Guo Y, Brown C, Ortiz C, Noelle RJ. Leukocyte Homing, Fate, and Function Are Controlled by Retinoic Acid. Physiol Rev 95: 125–148, 2015; doi:10.1152/physrev.00032.2013.—Although vitamin A was recognized as an “anti-infective vitamin” over 90 years ago, the mechanism of how vitamin A regulates immunity is only beginning to be understood. Early studies which focused on the immune responses in vitamin A-deficient (VAD) animals clearly demonstrated compromised immunity and consequently increased susceptibility to infectious disease. The active form of vitamin A, retinoic acid (RA), has been shown to have a profound impact on the homing and differentiation of leukocytes. Both pharmacological and genetic approaches have been applied to the understanding of how RA regulates the development and differentiation of various immune cell subsets, and how RA influences the development of immunity versus tolerance. These studies clearly show that RA profoundly impacts on cell- and humoral-mediated immunity. In this review, the early findings on the complex relationship between VAD and immunity are discussed as well as vitamin A metabolism and signaling within hematopoietic cells. Particular attention is focused on how RA impacts on T-cell lineage commitment and plasticity in various diseases.

I. INTRODUCTION

Nearly a century ago, vitamins were recognized as essential nutrients for human health. Among various vitamins, vitamin A was found to be essential for embryogenesis (215) and host defense against various microbial infections (209). Developmental biologists have extensively studied the active form of vitamin A, retinoic acid (RA), and have mapped how gradients of RA control the most exquisite processes during embryogenesis. Given the known correlation between vitamin A deficiency (VAD) and increased susceptibility to infectious diseases (ID) among preschool children in developing countries (20, 49), immunologists have focused on the understanding of how vitamin A, through RA, controls immunity. Initial clues into the molecular mechanisms of the “anti-infective” impact of vitamin A were provided in 2004, when Iwata et al. (84) demonstrated that RA imprints the homing of leukocytes to the gut through the upregulation of α4β7and chemokine (C-C motif) receptor 9 (CCR9). Our own studies (11, 140) and those of others (31, 139, 181) demonstrated that RA could enhance T-cell commitment and Treg differentiation. These seminal findings focused the immunological community on understanding the role of RA in the regulation of gut immunity and tolerance. The use of small molecule inhibitors or agonists of RA signaling as well as genetic-engineered mouse models has expanded our knowledge of how RA controls immune responses besides its known function in controlling gut homing. Numerous studies have elucidated the essential role of RA in modulating immune cell “lineage commitment” in various disease models in a context-dependent manner. RA is now appreciated as a pivotal mediator in promoting tolerance or immunity depending on what type of cell is acted upon. In this review, we focus on the known mechanism of how RA is synthesized by various antigen-presenting cells and how this is translated into signals that govern leukocyte lineage commitment.

II. VITAMIN A DEFICIENCY AND IMMUNITY

Vitamins by definition are organic compounds that cannot be synthesized by mammals and thus have to be obtained from food sources (52). The recognition that vitamin A plays an important role in immunity dates back to the 1920s when Drs. Green and Mellaby observed their colony of dogs on a VAD diet succumbed to an infection that ravaged through the animal facility (127). Since that time, the idea that vitamin A was an “anti-infective vitamin” instead of “growth-producing vitamin” (62) grew in recog-
nition. Following this study, a small clinical trial was initiated in women with septicemia, in which only 2 out of 24 recovered without vitamin A treatment, and 5 out of 5 recovered with vitamin A treatment (128). An even larger clinical trial of puerperal sepsis reinforced the positive correlation between vitamin A treatment and clinical outcome by showing a statistical improvement in a wide range of ID of those on vitamin A-rich diets (63). At least 30 uncontrolled clinical trials led by Drs. Mellanby and Green demonstrated that vitamin A supplementation, in the form of cod-liver oil, reduced the morbidity and mortality caused by respiratory inflammation, measles, puerperal sepsis, and other ID (as reviewed in Ref. 165). A litany of studies have focused on the analysis of VAD and immunity, with the increasing knowledge of immunity and better-controlled experiments than in the early days. VAD animals are characteristic of a wide range of immunological changes, including pathological disintegration in mucosal area (2), decreased antibody (Ab) response, changes in lymphocyte populations, and T- and B-cell dysfunction, which can be restored by treatment with retinol, a derivative of vitamin A (154).

The importance of vitamin A in human disease is highlighted further by the increased burden of ID in VAD children and adults. Presently, VAD is still a common health problem in some developing countries and a leading cause of childhood morbidity and mortality. An estimated 250 million children under the age of 5 yr have VAD (190), defined as serum retinol <0.7 μM (37), and a further 120 million are estimated to have significant VAD in the absence of clinical signs such as xerophthalmia. Malnourished children have increased susceptibility to diarrhea due to ID (49) and an increased risk of acute respiratory infections (20) and measles (54, 122). Some clinical data also indicate a correlation between VAD and the disease progression (166), mortality (166), and maternal-infant disease transfer (64, 167). Infection also leads to enhanced vitamin A depletion (21). Since the first randomized and controlled trial in the 1980s, a number of clinical trials in children from endemic areas have demonstrated a dramatic reduction in severity of diarrheal disease and measles morbidity with vitamin A supplementation (78, 81, 172). In addition, supplementation has led to a lower incidence of respiratory tract infections in some studies (9). Of note, high-dose vitamin A supplementation even reduced mortality and morbidity in children without clinical evidence of VAD (32, 78). Based on these clinical outcomes, the World Health Organization recommends that preschool children in developing countries receive high-dose vitamin A supplementation every 4–6 mo, often administered at the time of vaccination for clinical convenience.

In addition to the role of vitamin A sufficiency to efficiently fight against infectious diseases, evidence also suggests that vitamin A supplementation can improve vaccine efficacy (92). The study by Sudfeld et al. (180) showed that at least two doses of 200,000 IU (60 mg retinol equivalent) for children ≥1 yr of age and 100,000 IU (30 mg retinol equivalent) for infants reduced measles mortality by 62%. In the study, vitamin A is administrated orally in an oil-based preparation of retinyl palmitate or retinyl acetate. However, the relationship of vitamin A supplementation and mechanistic impact on immunity is not always apparent. Chicks fed on a high-vitamin A diet had a significantly lower primary IgG and IgM Ab response to keyhole limpet hemocyanin (KLH), and also a lower secondary IgG response. In addition, administering retinyl palmitate did not change the Ab response in low-, regular-, or high-vitamin A diet-fed chicks (108). VAD diminished and high-level vitamin A diet enhanced the development of experimental asthma in mice (162). Furthermore, vitamin A supplementation proved unpredictable in some clinical trials. In a clinical trial including 471 at-birth children, vitamin A supplementation (50,000 IU retinyl palmitate in vegetable oil) was associated with lower tumor necrosis factor (TNF-α) and interleukin (IL)-10 production in girls without and boys with diphtheria-tetanus-pertussis vaccination, indicating a complicated interaction between vitamin A sufficiency, gender, and vaccination (88).

As discussed above, the early epidemiological data and effectiveness of vitamin A supplementation to boost immunity shed light on a key role of vitamin A for development of host defense and opened the door to a wealth of research into the mechanisms through which vitamin A regulates immune responses. On the other hand, the insignificant improvement by vitamin A or RA supplementation in some diseases makes it clear that vitamin A effects are not always predictable.

The importance of vitamin A to the ontogenic development of immune system was recognized in 1930s, when VAD children were found to have atrophic thymi, spleens, and lymphoid tissues (15). Numerous studies in mice have demonstrated that vitamin A causes this impact on immune system through various immune cell components, such as NK cells (39), T cells (110), and B cells (16). Recently, the importance of vitamin A in development of immune system was further understood at the molecular level by van de Pavert et al. (197). In this study, the authors established that the secondary lymphoid organ formation during embryogenesis depends on hematopoietic cell-autonomous RA signaling in utero, by control of a subset of type 3 innate lymphoid cells (ILC3) differentiation. Therefore, maternal vitamin A intake controls the efficiency of immune response in the adult offspring. It further illustrated the essential role of vitamin A in the development of efficient immune system.

III. VITAMIN A METABOLISM AND RA SYNTHESIS

Both genetic and pharmacological approaches have been applied to elucidate the pathway of metabolizing vitamin A
to RA. As retinoids (all vitamin A derivatives) cannot be synthesized de novo, the only source is diet derived. Earlier studies have illustrated the major proteins and enzymes involved in retinol storage are in the liver and the uptake of vitamin A is in the intestine (reviewed in Ref. 33). In mammals, retinol is the major circulating retinoid. Retinol is typically bound to retinol-binding protein 4 (RBP4) and transthyretin (TTR), and taken up by target tissues (10). Previous studies demonstrated that retinol-RBP-TTR complex (holo-RBP) uptake was facilitated by STRA6 (stimulated by retinoic acid gene 6) in various tissues (93). However, recent studies in stra6-null mice illustrated that STRA6 facilitates retinol uptake in the eye but couples holo-RBP to cell signaling in other tissues (12). Two sequential reactions are then carried out for the conversion of retinol to RA. First, retinol is converted by cytosolic alcohol dehydrogenase (ADHs, including ADH1, ADH5, and ADH7) (133) and microsomal retinol dehydrogenase (RDH) (158) to retinaldehyde. Adh1−/− mice are more sensitive to vitamin A embryotoxicity (134), but reveal no obvious phenotype when maintained on vitamin A-sufficient diet (38). In contrast, Rdh10 loss-of-function is lethal between embryonic day 10.5 (E10.5) and E14.5 and exhibits abnormalities characteristic of RA deficiency (158). These studies indicated that ADHs may control the removal of excessive retinol, whereas RDHs oxidize retinol to retinaldehyde (also called retinol). Second, retinaldehyde is irreversibly converted to all-trans-retinoic acid (ATRA) by three retinaldehyde dehydrogenases, RALDH1 (65), RALDH2 (43, 144) and RALDH3 (182) (encoded by Raldh1–3, respectively), in mice. In this article, RA refers to ATRA unless specified otherwise. Of note, RALDH2 can also catalyze the dehydrogenation of other aldehydes, such as octanal and decanal, even if less efficiently compared with retinaldehyde (208).

Even though vitamin A has long been known to be essential for a competent immune system, the underlying mechanisms whereby RA regulates immunity are still incompletely understood. Substantial efforts have focused on the metabolism and synthesis of RA by various APCs for the homeostatic regulation of immune migration and differentiation of various hematopoietic compartments.

