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Review

Regulation of CD8$^+$ T cell functions by RARγ

Claire Gordy, Ivan Dzhagalov, You-Wen He*

Department of Immunology, Duke University Medical Center, Durham, NC 27710, United States

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ABSTRACT

Retinoic acid plays a key role in the development and function of the immune system; however, the contribution of each of the three retinoic acid receptors (RARs) to the T cell immune response is not yet well understood. Of these receptors, both RARα and RARγ are expressed in T lymphocytes. While possible functional redundancy thus complicates understanding of the role of each receptor in T cells, emerging data suggest that RARα and RARγ function differently in thymocyte development and that RARγ is required for both primary and secondary CD8$^+$ T cell immune responses.

1. Introduction

The role of vitamin A in immunity has long been appreciated, with reports as early as the 1920s that vitamin A deficiency causes atrophy of lymphoid organs [1]. The importance of vitamin A in preventing infection has been recognized in numerous studies, and vitamin A supplementation is considered one of the major public health measures worldwide [2]. That vitamin A derivatives (referred to as retinoids) can modulate cell-mediated immunity was reported in the 1970s; however, the complex mechanisms by which retinoids regulate T cell development and function are only beginning to be elucidated [3]. This review will focus on the effects of retinoids, and in particular, retinoic acid receptor γ, on CD8$^+$ T cells.

2. RAR and RXR families

Vitamin A (retinol) is intracellularly metabolized to form compounds known as retinoids. Derivatives of vitamin A, including all-*trans*-retinoic acid and 9-*cis*-retinoic acid, are recognized by members of two families of nuclear receptors: the retinoic acid receptors (RARs) and retinoic acid X receptors (RXRs) [4]. The three members of the RAR family, RARα, RARβ, and RARγ, are encoded by distinct genes, and bind both all-*trans*-retinoic acid and 9-*cis*-retinoic acid, while members of the RXR family bind only 9-*cis*-retinoic acid [5–9]. Each of the three RAR subtypes are present as multiple isoforms differing in the 5′-untranslated region and N-terminal A region generated from differential promoter usage and alternative mRNA splicing [10–15].

Heterodimers formed between RARs and RXRs regulate transcription in a ligand-dependent manner. RAR/RXR heterodimers constitutively bind retinoic acid response elements (RAREs), and in the absence of ligand, recruit corepressor proteins to inhibit transcription of target genes [16–26]. Upon RAR ligand binding, corepressors are displaced, allowing heterodimers to instead recruit coactivators, resulting in transcriptional activation [24–26].

3. RAR expression in lymphocytes

During embryonic development, RARα is nearly ubiquitously expressed, while the expression patterns of RARβ and RARγ are much more limited [27,28]. In adult mice and humans, RARγ expression is largely restricted to the skin, while RARβ is expressed in the cerebral cortex, prostate, and kidneys [29–33].

The expression of the various RAR isoforms has been examined in human and mouse lymphocytes and is summarized in Tables 1 and 2. RARα1 and RARγ1 are constitutively expressed in human T and B cells, while RARβ2 expression is only detected following treatment with all-*trans*-retinoic acid [34]. RARγ2 is expressed only at low levels in human lymphocytes, and its expression is not induced by all-*trans*-retinoic acid, while RARβ1 and RARβ3 are not expressed in T or B cells under any conditions [34]. Similarly, RARα and RARγ, but not RARβ, are expressed by T cell hybridomas as well as murine thymocytes and thymic stromal cells [35–37]. Expression of RARα begins in thymocytes by day 17 of embryonic development, while RARγ is not expressed, or is expressed only at very low levels prior to birth [38].

Abbreviations: RAR, retinoic acid receptor; RXR, retinoic acid X receptor; RARE, retinoic acid response element; DP, CD4$^+$ CD8$^+$ double positive; SP, single positive; RALDH, retinaldehyde dehydrogenase; DN, double negative; rLmOVA, OVA-expressing *L. monocytogenes*; IEL, intestinal epithelial lymphocyte; LPL, lamina propria lymphocyte.

* Corresponding author at: Box 3010, Department of Immunology, DUMC, Durham, NC 27710, United States. Tel.: +1 919 613 7870; fax: +1 919 684 8982.
E-mail address: he000004@mc.duke.edu (Y.-W. He).

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Table 1
Expression of RARα, RARβ, and RARγ in thymocytes, peripheral T cells, and thymic stromal cells

	RARα	RARβ	RARγ
Fetal thymocytes	+	–	–
Postnatal thymocytes	+	–	+
Mature CD4 T cells	+	–	+
Mature CD8 T cells	+	–	+
Thymic stroma	+		+

Postnatal RARγ expression is differentially regulated throughout T cell development. $CD4^+$ $CD8^+$ double positive (DP) thymocytes express low levels of RARγ [39]. While expression increases in both $CD4^+$ and $CD8^+$ single positive (SP) thymocytes, $CD8^+$ SP thymocytes express two- to three-fold more RARγ than $CD4^+$ SP thymocytes [39]. Expression continues to increase in mature $CD4^+$ T cells; however, their expression level remains less than that of mature $CD8^+$ T cells [39].

4. RARγ and T Cell development

Vitamin A deficiency has long been known to cause thymic atrophy [1]. Further evidence for a role for retinoids in the thymus is provided by the observation that endogenous RAR ligands are generated from vitamin A in the thymus through a retinaldehyde dehydrogenase (RALDH)-dependent metabolic pathway [40]. Based on the results of both *in vitro* and *in vivo* studies, several potential functions for retinoic acid in thymocyte development have been proposed.

