy as therapeutic modalities in the treatment of syndromes in which retinoid imbalances may be involved. In summary, the potential of RA to transform from an essential to pathological mediator of immune responses raises many questions on how vitamin A metabolism affects disease. Furthermore, because retinoids are prevalent in clinical settings (de Lera et al., 2007), an additional consideration in their use may be the patient's risk factors for inflammatory disease.

ACKNOWLEDGMENTS

This work was supported by the Division of Intramural Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, and the Office of Dietary Supplements. We thank E. Wohlfert and T. Hand for helpful discussions and critical reading of the manuscript.

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