A. RA Synthesis in the Gut

While ADHs are widely and constitutively expressed by multiple cell types, RALDH expression is dynamically regulated in a temporally and spatially restricted manner. Expression of RALDH is viewed as a key enzyme defining the ability of specific cell types or tissues to produce RA (43, 65, 144, 182). The discovery that RA induces the migration of leukocytes to the gut (84, 137, 223) has focused much of the attention of RA synthesis to mucosal sites. As such, researchers have begun to elucidate the factors and cells that govern the expression of RALDHs and RA synthesis in immune relevant cells in the gut and in the periphery. In situ hybridization and immunocytochemical analysis have confirmed RALDH1 expression in epithelial cells of mucosa, including small and large intestines (55). In the small intestine, epithelial cells express Raldh1 and produce RA constitutively by taking retinol from food sources (14, 55, 104, 192). Further studies found that monocyte-derived dendritic cells (MoDC) differentiated with RA or the supernatant of human intestinal epithelial cells acquire the ability to produce RA and induce regulatory T cell (Treg) differentiation in the gut (80). It has also been shown that direct contact between dendritic cells (DC) and intraepithelial lymphocytes (IELs) endowed the former to express Raldh2 and produce RA (47, 79). Many studies have suggested that CD103+ DCs in the small intestine are responsible for the high level of RA in mesenteric lymph node (MLN) (RALDH2), Peyer’s Patch (PP) (RALDH1), and lamina propria (LP) (RALDH2) of the small intestine (84). Recent studies have shown that not only are there more CD103+ DCs in murine small intestine lamina propria (SI-LP) and draining MLN than in other lymphoid tissues, but CD103+ DCs in SI-LP and MLN showed higher Raldh2 expression and activity (132).

Our recent study demonstrated that both the expression and enzymatic activity of RALDH1 and RALDH2 in donor DCs and macrophages of MLN and LP lymphocytes were elevated in graft-versus-host-disease (GVHD) mice (5). The increase of RA synthesis in active GVHD mice is responsible for donor T cells differentiation into pathogenic Th1/Th17 cells and their migration to the intestine for disease progression.

Another study showed that RALDH2 is also expressed in MLN stromal cells, consistent with their ability to imprint proliferating T cells with α4β7 and CCR9. This study suggested that RA-producing stromal cells and DCs in MLN warrant the gut-tropism of activated T cells in the mucosa area (131). In one elegant study of implanting MLN and peripheral LN fragments into the mesenteric region of host rat and mouse, it was established that stromal cells in MLN but not in peripheral LN provided a unique microenvironment allowing for the acquisition of a gut-tropic phenotype of T and B cells during antigen-induced activation (3, 72). In addition to gut mucosa, CD103+ DCs was also found in cutaneous and lung-draining LN, suggesting an important role in controlling T cell homing in those sites (67). A recent study demonstrated that lung CD103+ and CD24+CD11b+DCs express RALDH activity and can induce gut-homing receptors α4β7 and CCR9 (156). Even though no specific RALDH was identified, T cells primed by lung DCs via intranasal immunization efficiently induced migration to the gut and control enteric challenge of highly pathogenic Salmonella. While these correlations of expression and function within the CD103+ DC subset are compelling, it remains to be determined whether RA synthesis
by these cells are causally imperative for the function of RA in the gut.

**B. RA Synthesis in the Periphery**

Various peripheral DC subsets acquired RA-producing potential in both allogenic skin transplantation (151) and melanoma model (69). As such, our studies have established that RA is a critical immune mediator outside the gut, in sustaining and controlling leukocyte differentiation in the periphery. RA production by DCs in peripheral tissues and lymphoid organs is consistent with the corresponding role of RA in regulating adaptive immunity in periphery. Furthermore, CD8α+CD8β– and CD8α+CD8β+ but not CD8α–CD8β– plasmacytoid DCs (pDC) were able to produce RA, and induce the conversion of naive CD4+ T cells to Tregs in combination with transforming growth factor (TGF)-β in vitro and in vivo during lung inflammation-mediated airway hypersensitivity (109).

As mentioned in section II, the intake of retinoids by the mother determines the development of secondary lymphoid organs during embryogenesis through the regulation of lymphoid tissue inducer (LTi) cells and lymphoid organs during embryogenesis. RA production by BMDCs in peripheral tissues and lymphoid organs is consistent with the corresponding role of RA in regulating adaptive immunity in periphery. Furthermore, CD8α+CD8β– and CD8α+CD8β+ but not CD8α–CD8β– plasmacytoid DCs (pDC) were able to produce RA, and induce the conversion of naive CD4+ T cells to Tregs in combination with transforming growth factor (TGF)-β in vitro and in vivo during lung inflammation-mediated airway hypersensitivity (109).

No studies have yet reported RALDHs expression in T cells or RA synthesis by activated T cells in vitro or in vivo. The exclusive synthesis of RA by various APC subsets and impact on T cell differentiation raised the question of whether RA, as a lipophilic molecule, permissively diffuses across cell membranes of DCs and T cells during antigen presentation and thus determines the phenotype of corresponding T cells. Most likely, RA-producing DCs (or other APCs), in response to different stimuli, can generate an RA-rich microenvironment, which is also rich in stimuli-specific cytokines/chemokines, and determine the T-cell lineage commitment. Lineage-specific deletion of Raldh(s) will facilitate the understanding of the role of each RALDH in DCs or macrophages in the regard of determining T-cell differentiation and migration. Such studies may lead to the therapeutic inhibition of RALDH activity in specific cell subsets for control of disease progression. For instance, Crohn’s disease and ulcerative colitis are driven by enhanced recruitment of effector macrophages, neutrophils, and effector T cells (159). Thus RALDH inhibition may block effector T-cell recruitment into the intestine and ameliorates the disease progression.

**C. Regulation of RA Synthesis in the Immune System**

Interest in understanding what inflammatory mediators control RA synthesis by the immune system has been paramount. The prominent targets for regulation involve the transcriptional regulation of each of the RALDHs as well as the expression of the RA catabolic pathways, involving mainly cytochrome P-450, CYP26 enzymes (CYP26A1-C1), and possibly retinal reductases (89). The following section focuses on our current understanding of the molecules involved in the expression and activity of RALDH1–3 and CYP26 in different immune cells.

1. **Granulocyte-macrophage colony stimulating factor and IL-4**

Granulocyte-macrophage colony stimulating factor (GM-CSF) and IL-4 have been shown to synergistically induce Raldh2 expression in both bone marrow-derived DCs (BMDC) and splenic DCs, thus enabling them to produce RA (6). Even though 1 μM ATRA minimally induced Raldh2 expression in BMDC or splenic DC, it moderately enhanced GM-CSF and/or IL-4-induced Raldh2 expression on BMDCs. The authors proposed a positive feedback loop of RA production in the intestine. That is, GM-CSF produced by CD11c–F4/80+ macrophages in the intestinal tissues induces Raldh2 expression and RA production by DCs. Raldh2+ DCs then provide RA to macrophages and induce further GM-CSF production. A recent study further elucidated that RA controls the generation of gut-tropic migratory DC precursors in the bone marrow (223). Therefore, high RA in the intestinal microenvironment is maintained through the generation of gut-homing DC precursors in bone marrow and further enhancement of RA production by GM-CSF in the intestine. Consistent with the finding that GM-CSF and IL-4 induced Raldh2 expression on BMDCs, IL-4 was shown to induce Raldh2 expression in MLN-DC (50).

2. **TLR signaling**

The RA-producing ability of CD103+ DCs in the gut mucosa is dependent on MyD88 signaling, in particular TLR2-mediated signaling (48, 206). TLR2 signaling also stimulated RALDH2 and IL-10 expression on splenic DCs, thus inducing Treg development but suppressing Th1/Th17 cell differentiation (119). Another study showed that TLR5 ligand, flagellin, specifically stimulated CD103+ LP-DCs to produce RA (194). The MyD88 and TLR signaling-dependent RALDH2 expression indicate the potential ability of both commensals and pathogens to induce RA synthesis by gut-associated DCs. Consistent with this, infection with
3. 4-1BB and prostaglandin E2

The pathogen-specific induction of RA synthesis (17) was observed following infection with lymphocytic choriomeningitis virus (LCMV), highlighting RALDH2 induction was observed following infection with the helminth *Schistosoma mansoni*, which provokes Th2 response, led to massive RALDH2 upregulation within alternatively activated macrophages (17). Interestingly, human mast cell-derived IL-3 can induce RALDH2 expression in basophils, thus inducing Th2 response in allergic diseases (174). However, no RALDHs induction was observed following infection with lymphocytic choriomeningitis virus (LCMV), highlighting the pathogen-specific induction of RA synthesis (17).

4-1BB+CD103+ DCs in MLN displayed higher RALDH activity than 4-1BB-CD103+ DCs. 4-1BB stimulation allowed TLR2-pretreated splenic DCs to be more potent Treg inducers. The essential role of 4-1BB for steady-state RALDH2 expression on MLN-DCs was confirmed further by weak RALDH2 expression and Treg-promoting activity in MLN-DCs of 4-1BB-deficient mice (105). As it is essential for the gut-associated lymphoid organs to fight against foreign pathogens while maintaining tolerance towards food antigens, there needs to be negative regulation of RA synthesis by MLN DCs, thus eliciting anti-inflammatory response. Prostaglandin E2 was indeed observed following infection with *Shistosoma mansoni*, highlighting the pathogen-specific induction of RA synthesis (17).

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4. Wnt signaling

β-Catenin-stimulated Wnt signaling was upregulated in LPDCs compared with splenic DCs. Further studies illustrated that β-catenin is required for steady-state *Raldh1* and *Raldh2* expression at mRNA level in intestinal DCs and macrophages. Therefore, specific deletion of β-catenin in DCs led to lower Treg but higher Th1/Th17 differentiation, and the host is more prone to inflammatory bowel disease (120).

We summarize the different molecules for stimulating/maintaining RA synthesis in various cells in the gastrointestinal (GI) tract in **Figure 1**.

5. RA catabolism

To maintain RA tissue concentrations and gradients, regulation of catabolism is coupled with synthesis. A family of Cyp26A1-C1 can transform ATRA into more polar metabolite 4-hydroxy-RA or 4-oxo-RA, which is subject to further metabolism and elimination from cells (117, 183, 212). A recent study suggested that dehydrogenase/reductase-3 may also be involved in the catabolism of RA by reducing the level of all-trans-retinal (89). Thus the expression and activity of RALDHs, CYP26, and dehydrogenase/reductase-3 in combination determine the intracellular and extra-cellular RA levels. Cyp26A1 and Cyp26B1 show specific activity for ATRA (211, 213), whereas Cyp26C1 shows activity for both ATRA and 9-cis-RA (186). During early embryogenesis, RA levels decrease in the regions where CYP26 are expressed, thus Cyp26a1−/− or Cyp26b1−/− mice die in utero or immediately after birth (1, 219). The key role of CYP26 in regulating RA concentration was also demonstrated in immune cells. For instance, peroxisome proliferator-activated receptor gamma (PPARγ) agonists induced human DCs to produce RA, which then leads to the upregulation of CD1d, an antigen-presenting glycoprotein. This effect was enhanced by a CYP26 inhibitor (R115866), illustrating the essential role of CYP26 in RA catabolism in the immune system (184).