First, retinoic acid inhibits TCR-mediated thymocyte apoptosis *in vitro* [41]. In agreement with this finding, retinoic acid has been shown to inhibit negative selection of thymocytes. All-*trans*-retinoic acid or 9-*cis*-retinoic acid supplementation results in the development of increased numbers of SP thymocytes in fetal thymic organ culture [38]. This increase in mature thymocytes appears to be due to inhibition of negative selection, as all-*trans*-retinoic acid supplementation blocked superantigen-mediated deletion of $CD4^+$ $V\beta3^+$ thymocytes and partially prevented the SEB-mediated deletion of $V\beta6^+$ and $V\beta8^+$ thymocytes [38]. Similarly, retinoic acid inhibits negative selection of H-Y TCR transgenic thymocytes cultured with male thymic stromal cells, with 9-*cis*-retinoic acid showing a 10-fold more potent effect than all-*trans*-retinoic acid [42].

These effects appear to be mediated by an RARα/RXR-dependent pathway, and antagonized by an RARγ-dependent pathway. The greater potency of 9-*cis*-retinoic acid suggests that RXRs may be the relevant receptor in inhibiting thymocyte death; however, although addition of an RXR-specific agonist decreased the concentration of all-*trans*-retinoic acid required for inhibition of apoptosis, very high doses of this agonist were required to replicate the effects of 9-*cis*-retinoic acid [43]. A role for RARα was demonstrated by the ability of RARα-specific agonists to efficiently inhibit activation-induced thymocyte apoptosis both *in vitro* and *in vivo* [43]. Furthermore, addition of RARα-specific antagonists blocked the protective effects of retinoic acid [43]. RARγ-specific agonists, on the other hand, enhanced activation-induced thymocyte apoptosis, and RARγ-specific antagonists reduced the concentration of all-*trans*-retinoic acid necessary for inhibition of apoptosis [43]. Together, these results suggest that RARα is required for retinoic acid-mediated inhibition of apoptosis, and that RXR ligand binding counteracts antagonism from RARγ.

Table 2
Relative expression levels of RARγ throughout T cell development

	RARγ
DP thymocytes	+
$CD4^+$ SP thymocytes	++
$CD8^+$ SP thymocytes	++++
Mature $CD4^+$ T cells	+++
Mature $CD8^+$ T cells	++++

The ability of RARγ to cause thymocyte apoptosis has been documented both *in vitro* and *in vivo*. In contrast to observations in TCR-stimulated thymocytes, addition of either all-*trans*-retinoic acid or 9-*cis*-retinoic acid to unstimulated cultures of either whole thymocytes or sorted DP thymocytes results in increased apoptosis [36]. This increase in apoptosis appears to be mediated by RARγ, as RARγ-specific agonists, but not RARα-specific agonists were able to replicate the effects of retinoic acid [36]. The ability of an RARγ-mediated pathway to cause thymocyte apoptosis was confirmed *in vivo*, as treatment of mice with an RARγ-specific agonist resulted in rapid thymic involution largely due to loss of DP thymocytes [36].

The mechanism by which RARγ enhances apoptosis has not been examined in thymocytes. However, studies in T cell hybridomas suggest that RARγ ligation results in nur77-mediated upregulation of cell surface FasL, while RARα ligation inhibits FasL expression by blocking the ability of nur77 to bind DNA, and also inhibits activation-induced up-regulation of the proapoptotic Bcl2 family member Bim [37,44,45].

The role of RARγ in thymocyte development has been further examined using genetic models allowing either overexpression or deletion of RARγ. Notably, no changes in thymic cellularity or apoptotic rates were reported in either model, possibly due to differences in concentration of exogenous ligand used in previous studies from physiologic levels present in the thymus [39,46].

Mice expressing a human RARγ transgene in T cells display alterations in thymocyte development, with an increase in CD8 T cells in both the thymus and the periphery [46]. These results, along with the increased expression of RARγ observed in $CD8^+$ SP thymocytes, suggest that RARγ may play a role in the CD4/CD8 lineage decision. However, T cell development is unaffected in the absence of RARγ. Mice lacking RARγ in hematopoietic cells have normal thymic cellularity, and the frequency of DN1-DN4, DP, and $CD4^+$ and $CD8^+$ SP thymocytes are similar to those in wild type mice [39]. Furthermore, normal numbers of mature $CD4^+$ and $CD8^+$ T cells were observed in the periphery [39]. While these data indicate that RARγ is not required for normal T cell development, it is possible that the presence of RARα may compensate for loss of RARγ in some functions. To better understand the function of each receptor in T cell development, analysis of RARα-deficient and RARα/RARγ double-deficient mice, along with *in vivo* administration of selective inhibitors of each RAR will be necessary.

5. RARγ and the CD8 T cell response

Early studies demonstrated that although high doses of retinoic acid cause a dramatic reduction in thymic and splenic cellularity, lower doses enhance cell-mediated immune responses [3]. *In vivo* treatment with low doses of retinoic acid resulted in a 10-fold increase in cytotoxicity of splenocytes, but did not enhance mitogen-induced splenocyte proliferation [3]. While the functions of retinoic acid in cell-mediated immunity have not been completely elucidated, RARγ has been implicated in promoting the $CD8^+$ T cell response.

Although the absence of RARγ has no effect on T cell development, $CD4^+$ T cell polarization, or T-dependent humoral immune response, mice lacking RARγ in hematopoietic cells (referred to hereafter simply as RARγ-deficient) mount a defective $CD8^+$ T cell