The classical RA response element (RARE) in the Cyp26a1 promoter region and a G-rich element synergistically allow for RA-induced Cyp26a1 expression (111, 112). In contrast, Takeuchi et al. (188) found that expression of Cyp26b1 instead of Cyp26a1 or Cyp26c1 can be induced by RA in naive T cells upon activation, indicating an indirect regulation of Cyp26b1 by RA in T cells. This study also illustrated that RA-induced Cyp26b1 expression was suppressed by TGF-β, but enhanced by proinflammatory cytokines IL-12, IL-4, and TNF-α. These findings are consistent with the effect of RA in 1) suppressing IL-12-mediated Th1, 2) enhancing IL-4-mediated Th2 response (83), and 3) enhancing TGF-β-induced Treg differentiation (11, 139, 181). To better understand the role of CYP26 in regulating regional RA level and thus differentiation or homing phenotype of immune cells, clear expression patterns of Cyp26 isoforms across hematopoietic cell lineages need to be defined. Also, conditional overexpression of specific Cyp26 isoforms in specific immune cell lineages will provide a better understanding of whether CYP26 activity determines cellular RA level in vivo. Due to the side effects of RA isomers in therapeutic applications, CYP26 inhibitors were synthesized and evaluated in preclinical models (60). The small molecules can potentially target certain specific cells through drug-Aβ conjugates (193).

IV. RA SIGNALING PATHWAY

There are three different retinoic acid receptor (RAR) subtypes: RARα, RARβ, and RARγ (44, 222), each consisting
of at least two isoforms generated by alternative splicing at the NH₂ terminus (58, 91, 106). There are three major domains in RARs required for its function, including NH₂-terminal domain (NTD), central DNA-binding domain (DBD), and COOH-terminal ligand binding domain (LBD) (27). RARs form heterodimer with retinoid X receptor (RXR) to regulate target genes expression. RAR-RXR heterodimer binds to RARE composed of two direct repeats of a core hexameric motif, PuG (G/T) TCA separated by 5 bp (DR5), 2 bp (DR2), or 1 bp (DR1) to regulate target gene transcription (8). The subsequent ligand-induced conformational changes in LBD direct the dissociation of corepressors and association of coactivator complexes, thus inducing gene transcription (27). Specifically, in the absence of retinoid ligand (such as RA), RAR-RXR heterodimer recruit and maintain corepressors to the promoter region of target genes, prevent other transcriptional machinery such as RNA polymerase binding to the promoter, and thus repress the transcription of these genes. On the other hand, however, RA binding to the RAR-RXR heterodimer releases the corepressors from the promoter, and recruits the coactivators and the transcription machinery so that target genes can be transcribed. ATRA can bind to RAR, but 9-cis-RA can bind to both RAR and RXR (57, 101, 224).

In addition to functioning through RAR-RXR heterodimer, RA was also shown to be a ligand for PPARdelta. The inconsistency between the prosurvival effect of RA and the proapoptotic function through RAR led to the discovery of PPARdelta as another receptor for RA signaling in specific cell types (161). Another study also illustrated that PPARdelta activation by RA leads to expression of the insulin-signaling gene PDK1, thus stimulating lipolysis and reducing triglyceride content (13).

In the immune system, some direct evidence has shown that RA can regulate target genes expression at transcription level (188). The cross-talk between RARα and nuclear factor of activated T cells, cytoplasmic, calcineurin-dependent 1 (NFATc1)/NFATc2 proved essential for CCR9 induction in response to transient TCR stimulation (148). In addition to the direct impact of RA on various genes transcription, RA was found to control microRNAs in different T-cell subsets, which enables RA to function through posttranscriptional regulation of multiple genes (86, 187). Therefore, given the large number of potential RAREs in the genome, RA may (in)directly regulate target genes through various mechanisms.

Various genetic models have been used to elucidate the role of RA in controlling the immune response from whole body
to cellular and molecular level. DR5-luciferase mice have been used to visualize immune stimulation-induced RA signaling at both the whole body and cellular levels (5, 69, 151, 183). Various studies have used transcriptional profiling to determine the target of RA signaling in T cells (76), DCs, and other cell subsets. Conditional deletion of RARs in specific tissues or cell subsets has also been used to reveal the role of various RARs in controlling different aspects of immune response. In addition, overexpression of a dominant negative RARα (dnRARα) has been used to reveal the function of RA signaling in controlling T-cell differentiation (4, 5, 69, 151). This serves the purpose of recapitulating VAD in the immune system. The following section will focus on the known mechanism of RA signaling in control of T-cell differentiation and lineage commitment.

V. RA IN REGULATION OF T CELLS

A. RA Regulates T-Cell Homing Potential

The essential role of RA in gut immunity had led researchers to investigate the role of RA in the gut-tropism for T cells, an essential component in gut immunity. The interaction between integrin α4β7 and mucosal addressin cell adhesion molecule-1 (MAdCAM-1) is essential for T-cell homing to intestine endothelium (19), whereas CCR9 mediates T-cell migration to intestinal epithelial cells via interaction with chemokine (C-C motif) ligand 25 (CCL25) (100). T cells gain α4β7 and CCR9 expression during antigen stimulation by MLN-DC or PP-DC, or αCD3 and αCD28 stimulation in the presence of RA, while downregulating E- and P-selectin ligands simultaneously (84). The essential role of RA in regulating T-cell gut-tropism was further demonstrated by the fact that α4β7+ memory/activated T cells were significantly reduced in lymphoid organs and depleted of intestinal LP in VAD mice (84).

Multiple studies have confirmed the direct effect that RA imposes on α4β7 and CCR9 expression in both CD4+ and CD8+ T cells. Using in vitro culture system, Mora et al. (136) found that CD8+ T cells activated by DCs from peripheral LN gain skin-homing tropism by expressing E- and P-selectin ligands by default, while RA-producing DCs from the intestine allows for α4β7 and CCR9 expression (136). Other groups proved that RA induced α4β7 and CCR9 expression on CD4+ T cells while inducing simultaneous FoxP3 expression (11, 139, 181).

1. RA regulation of α4β7

Kang et al. (90) found that RA treatment enhanced transcription of both integrin α4 and CCR9 on T cells, whereas both integrin αE and the constitutive expression of β7 can be enhanced by TGF-β. Both integrin α4 and αE bind to β7. αEβ7 binds E-cadherin on epithelial cells (25). Even though there are seven potential RAREs in α4 promoter region, only one RARE was confirmed by the authors through binding to RARα in ChIP assay. Therefore, coordination between TGF-β and RA determines T cell migratory ability. Another study further demonstrated that increasing the relative amount of α4 to integrin β1 is essential for RA-induced α4β7 expression, as α4 predominantly binds to β1 in both naive and activated CD4+ T cells and only binds to β7 when highly expressed (41). α4β1 is involved in leukocyte migration to mucosal tissues, bone marrow, splenic follicles, and inflamed tissues (71, 199). The active participation of β1 in RA-regulating α4β7 expression on T cells proved to be physiologically important as β1+/− mice showed lower memory CD4+ T cells in bone marrow but elevated in PP.

2. RA regulation of CCR9

By measuring CCR9 mRNA level in activated CD4+ T cells in the presence of RA, and assessing the binding between NFAT and RAR-RXR complex in CCR9 promoter regions, Ohoka et al. (148) demonstrated that transient TCR-mediated signaling and CD28 costimulatory signals induced transcription factor NFATc1 and NFATc2 nuclear translocation. NFATc2 enhanced but NFATc1 inhibited RAR-RXR binding to RARE in CCR9 promoter region (148). Only transient T-cell activation allows for CCR9 upregulation as prolonged TCR signaling retains inhibitory NFATc1 in the nucleus. This finding confirms that the cross-talk between RAR-RXR and other TCR-signaling-induced transcription factors is essential for the delicate regulation of RA-responsive genes expression. Another elegant study by Agace group (183) showed that high antigen dose decreased RA-induced CCR9 expression on activated CD8+ T cells, but induced E-selectin ligand expression. High antigen dose signal seems to function independently of or downstream of RA signaling. Thus T-cell receptor (TCR) signaling strength seems to determine CCR9 expression level. It remains unknown how RA signaling and TCR activation pathway cooperatively determine the tissue-tropism of CD8+ T cells.

Of note, CCR9 is constitutively expressed in naive CD4+CD8+ and CD8+ thymocytes, and on a subset of CD8+ T cells exiting the thymus into lymphoid organs (24). It remains unknown whether the steady-state CCR9 expression in these cells is regulated by RA signaling. There have not been studies addressing the mechanism of how CCR9 expression is lost on naive CD8+ T cells activated in vitro in the absence of RA.

The essential role of RA in inducing α4β7 and CCR9 expression was not only demonstrated pharmacologically by administration of specific RARs agonists/antagonists (including RA), but also by genetic approaches to manipulate RA signaling mediated in whole mouse or specific cell types. In line with previously published data using RARα antagonist, both our lab (68, 69, 151) and Hall et al. (70) showed...
that intrinsic RA signaling is required for gut-imprinting of T cells. Downregulation of RA-induced α4β7 and CCR9 expression was observed in both dnRARα-expressing and RARα-deficient CD8+ T cells and illustrated the requirement of RARα-RXR for CCR9 upregulation (68). In line with our studies, previous research showed that RARγ deficiency did not lead to any loss of RA-induced α4β7 or CCR9 expression on CD4+ or CD8+ T cells when activated in vitro (82).

B. RA-Dependent Induction of FoxP3 Treg Cells to Mediate Tolerance

It has been proposed that constitutive RA presence in the gut is essential for tolerance to commensal microbes and dietary antigens by inducing Tregs to dampen the potential proinflammatory responses. To maintain immunity against foreign pathogens versus tolerance against food antigens, gut-associated DCs or other APCs have to distinguish, process, and present various antigens to induce inflammatory T-cell response or Treg differentiation, respectively.

1. Gut-associated Treg differentiation

Numerous studies have demonstrated that RA in combination with TGF-β can induce naive CD4+ T cells differentiation into stable FoxP3+ T cells both in vitro and in vivo (11, 31, 139, 181). Benson et al. (11) demonstrated that in vitro RA promoted induced TGF-β-dependent generation of inducible Tregs (iTreg) generation even in the presence of high-level costimulation. In vivo, these Tregs were very stable under CFA-driven infection (11). We will refer to Tregs generated in the presence of RA as RA-Tregs. Another group reported that oral administration of protein antigen or lymphopenic host induced conversion of naive CD4+ T cells into Tregs in the small intestine. This induction was dependent on RA produced by LP-DCs, because RAR-specific antagonists LE540 and LE135 inhibited RA-induced FoxP3 expression (181). Further studies elucidated that different types of APCs are responsible for inducing RA-dependent Treg differentiation. It was observed that CD103+ and not CD103- MLN-DCs promoted the conversion of naive T cells into FoxP3+ Tregs. This conversion was dependent on both TGF-β and RA (31). Further study showed that indeed bile retinoids could also imprint intestinal CD103+ but not CD103- DCs with the ability to generate gut tolerance (85). Consistently, CD103+ DCs showed higher transcription of genes related to the activation and secretion of TGF-β (tgfb2, plat, itbp3, and Raldh2) than CD103- DCs (31). The addition of RA to CD103- DCs partially allowed these APCs to induce Foxp3 expression in T cells, indicating that RA is not the unique factor produced by these APCs for inducing Treg conversion.

A recent study showed that a deficient production of GM-CSF [encoded by colony-stimulating factor (Csf2)] from granulocyte-macrophages can affect the phagocytic functions of APCs, leading to reduced Treg numbers and impaired oral tolerance (138). Exogenous GM-CSF can restore the immunoregulatory potential of Csf2<sup>-/-</sup> APCs ex vivo. Notably, blockade of RA abrogated the ability of exogenous GM-CSF to rescue Treg induction in vitro by Csf2<sup>-/-</sup> APCs, whereas addition of RA rescued Treg induction in these cultures. Consistent with these findings, injection of a RAR antagonist compromised GM-CSF-mediated rescue of Tregs in Csf2<sup>-/-</sup> mice in vivo, whereas injection of RA restored Treg frequency in Csf2<sup>-/-</sup> mice in vivo.

The studies mentioned above describe the importance of RA in promoting the conversion and stability of Tregs. However, RA can additionally inhibit the differentiation of inflammatory Th17 cells. Mucida et al. (139) showed that TGF-β induced Th17 differentiation in the presence of IL-6, but RA inhibited Th17 differentiation allowing Treg differentiation. Exogenous RA was shown to inhibit induction of Th17 cells in vivo using an infection model, whereas injection of RAR antagonists resulted in a decrease in Foxp3<sup>+</sup>Treg cells in the LP (139). In addition, another study found that intestinal macrophages, by secreting large amounts of IL-10 and RA, might limit their own production of IL-6 and IL-23. Thus, in the TGF-β-rich intestinal microenvironment, LP macrophages would continuously favor the generation of Treg cells (40).

In addition to the prominent role that RA plays in gut tolerance, it also plays an important role in the immune privilege of the eye. Retinal pigment epithelial cells were found to produce both TGF-β and RA, thus converting bystander CD4<sup>+</sup> T cells into FoxP3<sup>+</sup> Treg. Both pretreatment with RAR antagonists and silencing of RAR function in retinal pigment epithelial cells prevented Treg conversion. As anticipated, experimental autoimmune uveitis developed in VAD but not in vitamin A-sufficient mice (94).

2. Role of RA in the induction of FoxP3<sup>+</sup>CD8<sup>+</sup> Treg

The first observation of RA-induced Foxp3 expression in CD8<sup>+</sup> T cells was reported by Kishi et al. using an autoimmune diabetes model (98). The authors generated islet-specific CD8<sup>+</sup>Foxp3<sup>+</sup>CD103<sup>+</sup> Tregs by stimulating naive CD8<sup>+</sup> T cells with splenic DCs in the presence of the specific peptide, TGF-β, and RA in vitro. The CD8<sup>+</sup>Foxp3<sup>+</sup> Tregs generated in vitro inhibited diabetogenic CD8<sup>+</sup> T cell proliferation in vitro and completely prevented diabetes onset when transferred together with diabetogenic splenocytes from non-obese diabetic (NOD) mice. Using a transgenic mouse model, which express hemagglutinin (HA) antigen on intestinal epithelial cells, Fleissner et al. (53) found that CD8<sup>+</sup>Foxp3<sup>+</sup> cells can be induced in the MLN. These CD8<sup>+</sup>Foxp3<sup>+</sup> cells suppressed both CD4<sup>+</sup> and CD8<sup>+</sup> T cell proliferation (53). Further studies illustrated that RA in...
combination with TGF-β can induce FoxP3 expression on HA-specific CD8+ T cell when stimulated by MLN DCs in the presence of HA peptide. In the presence of IL-2, TGF-β, and RA, donor DCs induced and expanded alloantigen-specific CD8+FoxP3+ suppressive cells with enhanced expression of CCR4, CTLA4 and CD103. The induced CD8+FoxP3+ cells protected skin allografts in vivo via suppressing the upregulation of costimulatory molecules on DCs, and the production of proinflammatory cytokines by both CD4+ and CD8+ T cells (107). Even though FoxP3+CD8+ Tregs are not yet well recognized as a major suppressive population, the above studies illustrate that RA-dependent induction of FoxP3 expression was similar in CD4+ and CD8+ T cells, highlighting its role in maintaining immune tolerance.

3. RA-dependent induction of human Tregs

There have been contradictory findings in the regard of how RA regulates FoxP3 induction in human cells. Studies using human peripheral blood cells proved that ATRA and TGF-β converted naive but not memory CD4+ T cells into stable and suppressive FoxP3+ Tregs. Furthermore, ATRA treatment enhanced the suppressive capability of natural Tregs, reinforcing the key role that RA plays in tolerance mediated by both natural and induced Tregs (205). Lu et al. (115) found that RA enhanced and stabilized FoxP3 expression in CD4+CD45RA+ naive human T cells activated with suboptimal αCD3 and αCD28 in the presence of IL-2 and TGF-β in vitro. These induced Tregs showed suppressive activity in vitro and prevented immunodeficient mice from human-anti-mouse GVHD (115). Another group revealed the synergistic effect between ATRA and rapamycin in maintaining FoxP3 expression and suppression activity of human Tregs (59). ATRA alone only maintains transient FoxP3 expression during the expansion of Tregs purified from adult human peripheral blood. This study suggests the potential requirement for both rapamycin and ATRA in generating a large number of stable Tregs for therapeutic infusion into patients.

4. Molecular mechanism of RA-induced FoxP3 expression

The molecular mechanism of how RA controls FoxP3 expression has been investigated at transcriptional and epigenetic levels. It is well established that the enhancement of FoxP3 expression by RA depends on TGF-β, as RA alone is insufficient to induce Foxp3 expression (11, 31, 139). Studies using neonatal mouse cells and luciferase reporter assays showed that TGF-β triggered the activation of Foxp3 gene, via the binding of Smad3 and NFAT to an enhancer in the intronic conserved non-coding sequence (CNS1) (195). Recent reports showed that CNS1 contains binding sites for transcription factors NFAT, Smad3, and RAR/RXR. Further studies revealed that RA enhances TGF-β signaling by increasing the expression and phosphorylation of Smad3 (146, 217). Indeed, RA actions involve the binding of RAR/RXR to CNS1. This leads to the increased histone acetylation in the region of the Smad3 binding site and increased binding of phosphorylated Smad3, leading to increased FoxP3 expression (218).

However, other researchers reported that RA could induce FoxP3 expression even in CD4+ T cells isolated from Smad3 or Smad2 knockout mice. These observations indicate that RA upregulates an alternative signaling axis of TGF-β, leading to ERK1/2 rather than Smad2/3 activation (114). The contradictory findings may be due to redundant or overlapping signaling pathways in the control of FoxP3 transcription. These findings are also consistent with recent observations that Smad3 binding to CNS-1 is dispensable for FoxP3 expression except in the gut (160).

Recent studies by two different groups have shown that miR10a expression is a signature of RA-induced Tregs. One study showed that miR10a is highly expressed in natural Tregs compared with other T-cell subsets and can be induced by TGF-β and RA. MiR10a in these RA-induced Tregs can target both Bcl-6 and Ncor2, and attenuate the conversion of inducible Tregs into follicular helper T cells (187). Another study confirmed that miR10a is selectively upregulated in TGF-β-induced Tregs only in the presence of RA, and allows for stable FoxP3 expression; however, genetic ablation of miR10a had no impact on FoxP3 expression. These studies open the question as to how RA regulates various microRNAs, which have been discovered to regulate various aspects of the immune response. It will be interesting to elucidate the pathways that RA regulates through fine-tuning of miRNAs so that it may determine the stability and plasticity of various T-cell subsets.

C. RA in Regulation of Other Aspects of T-Cell Differentiation

1. Th17 differentiation

The impact of RA on Th17 survival and differentiation is highly RA concentration dependent. Various studies have shown that RA suppresses Th17 differentiation while promoting TGF-β-dependent Treg differentiation (139, 217). Xiao et al. (217) showed that RA inhibited IL-6R, interferon (IFN) regulatory factor 4 (IRF4), and IL-23R, which are all required for Th17 differentiation, induced by TGF-β during Th17 differentiation, thus suppressing Th17 development (217). Additional studies addressed the molecular mechanism of how RA regulates Th17 differentiation. In naive mice, the steady-state ATRA in mouse sera is 1–10 nM (1–10 μg in 25 g adult mice) (126, 143). Therefore, we propose that exogenous RA above 100 nM should be considered a pharmacological concentration. The following discussion will focus on how the physiological and pharma-
A detailed study demonstrated that flagellin-activated LP-DCs produce RA to promote both Th17 and Th1 cells differentiation in vitro (196). IL-17 instead of IFN-γ production is inhibited by RA in TK340, illustrating the specific role of endogenous RA produced by LP-DCs in driving Th17 differentiation. Unsurprisingly, 1 nM RA is sufficient to promote Th17 differentiation in the presence of splenic DCs. The reduced IL-17 and IFN-γ production by 10 μM RA (~1,000-fold higher than physiological concentration) may indeed be caused by high toxicity. Similarly, Elias et al. (51) also showed that 1 μM Pan-RAR agonist, 4-[E-2-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)-1-propenyl]benzoic acid (TTNPB), inhibited IL-17 differentiation in vitro (51). We suggest that the concentration may have been too high for evaluating the physiological role of RA in Th17 differentiation. Th17 cells are decreased in the SI of VAD mice, whereas DCs isolated from MLN, PP, or SI-LP of VAD mice, enhanced Th17 cells differentiation in vitro (26). The discrepancy ascribed to the reduced commensal microbes in the gut of VAD mice, highlighting that Th17 differentiation is fine-tuned by RA in the gut via various mechanisms. Another study further argued against the general conclusion that RA suppressed Th17 cell differentiation, illustrating that although slightly reduced, Th17 differentiation still occurred in the presence of <30 nM RA, and these Th17 cells generated in vitro proved proinflammatory in vivo and migrated to the intestine (203). In the same study, pharmacological levels of RA (>30 nM) inhibited Th17 differentiation in vitro. In other words, the experimental outcome is largely dependent on the concentration of RA used in the culture. Therefore, the controversy between various studies may be due to the concentration of RA and other cytokines applied. To more accurately assess the role of RA in Th17 differentiation fate, new insights should be gained through the analysis of Th17 response via genetic blockade of RA signaling. In this regard, Hall et al. (70) and our group reported deficient Th17 cell differentiation in RARα-deficient CD4+ T cells. In the study of Hall et al. (70), defective TCR signaling was observed in the RARα-deficient CD4+ T cells, too. It remains unknown whether RARα deficiency has any impact on RORγT expression per se.

2. Th1/Th2 differentiation

Earlier studies revealed that VAD mice display excess Th1 and insufficient Th2 response via various mechanisms. One study followed to demonstrate that the RXR but not RAR signaling pathway activation enhanced Th2 development (177). These authors claimed that 9-cis-RA but not ATRA supplementation enhanced Th2 development from naive Th0 cells isolated from D011.10 TCR transgenic mice. Further studies elucidated that RXR agonist AGN194204 but not RAR agonist TTNPB promoted Th2 development even under αCD3 and αCD28 stimulation in the absence of APC. lwata et al. (83) demonstrated that RA at ≥1 nM directly suppressed Th1 but enhanced Th2 differentiation. This effect can be mimicked by RAR agonists Am80 and Tp80, but not RXR agonists HX600 or T2335. These findings reinforced the role of RAR-dependent RA signaling in promoting Th2 but suppressing Th1 development (83). In human T cells, RA, RARα agonist AM580, and not RARβ/ RARγ agonists decrease IFN-γ and TNF-α production while promoting IL-4, IL-5, and IL-13 synthesis (35).

3. CD8+ T-cell differentiation

In an oral immunization model of Listeria monocytogenes, Huang et al. (77) found that RA and TGF-β produced by MLN DCs enhanced the expression of CD8α on activated CD8β+ T cells. CD8α can interact with a nonclassical MHC molecule thymus leukemia antigen induced on DCs so that high-affinity memory precursor cells are geared towards the gut-imprinting effector cells (77). This study supports the concept of introducing RA into a vaccination to induce highly Ag-sensitive CD8+ memory cells in the intestine for defense against mucosal pathogens. Our own studies have illustrated that RA in the periphery is required for CD8+ T cell survival during expansion, thus allowing for the optimal antigen-specific CD8+ T-cell expansion and accumulation (69). Our further study suggested that RARα is the major isof orm in determining CD8+ T-cell survival during systemic Listeria monocytogenes infection (68).

Taken together with what has been discovered about Th17, Th1/Th2, and CD8+ T-cell differentiation, the contradictory observations seem to be due to the usage of various agonists/antagonists in experimental contexts. Caution should be taken when conclusions are drawn from studies with ~10-fold above mouse sera RA. In vitro, the presence or absence of APC in culture may also influence the final outcome of RA signaling on T-cell differentiation phenotype. More genetic approaches should be harnessed to understand whether RA signaling-deficient CD4+ T cells are less efficient in differentiation into Th2 cells.

D. RA in Regulation of T-Cell Activation and Effector Functions

Emerging data suggest that RA is critical for the development of T-cell-mediated immunity. Hall et al. (70) have discovered that RA is essential for TCR signaling in early CD4+ T-cell activation and controls CD4+ T-cell proliferation and differentiation into Th1 and Th17 cells in vitro. In this study, the authors used T cells from RARα knockout (KO) mice and showed that RARα-deficient CD4+ T cells are defective in calcium influx and ERK phosphorylation and displayed insufficient activation. The RARα KO mice showed diminished Th1 and Th17 responses in Toxoplasma gondii infection and failure to control the infection.
Our studies using another transgenic model by selectively overexpressing a dominant negative RARα (dnRARα) in T cells have demonstrated that the RA signaling-deficient CD4+ T cells can proliferate, but fail to differentiate into Th1 and Th17 cells in vivo (151). The differentiation failure accounts for the delay in allogenic skin graft rejection. The discrepancy between the two studies is presently unclear and is likely due to the means with which RAR signaling was ablated.

E. RA Regulates T-Cell Survival

In addition to the requirement of RA for the development of effector T-cell immunity, our own studies have shown that RA is essential for the survival of effector CD8+ T cells during expansion phase (69). In both tumor and neo-antigen immunization model, our studies showed for the first time that in vivo, RA signaling is essential for CD8+ T cells survival during expansion at effector phase. Since RA signaling in these studies was selectively disrupted in CD8+ T cells, it proves that it is an intrinsic need of T cells to trigger RA signaling pathway to survive. As anticipated, RA signaling-sufficient CD8+ T cells are essential to control tumor growth. This was confirmed Listeria monocytogenes infection model, in which we demonstrated that RARα-deficient CD8+ T cells failed to accumulate and control bacterial expansion in the spleen (68).

In addition to RARα, RARγ has also been implicated in CD8+ T-cell biology. It has been shown that overexpression of human RARγ, under control of the Lck promoter, elevated the number of CD4+CD8+ single positive cells in the thymus. These data suggest that RARγ may be involved in CD8+ T-cell development in the thymus (152). However, when RARγ is deleted from hematopoietic compartment, it is without effect on CD8 ontogeny (82). Therefore, in our point of view, RARγ does not regulate CD8+ T-cell survival.

VI. RA REGULATION OF T-CELL IMMUNITY IN DISEASES

A. RA in T-Cell-Mediated Anti-tumor Immunity

1. Extrinsic effect of RA on anti-tumor T-cell immunity

Early studies have shown that a vitamin A-rich diet or RA treatment can enhance the frequency of tumor-specific cytotoxic effector killer T cells elicited by the vaccination with irradiated tumor cells (118). Other studies found that in both tumor-bearing mice (102) and cancer patients (220), high-dose RA treatment can induce the differentiation of immature myeloid-derived suppressor cells (MDSC) into mature DCs, thus diminishing the tolerance of T cells and generating greater T-cell responses against the tumor. An earlier study showed that liposomal RA treatment induced regression of murine acute promyelocytic leukemia in cooperation with adaptive immunity (210). In line with these findings, combination of a DNA vaccine and RA administration induced robust CD4+ and CD8+ T-cell immunity and enabled long-term survival in an acute promyelocytic leukemia mouse model, although ATRA alone failed to elicit pronounced T-cell immunity (56). While the studies above were not specifically designed to understand the role of RA in mediating enhanced anti-tumor T-cell immunity, they support the notion that ATRA may be important for the development of anti-tumor T cell response, thus contributing to the observed efficacy in tumor control.

2. Intrinsic effect of RA on anti-tumor T-cell immunity

Our own studies demonstrated that T cell-intrinsic RA signaling is essential for CD8+ T-cell expansion and differentiation to IFN-γ secretion (69) in response to tumor antigen. Our studies have shown that as tumors progress, there is a progressive increase in the tissue concentration of RA at the tumor site (69). One may question as to whether RA in the tumor microenvironment (TME) contributes to the tolerant TME by facilitating the development of Treg or is it essential for the development of T effector functions (11, 31, 139, 181). One may speculate that RA produced by host DCs in TME can indeed induce Treg conversion, given heightened suppressive cytokines such as TGF-β production by malignant cells (45, 191) or host APCs (28) at late stage of tumor growth. However, we did not observe dramatic Treg changes in TME when RA signaling was blocked by dnRARα overexpression in T cells. Additional effort should be given to the study of RA signaling-deficient Tregs in TME, to evaluate whether Treg functionality in TME may be indeed dependent on RA signaling. We summarize the function of RA in regulating various aspects of anti-tumor T-cell immunity in Figure 2.

B. RA in Anti-infection T-Cell Immunity

VAD in children leads to increased susceptibility to ID and thus higher morbidity and mortality (173). These epidemiological and clinical findings clearly established that RA is essential for host resistance to infection. T cells are an essential component in fighting against both primary infections and maintenance of long-term memory response against infectious agents (214). Presently, vitamin A supplementation is currently used in many developing countries to reduce childhood mortality (78, 81, 172). The essential role of both RA and T cells in immune responses against ID suggests that RA signaling may play a key role in the development of T-cell immunity against infection. However, while VAD is associated with increased susceptibility to ID, the effect of vitamin A supplementation on ID control has been beneficial (200)/neutral (141) or even deleterious.
mucosal CD8

Kaufman et al. (92) found that VAD diet diminished the frequency of CD8+ T cells and protected against challenge. Similarly, in VAD mice, retinyl palmitate or RA administration fully restored the vaccination-driven gastrointestinal CD8+ T-cell response and protection against challenge. In contrast, such an effect was not observed in Listeria monocytogenes-infected rats (163). Given the importance of adaptive T-cell immunity in host protection against infection, our discussion will focus on how RA regulates anti-infection T-cell immunity in different infectious diseases.

1. RA regulation of host resistance and CD8+ T-cell immunity

A) RA CONTROLS HOST RESISTANCE AND MUCOSA-RESIDENT CD8+ T-CELL IMMUNITY. Pharmacological and genetic approaches have been used to elucidate the function of RA in control of CD8+ T-cell responses to ID. The gut-homing tropism, FoxP3 induction, and survival effects that RA exerts on CD8+ T cells can all influence host susceptibility to ID. Kaufman et al. (92) found that VAD diet diminished the mucosal CD8+ T-cell response elicited by intramuscular immunization with recombinant adenovirus vaccine vector expressing OVA (rAd5-OVA). As a consequence, this abolished the protection against oral challenge of Listeria monocytogenes overexpressing OVA (92). As expected, retinyl palmitate or RA administration fully restored the vaccination-driven gastrointestinal CD8+ T-cell response and protection against challenge. Similarly, in VAD mice there was a reduced frequency of CD8+ T cells in the lower respiratory tract to a candidate human parainfluenza virus type 1 vaccine compared with control mice. This study suggested vitamin A (or RA) controls CD8+ T-cell response in mucosa other than the gut (157). Therefore, depending on specific infectious pathogens, RA signaling may impose quite different effects on systemic CD8+ T-cell response. It has been proposed that the gut-tropism of T cells imposed by ATRA has proven beneficial in eliciting more robust T-cell immunity in the mucosa and better control of both Salmonella (73) and LCMV infection (189). In both studies, more effector and memory CD8+ T cells were observed in the gut of ATRA-treated mice.

With the use of genetic models that allow conditional blockade of RA signaling, precise questions can be asked as to the role of RA on specific leukocyte lineages. In one study using transgenic mice in which RARγ is deleted from the hematopoietic compartment, defective primary and memory CD8+ T-cell response against Listeria monocytogenes was observed. It was proposed that this was due to a deficiency in macrophages secreting proinflammatory cytokines such as TNF-α and IL-6 (82). Interestingly, a recent study showed that RA signaling-deficient CD8+ T cells are more prone to the development of memory precursors instead of short-lived effectors in the context of vaccinia virus infection (4). Genetic deletion of specific RAR isoforms exclusively in T cells allowed for the dissection of RARα, RARβ, and RARγ in control of anti-infection CD8+ T-cell immunity. Our recent study demonstrated that RAR is essential for survival of CD8+ T cells in vivo following Listeria monocytogenes infection. In contrast, RAR deletion leads to modest deficiency in Ag-specific CD8+ T-cell expansion during infection. The defective survival of RAR-deficient CD8+ T cells leads to a deficiency in control of...
**Listeria monocytogenes** expansion in the spleen (68). No defects were observed in RARγ-deficient CD8+ T cells, further corroborating that lower TNF-α and IL-6 production by the RARγ-deficient macrophages caused defective CD8+ T-cell immunity in a host with RARγ-deficient hematopoietic compartment.

**2. RA regulation of host resistance and CD4+ T-cell immunity**

A) TH1/TH2 BALANCE. The balance between Th1 and Th2 responses during CD4+ T-cell differentiation is critical for infection control as it determines the humoral response against pathogens. Previous studies revealed that VAD environment induced constitutive expression of Th1 cytokines, and limited the humoral response to infection, thus diminishing the host ability to contain infection through antibody (Ab) production (23). Investigating *Trichinella spiralis* infection in VAD mice, Cantorna et al. (22) found higher IFN-γ production and lower frequencies of IL-5-producing Th2 cells, which can be restored by the addition of RA in vitro. These data suggest that RA inhibits Th1 and promotes Th2 differentiation and survival (22). Even in animals with normal serum levels of RA, RA was shown to enhance Th2 but inhibit Th1 responses during tetanus toxoid immunization resulting in higher IL-4:IFN-γ ratio and enhanced all anti-tetanus toxoid IgG isotypes. These data suggest that RA can be used as an adjuvant in generating more robust humoral response via Th2 skewing in vivo (116). However, despite the skewed Th2 response discussed above (22, 83), RA treatment of *Ascaris suum*-infected swine promoted profound and enhanced Th1, Th2, and Treg differentiation in the liver, albeit some may be an indirect impact from innate immune cells, such as more recruitment of eosinophils and more inflammatory cytokines produced thereafter (34).

B) TH1/TH17 RESPONSE. Previous studies using CD4+ T cells from both VAD and RARα KO mice showed increased frequencies of FoxP3+ Tregs, deficient proliferation, and differentiation into Th1 and Th17 cells in *Toxoplasma gondii* model, while no dramatic differences were reported in Th2 response (70). However, the lower Th1 and Th17 response in vivo led to impaired control of systemic *Toxoplasma gondii* systemically following oral infection, clearly demonstrating the essential role of intact RA signaling for protective CD4+ T-cell immunity. As RARα is also deleted APCs in these mice, such as DCs and myeloid cells, the defective CD4+ T-cell responses in vivo may be partially due to the intrinsic RA signaling deficiency in T cells. Therefore, additional studies in mice with lineage-specific control of RA signaling will help to address these issues. It also remains to be elucidated whether intrinsic RA signaling controls the balance between suppressive Tregs and inflammatory Th1/Th17 response in various ID circumstances. The immune system has apparently developed sophisticated mechanisms to control infection and avoid overt pathology by balancing the magnitude of the inflammatory response. No doubt, RA falls into the group of fine-tuning molecules to keep immune response in balance.

C) TREG REGULATION. RA also regulates host resistance and immunity via the enhancement of stable and gut-tropic Tregs. RA-Tregs are more stable than Tregs generated in the absence of RA (11). Due to concomitant α4β7 and CCR9 expression, RA-Tregs showed preferential migration to the gut (11, 181). RA-Tregs are thus also superior in protection against colitis due to the preferential migration to the gut (139). The role of RA in generating superior Tregs holds true during chronic helminth infection. Chronic helminth infection induced tolerogenic CD11clow DCs which induced RA signaling-dependent Treg generation, and modulated the immune response. Depletion of CD11c+ DCs favored the dominance of CD11clow DCs, leading to lower effecter CD4+ T-cell proliferation and effector cytokine production (170). RA treatment also enhanced FoxP3 but inhibited IL-17 expression in colonic biopsies from patients with ulcerative colitis and colon tissues ex vivo. In addition, RA also downregulated colon inflammatory responses and ameliorated trinitrobenzene sulfonic acid-induced murine colitis (7). RA-Tregs are also superior in resolving CD8+ T cell-mediated acute small intestine inflammation (129). This study highlighted that the selective migration of RA imposed on Tregs is key to their local suppressive function. All the above studies support that RA may ameliorate the infection-relevant pathology in the gut via generating highly stable and gut-residential Treg.

**C. RA and T-Cell Differentiation in Transplantation**

1. Suppression of effector response

During solid organ transplantation, donor alloantigens are processed and presented to CD4+ T cells, and induce the latter to activate and produce IL-2 and IFN-γ. The inflammatory cytokines can then induce cytotoxic CD8+ effecter T cells, B cells, and macrophages proliferation and differentiation, which can mediate allogeneic tissue and organ destruction (207). Even though numerous studies have confirmed that CD4+ and CD8+ effecter T cells mediate the rejection of allografts (169), there is always overregulation by Tregs (as reviewed in Ref. 202). Therefore, the balance between effecter T cells and Tregs determines the kinetics of rejection or acceptance of allogeneic tissues or organs. Direct regulation of RA on T cell allogetic in GVHD development has been reported. In a mouse model of acute GVHD, RARα-specific agonist ER-38925 administration dampened the cytotoxic T lymphocyte (CTL) response, and inhibited Th1-derived serum IL-12 and IFN-γ and macrophage-derived TNF-α. The substantial inhibition of antiallogenic T-cell responses in ER-38925-treated mice therefore prevented the development of acute GVHD (75).
2. RA promotes allogenic effector response

Our recent study revealed a novel function of RA as being essential for the development of T-cell effector response against an allogenic skin transplant. In a model where T cell-intrinsic RA signaling was ablated, deficient Th1/Th17 response led to significantly delayed rejection of allogenic skin grafts. Our failure to observe an overriding suppression of immunity by RA indicated the dominant role of endogenous RA in driving effector response than Treg development in this model. These studies provide insightful understanding of the function of RA in controlling the development of effector T cells in allogenic organ rejection.

3. RA regulates transplantation through Treg development

Despite the requirement of effector T cell-intrinsic RA signaling for allogenic skin rejection, the administration of RA agonists induces immune suppression in some circumstances. Prior to the discovery that RA can induce Treg development, a study in cardiac transplantation demonstrated the immunosuppressive efficacy of RARα agonist in organ transplantation (164). During acute cardiac transplantation, prophylactic treatment with RARα-specific agonist ER-38925 in combination with tacrolimus inhibited cytotoxic effector cells proliferation and alloantigen-specific inflammatory cytokines IL-2, IL-12 and IFN-γ production, and thus delayed the rejection of the mouse cardiac allograft (BALB/c→C3H/HeN). The inhibition of cytotoxic response may be due to the induction of Treg development in this model, although not stated by the authors. Another study revealed that combination of RA and TGF-β indeed attenuated acute cardiac rejection by promoting Treg and inhibiting Th17 differentiation (204).

Various studies have now proven that Tregs can be generated ex vivo, efficiently dampen the host immune response against graft-specific antigens, and thus prevent both acute and chronic allograft rejection in vivo (87, 216). Numerous studies have demonstrated the success of RA-Treg in the prevention of allogenic transplant rejection. Moore et al. (135) cultured naive T cells with allogenic APCs, TGF-β, IL-2, and RA to generate Tregs that were successful in preventing allogenic skin rejection, proving the suppressive function and potential for therapeutic application of RA-Treg in allogenic graft rejection (135). In a rat orthotopic tracheal transplantation model, adoptive transfer of RA-Tregs enhanced TGF-β and IL-10 production, whereas inhibited inflammatory cytokines IL-17, IFN-γ, IL-6, and MCP-1. RA-Tregs also inhibited the infiltration of effector T cells into the graft. The balance between anti- and proinflammatory response led to attenuated airway obliteration and graft fibrication, thus protecting rat trachea allograft rejection (168). More surprisingly, RA-induced CD8⁺ FoxP3⁺ Tregs were found to induce conventional CD4⁺ FoxP3⁺ Treg conversion and concurrently suppressed CD8⁺ T effector expansion in situ, thus protecting allogenic skin graft (107). Therefore, RA can induce FoxP3 expression on both CD4⁺ and CD8⁺ T cells, and promote immunosuppression required for allogenic grafts protection.

D. RA and T-Cell Differentiation in Autoimmunity

As RA regulates various aspects of T-cell function, it is not surprising that RA also modulates the progression of various autoimmune diseases through the regulation of CD4⁺ or CD8⁺ T cells. Specifically, RA can control the differentiation of CD4⁺ T cells into Th1, Th2, and Th17 which impact on the intensity and pathological manifestations of many autoimmune diseases. Furthermore, RA may also regulate the progression of autoimmune diseases via control of CD8⁺ T-cell differentiation. In the following section, we focus on how CD4⁺ T-cell differentiation is controlled by RA signaling towards immunity or tolerance, and thus determine the disease incidence and severity in different autoimmune diseases.

1. RA regulates experimental autoimmune encephalomyelitis

A) RA suppresses pathogenic T cells in experimental autoimmune encephalomyelitis. Early in 1991, long before the discovery of Tregs and Th17 cells, 13-cis-RA was demonstrated to prevent both active and passive experimental autoimmune encephalomyelitis (EAE) development through the inhibition of T-cell proliferation, IL-2 production, and effector function (123). The same therapeutic efficacy was observed in ATRA treatment of myelin basic protein (MBP)-specific cell-induced EAE. RA was found to increase IL-4 but decrease IFN-γ production, whereas interestingly, RA in combination with TGF-β agonist in promoting different suppressive Treg subpopulations targeting the rationale for retinoids in the treatment of multiple sclerosis (113). Further studies have revealed the role that RA plays in Th17 cells in the treatment of autoimmune diseases. One study showed that RA inhibited pathological Th1 and Th17 cells through downregulation of IRF4, IL6Rα, and IL23R without altering the frequency of Tregs, thus inhibiting EAE onset and progression (217).

B) RA promotes Treg in amelioration of EAE. The role of RA in promoting different suppressive Treg subpopulations makes the rationale that RA modulates the balance between inflammatory and tolerant response during the development of autoimmunity. A novel CD8αα⁺ Treg lineage derived from naive CD4⁺ T cells has been recognized lately as an important suppressive population generated in situ. Interestingly, RA in combination with TGF-β can induce CD8αα⁺ Tregs development from naive CD4⁺ T cells in
RUNX3-dependent manner, to protect mice against myelin oligodendrocyte glycoprotein-induced EAE (142). The timing of RA treatment, however, needs to be taken into account as long-term treatment also leads to other cytokine profile changes, which may result in effects opposite from what may be predicted from the disease onset. Studies showed that retinoids such as AM80, RARα-β-specific ligand, are effective during the early phase of EAE development. Such an effect was correlated with lower IL-10 production during disease progression. However, continuous administration failed to inhibit late EAE symptoms (99).

Despite the therapeutic efficacy of retinoids in treatment of EAE, more studies are warranted to illustrate whether RA signaling-deficient T cells still facilitate EAE progression. It is of great importance to understand the role of RA signaling in balance between effector and regulatory T cells during the disease progression for better control of time-dependent RA signaling.

2. RA regulates rheumatoid arthritis

Studies have revealed the essential role of RA in progression of rheumatoid arthritis (RA) via balancing between suppressive Treg and pathogenic Th1/Th17 cell differentiation. An early study in rheumatoid arthritis patients suggested that RA treatment decreased IFN-γ but not IL-2 or IL-4 production by CD4+ T cells from both healthy and rheumatoid arthritis patients. These results indicated the potential application of RA in treatment of rheumatoid arthritis (147). A recent study showed that RA treatment decreased Th17 and increased Tregs in a murine model of collagen-induced arthritis (CIA), leading to the inhibition of both incidence and progression of CIA (103). Further studies revealed that concurrent FoxP3 upregulation and IL-17 downregulation is independent of STAT3 or STAT5 signaling pathway, consistent with previous observations using conditional deletion of stat5 and stat3 (51). A very recent study demonstrated that all-trans-retinaldehyde (retinal) treatment reciprocally suppressed Th17 and promoted Treg development at both mRNA (decrease of IL-17 and IL-21 but increase of FoxP3, SOCS3, and IL-10) and protein level in DBA1/J mice with CIA, thus partially contributing to the attenuation of arthritis (150). It remains to be elucidated how retinal regulates the balance between Th17 and Tregs in CIA. All-trans-retinaldehyde demonstrated a function through phospholipase C1,4,5-trisphosphate/Ca2+ signaling independent of its conversion to RA in some cell types (29). However, the efficacy observed in the above study was likely via the conversion to RA. In addition to its direct impact on Th17 induction, RA was found to maintain the stability of natural Tregs and prevent Th17 differentiation. This occurs as a result of the downregulation of IL-6R expression and pSTAT3 activation (228). Further in vivo studies elucidated that adoptive transfer of RA-treated nTreg suppressed the progression of established CIA in DBA1 mice. However, untreated nTreg succumbed to Th17 differentiation in this chronic inflammatory environment and lost therapeutic efficacy. This proved true even if nTreg maintained stable FoxP3 expression in vivo and were originally isolated from CIA mice, indicating the therapeutic potential of RA in rheumatoid arthritis (228).

3. RA regulates uveitis

Eyes are one of the immune privileged sites in human body. Some studies have revealed the role of RA in maintaining the immune tolerant microenvironment in the eye through inhibition of Th1/Th17 response and promotion of Treg development. Immunization of C57BL/6 mice with human interphotoreceptor retinoid binding protein peptide 1 to 20 [IRBP(1-20)] elicited experimental autoimmune uveoretinitis (EAU). Keino et al. (95) reported that intraperitoneal RA administration dampened the severity of EAU when administrated at 200 μg/mouse (~20-fold above normal sera RA concentration, every other day) before the disease onset. No therapeutic efficacy was observed when RA is administrated at effector phase (95). Oral administration of Am80 at 3 mg/kg (~75 μg/25 g mouse, ~7-fold above naive serum RA level) every other day, inhibited the severity of EAU, through downregulation of IL-6R, IL-17A, and IFN-γ, and reciprocal upregulation of FoxP3 (96). The same effect of IL-6R upregulation on CD4+ T cells and antigen-specific Th1/Th17 response were observed in RA-treated mice as in EAE studies despite an unaltered Treg population.

Despite the therapeutic efficacy of RA and synthetic RAR ligands in EAU treatment, the molecular mechanism of ocular immune privilege was not fully understood until recently. The Caspi group was able to purify naive Foxp3+ CD4+ T cells from FoxP3-GFP mice, and convert them into highly suppressive Tregs using aqueous humor (AH) in synergy with TGF-β and RA. The Tregs also showed upregulation of CD25, GITR, CD103, and CTLA4 (226). More in-depth analysis revealed that AH stabilized TGF-β and RA-induced FoxP3 expression. This study indicated a dual role of RA in the eye: in vision and immune privilege. The same group further demonstrated for the first time that in vivo naive CD4+ T cells can be converted to Tregs in a RA-dependent manner in the eye during specific auto-antigen-induced proliferation. Cornea and retina proved to be the source of RA for Treg development as both are positive for all three Raldh isoforms expression and activity (227). More strikingly, either preimmunization of the host with IRBP(161-180) or priming of CD4+ T cells (CD44hiCD62L−) diminished RA-dependent Treg induction significantly, thus inducing severe uveitis. Regarding the source of RA, Kawazoe et al. (94) also demonstrated that retinal pigment epithelial cells produced RA and, in combination with TGF-β, induced bystander T cells conversion into Tregs, thus maintaining the ocular immune tolerance (94). This study further reinforced the key role of RA in prevention of autoimmune uveitis development.
All the above studies extended the important function of RA in both vision development and immune privilege (201). Such findings reinforced the importance of sufficient vitamin A intake in both vision development and the tolerant ocular microenvironment maintenance.

4. RA regulates T cell-mediated type 1 diabetes

Numerous studies have revealed the functional deficiency or decrease of Tregs in type I diabetes (T1D) patients (as reviewed in Ref. 18). Hence, manipulating the immune system to facilitate Treg expansion or shift from pathogenic effector T cells is predicted to be efficient in T1D treatment. Van et al. (198) showed the therapeutic efficacy of (500 µg/mouse, every other day) RA in T1D treatment. In their study, RA treatment of nonobese diabetic (NOD) mice at 10 wk of age, when the destructive insulitis already occurred, increased systemic Treg accumulation, decreased activated CD8⁺ effector T cell and Th1 cell infiltration into the islet (198). This combinatorial effect on T cells led to the regression of diabetes. Of note, the efficacy was completely dependent on Treg increase as depletion of CD4⁺CD25⁺ T cells impaired the efficacy. This study sets the foundation that RA-induced Tregs are essential for suppressing the development of T1D even though the direct inhibition of ATRA on effector T cells proliferation cannot be excluded.

A subsequent study tested the efficacy of retinoids, etretinate, and ATRA in three different preclinical models of human T1D (179). In streptozotocin-induced diabetes in CBA/H mice, both drugs suppressed the diseases progression in terms of decreasing hyperglycemia, concomitant with lower CD4⁺CD25⁺ effector T cells in the periphery. ATRA also decreased expression of IFN-γ and IL-17 at mRNA level and secretion of IL-6, IFN-γ, and IL-17 upon restimulation ex vivo. The authors observed the same effect as in previous studies (198) when RA was administrated in spontaneous diabetes developed in NOD mice. However, no efficacy was observed when cyclophosphamide was administered to accelerate diabetogenesis, highlighting the Treg-dependent ATRA efficacy. The discovery of ATRA-induced CD8⁺ Tregs led to the finding that CD8⁺FoxP3⁺ Tregs generated in vitro were suppressive in vivo and effective in T1D treatment (98).

More importantly, recent studies shed light on the importance of conditioning DCs to promote Treg development via RA production in T1D treatment. Immunization with Schistosoma mansoni soluble antigen preparations was known to protect NOD mice from T1D development (30). Interestingly, α-1, a well-characterized glycoprotein in S. mansoni antigen, was demonstrated to induce both TGF-β and RA production by DCs and induce Treg development in vitro (221). The mechanism emphasized the intrinsic requirement of RA-induced FoxP3 expression in the therapeutic efficacy of S. mansoni antigens in NOD mice.

5. RA regulates other autoimmune diseases

In addition to the well-known autoimmune diseases mentioned above, other studies have elucidated the mechanism of RA signaling in control or treatment of other autoimmune diseases. Sobel et al. (171) reported that CD4⁺ T cells in systemic lupus erythematosus patients are defective in both TGF-β and RA signaling, indicating the proinflammatory environment caused by Treg decrease. In another study on experiment autoimmune myositis, Ohyanagi et al. (149) found that oral administration of Am80 increased IL-4, IFN-γ, and IL-10 but not IL-17 production by T cells, and decreased T-cell infiltration into the muscle, leading to ameliorated inflammation. The multifaceted function of RA in T-cell differentiation and migration makes it a good candidate for the treatment of a wide spectrum of autoimmune diseases.

Despite the rapid progress in understanding both the mechanism and therapeutic application of RA in control of autoimmune diseases, additional insights are critical. At this time, we do not have a clear mechanistic understanding of the molecular impact of RA on key cellular elements (T, B, myeloid) in vivo and how it governs the development of tolerance and autoimmunity.

E. RA in T-Cell Regulation for Allergy Response

The promiscuous impacts of RA on T-cell differentiation make it an attractive mediator in control of allergy. Early studies established that T cells in VAD hosts were skewed towards IL-10⁺ Tregs but diverted from IFN-γ⁺ Th1 memory cells without any impact on IL-4 production (176). In the following section, we will discuss the role of RA signaling in the control of allergic response via regulation of different T-cell subsets.

1. RA regulates allergy response via Th1/Th2

Various studies have revealed that RA may affect allergy response by altering both Th1 and Th2 response. Studies have shown that both ATRA and 9-cis-RA induced Th2 cytokines IL-4 and IL-5 production by human CD4⁺ T cells, while inhibiting Th1 cytokine IFN-γ production (36). As discussed above, the timing of ATRA administration determines the impact on allergy progression, as CD4⁺ T-cell differentiation status is different at the onset and progression stage. For instance, liposomally encapsulated ATRA increased IgE response, bronchoalveolar lavage (BAL) eosinophilia, and accumulation of IL-5, TGF-β1, and CCL1 when given at the time of systemic sensitizing injections for asthma manifestation. However, such variables were not affected when administered at the time of intranasal challenges (121). In both cases, T cells were prone to IL-4/IL-5⁺ Th2 but not IFN-γ⁺ Th1 cells in vitro,
exacerbating allergic and inflammatory response. The different observations at different time points suggest that the impact on T-cell differentiation, for instance, is dependent on the priming status (plasticity) of these cells and their migration into the lung. This was consistent with previous reports showing that in ATRA-treated acute promyelocytic leukemia patients, there was a hyperleukocytic response associated with asthmalike respiratory syndrome (66, 74), eosinophilia, basophilia and hyperhistaminemia, peripheral edema, and so on (74).

2. RA regulates allergy through Tregs

More recent studies have focused on the role of RA-Treg in regulation of T-cell-mediated allergy. The ability of ATRA to induce FoxP3 expression was not only demonstrated in naive CD4+ T cells (11, 139, 181), but also proven true in Th2 memory cells generated in vivo. Kim et al. (97) discovered that OVA-specific Th2 memory cells generated in vivo can be converted into functional FoxP3+ Tregs in the presence of TGF-β and ATRA ± rapamycin under both Ag-specific stimulation and polyclonal stimulation (97). The “converted” Tregs downregulated GATA3 and IRF4; produced little IL-4, IL-5, and IL-13; and migrated efficiently into the lung. Thus Tregs efficiently suppressed Th2-mediated airway hyperreactivity, including eosinophil and IgE response. This finding supported the therapeutic potential of RA in allergy treatment.

In addition to pharmacological approaches, genetic-engineered mouse models were also used to elucidate molecular mechanisms of how endogenous RA signaling regulates allergic response. In complete Aspergillus allergen-immunized mice model, epithelial metalloproteinase 7 (MMP7) activated IL-25 to promote Th2 cells differentiation, but inhibited RALDH1 and thus Treg development (61). Therefore, MMP7−/− mice showed higher RALDH1 expression, lower IL-4 and IL-5 in bronchoalveolar fluid, and dampened airway hyperreactivity. As such, the RALDH inhibitor Citral restored asthma in MMP7−/− mice, whereas ATRA administration attenuated the asthma phenotype with a concomitant increase in Treg.

The key role of RA in modulating allergy via Treg induction was also demonstrated in Th17-dominated chronic allergic airway inflammation. During mucosal sensitization with OVA and TNF-α, prolonged ATRA treatment enhanced Treg activity in the lung and consequently ameliorated Th17-mediated disease, reinforcing the therapeutic potential of ATRA in asthma treatment by modulating Th17-Treg balance (225). Of note, ATRA concentration needs to be taken into account for allergy treatment. In both OVA immunization-OVA challenge and house dust mite immunization-house dust mite challenge models, different doses of ATRA changed the allergy response differentially (124). Even though administration of ATRA at different doses (50, 500, and 2,500 μg) all enhanced eosinophil recruitment to the BAL, fewer eosinophils were recruited into BAL of 2,500 μg ATRA-treated mice than those treated at lower doses. In addition, total IL-5 and IgE in BAL were decreased in 2,500 μg ATRA-treated but increased in lower dose-treated hosts. Given that 50 μg is about fivefold higher than the total RA concentration in the whole mouse body, 500 and 2,500 μg (~50- and ~500-fold higher than physiological concentration, respectively) may indeed have some off-target effects and/or even toxicity. The lower eosinophil counts in 2,500 μg treated group may be due to high toxicity. Therefore, lower pharmacological doses of ATRA enhance Th2 response in vivo. The global adverse effect of high-dose treatment needs to be taken into account when evaluating the application of RA in allergy treatment.

More studies are certainly needed to understand the regulatory role of RA in Th2 or Th17-dominant allergy disease. The recruitment, proliferation, differentiation, and activity of various T-cell subsets are the key components that RA signaling may impose impact on during both disease development and treatment.

F. RA and T Cell-Dependent Humoral Response

As discussed above, VAD leads to increased susceptibility to ID among young children, causing higher mortality and morbidity (173). B cells and humoral response are vital to host protection and vaccination against many infectious pathogens. There are two types of Ab response, namely, T-dependent and -independent response, depending on the specific Ag. Due to the wide-sectrum features that RA regulates in T-cell differentiation, RA may also modulate humoral response via the pathway of T-cell differentiation. For researchers interested in the direct impact of RA on B cells, B-cell differentiation, memory B-cell generation, and IgA class switching, we recommend other review articles (155).

VII. CONCLUDING REMARKS

Numerous studies have elucidated the mechanism of how vitamin A controls the immune response through RA signaling. During embryogenesis, maternally derived RA is essential for the development of secondary lymphoid organs (197). In adult mammals, RA controls various immune cells differentiation and thus the ultimate outcome of immune response and host protection (84, 137). The dual role of RA promoting tolerance (11, 31, 139) and immunity (69, 70, 151) in different disease contexts points to the key notion that RA functions as a regulator of immune response. The overall impact of vitamin A deprivation or RA signaling ablation depends on the microenvironment in which other cytokines/chemokines...
may interact with RA-mediated signaling to determine the cell differentiation fate.

Although Iwata et al. (84) provided the initial clues that the “anti-infective” effect of vitamin A is conveyed through the upregulating impact of the gut-homing molecules α4β7 and CCR9, the impact of RA on immunity is far more profound. In the periphery, RA controls the specification and fate of Th1s, Th17s, and Th2s. In this regard, many questions remain to be answered. First, does RA signaling control the plasticity and stability of effector T cells during differentiation as it stabilizes FoxP3 expression in Tregs? That is, is RA signaling required for stable expression of GATA-3, T-bet, and RORγT expression possibly through epigenetic modification? Second, what is the difference between signaling pathways induced by physiological and pharmacological RA? Caution should be taken in the interpretation of data acquired through the administration of high-dose ATRA or 9-cis-RA due to possible toxicities. Third, what other transcription factors cooperate with RARs in determining the lineage commitment of different immune cells? Fourth, what genes in T cells are directly or indirectly regulated by RA that impact on their function and fate? To answer these two questions, advanced proteomic and genomic analyses need to be harnessed. Chromatin-immunoprecipitation (ChIP)-Seq analysis will help to identify novel target genes during T-cell differentiation. These mechanistic studies will lead to a better understanding of how RA exerts such profound control over the polarity of the immune response.

ACKNOWLEDGMENTS

Address for reprint requests and other correspondence: R. J. Noelle, Norris Cotton Cancer Center, One Medical Center Dr., Rubin Bldg., Rm. 730, Lebanon, NH 03755 (e-mail: RJJ@Dartmouth.edu) or MRC Centre for Transplantation, King’s College London, 5th Floor Tower Wing, Guy’s Hospital, London SE1 9RT, UK (e-mail: Randolph.Noelle@kcl.ac.uk).

GRANTS

This work was supported by National Institutes of Health Grant 1R01AT005382.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

REFERENCES


1. Ross AC, Chen Q, Ma Y. Vitamin A and retinoic acid in the regulation of B-cell
2. Nolting J, Daniel C, Reuter S, Stuelten C, Li P, Sucov H, Kim BG, Letterio JJ, Kret-
schmer K, Kim HJ, von Boehringer H. Retinoic acid can enhance conversion of naive
T cells into regulatory T cells independently of secreted cytokines. J Exp Med 206: 2131–2139,
2009.
3. Ohoka Y, Yokota A, Takeuchi H, Maeda N, Iwata M. Retinoic acid-induced CCR8
expression requires transient TCR stimulation and cooperativity between NFATc2
and the retinoic acid receptor/retinoid X receptor complex. J Immunol 186: 733–744,
2011.
ameliorates experimental autoimmune myasthenia, with modulation of Th cell differen-
SK, Park SH, Kim HY, Cho ML. Retinoid attenuates inflammatory arthritis by regulatory
regulation of IL-17-producing T cells and Foxp3(+) regulatory T cells and the inhibi-
Nowak E, Blomhoff R, Sockanathan S, Chandraratna RA, Dmitrovsky E, Noelle RJ. A
retinoic acid-dependent checkpoint in the development of CD4(+) T cells.
7. Pohl J, Laface D, Sands JF. Transcription of retinoic acid receptor genes in transgenic
DE. Retinoid treatment of experimental allergic encephalomyelitis. IL-4 production
9. Ross AC. Vitamin A deficiency and retinoid repletion regulate the antibody response
to bacterial antigens and the maintenance of natural killer cells. Clin Immunol Immun-
10. Ross AC, Chen Q, Ma Y. Vitamin A and retinoic acid in the regulation of B-cell
11. Ruane D, Brane L, Reis BS, Cheong C, Poel J, Do Y, Zhu H, Velinon K, Choi JH, Studt
N, Mayer L, Lavelle EC, Steinman RM, Mucida D, Mehandru S. Lung dendritic cells
induce migration of protective T cells to the gastrointestinal tract. J Exp Med 210:
frequencies and heightened CD103 expression among virus-induced CD8(+) T cells
in the respiratory tract airways of vitamin A-deficient mice. Clin Vaccine Immunol 19:
JK, Staeling-Hampton K, Trainor PA. RDH10 is essential for synthesis of embryonic
retinoid acid and is required for limb, craniofacial, and organ development. Genes Dev
15. Schlenner SM, Weigmann B, Ruan Q, Chen Y, von Boehminger H. Smad3 binding to
the foxp3 enhancer is dispensable for the development of regulatory T cells with
16. Schlug TT, Berry DC, Shaw NS, Travis SN, Noy O. Opposing effects of retinoic acid on
cell growth result from alternate activation of two different nuclear receptors. Cell
17. Schuster GU, Kenyon NJ, Stephens CB. Vitamin A deficiency decreases and high
dietary vitamin A increases disease severity in the mouse model of asthma. J Immunol
18. Seguin-Devaux C, Hanniot D, Dailloux M, Latger-Cannard V, Zannad F, Mertes PM,
Longrois D, Devaux Y. Retinoic acid amplifies the host immune response to LPS
through increased T lymphocytes number and LPS binding protein expression. Mol
Prevention of acute and chronic allograft rejection by a novel retinoic acid receptor-
1999.
21. Semba RD, Graham NM, Cailla FA, Margolick JB, Clement L, Vlahov D. Increased
mortality associated with vitamin A deficiency during human immunodeficiency
22. Semba RD, Miotti PG, Chipangwii JD, Liomba G, Yang LP, Saah AJ, Dalabatza GA,
Hoover DR. Infant mortality and maternal vitamin A deficiency during human
Foxp3(+) regulatory T cells induced by TGF-beta, IL-2 and all-trans retinoic acid
attenuate colitibative bronchiolitis in rat trachea transplantation. Int Immunopharmacol
24. Shizuru JA, Gregory AK, Chao CT, Fathman CG. Ileal allograft survival after a single
25. Smith KA, Hochwelder K, Hammerler HG, Boon L, MacDonald AS, Maizels RM.
Chronic helminth infection promotes immune regulation in vivo through dominance
Hoover DR. Infant mortality and maternal vitamin A deficiency during human
27. Smith KA, Hochwelder K, Hammerler HG, Boon L, MacDonald AS, Maizels RM.
Chronic helminth infection promotes immune regulation in vivo through dominance
28. Solob ES, Brusko TM, Butfiloski EJ, Hou W, Li S, Cuda CM, Abid AN, Reeves WH,
Morel L. Defective response of CD4(+) T cells to retinoic acid and TGFbeta in
of vitamin A supplementation on childhood mortality. A randomised controlled com-
31. Spiegel N, Didichenko N, McCaffery P, Langen H, Dahinden CA. Human basophil
activation by mast cell-derived IL-3 express retinoldehyde dehydrogenase-II and pro-
32. Stansfield SK, Pierre-Louis M, Lerebours G, Augustin A. Vitamin A supplementation
and increased prevalence of childhood diarrhoea and acute respiratory infections.


