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Small-molecule ROR γ t Antagonists: One Stone Kills Two Birds

Chao Zhong^{1,2} and Jinfang Zhu^{1,*}

¹Molecular and Cellular Immunoregulation Unit, Laboratory of Immunology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892, USA, USA

²Institute of Systems Biomedicine, School of Basic Medical Sciences, Peking University Health Science Center, Beijing, China

Abstract

Although small-molecule ROR γ t antagonists suppressing Th17 cells are widely reported, the effect of these molecules on other ROR γ t-expressing cells is unknown. A new study reported that ROR γ t inhibition in CD4⁺CD8⁺ thymocytes resulted in skewed T cell repertoire, contributing to reduction in frequency of self-reactive T cells and resistance to autoimmunity.

Interleukin (IL)-17-producing T helper (Th17) cells are critically involved in many forms of organ-specific autoimmune disorders and chronic inflammations. IL-23 is essential for the development and/or activation of Th17 cells. Therefore, IL-23 and IL-17, as well as their receptors, have been considered as therapeutic targets for treating Th17-mediated diseases¹. In fact, antibodies against IL-23p19, IL-23p40, IL-17A and IL-17RA have shown promising efficacy in treating many autoimmune diseases, including psoriasis, ankylosing spondylitis, and multiple sclerosis, in clinical trials. However, blockage of IL-17A or IL-17RA is ineffective in treating Crohn's disease. This is possibly because that besides IL-17A, several other effector cytokines, such as IL-17F, IL-22, and GM-CSF, produced by Th17 cells may also play critical roles in inflammation; blockade of one particular cytokine may not be sufficient to repress Th17-mediated inflammation.

Th17 cell differentiation is regulated by a group of important transcription factors, including truncated form of retinoic acid receptor-related orphan nuclear receptor γ (ROR γ t), signal transducer and activator of transcription 3 (STAT3), Interferon regulatory factor 4 (IRF4), and BATF. Among these critical factors, only ROR γ t is specifically expressed by Th17 cells among the T helper effector cells and thus is regarded as the master regulator of Th17 cells². Similar to other members of the nuclear hormone receptor family, ROR γ t has DNA- and ligand-binding domains (DBD and LBD)³. Given that ROR γ t is the master regulator of Th17 cells, and that its activity could be modulated by cost-effective small molecules, inhibiting ROR γ t's transcriptional activity by targeting either domain could be a superior

*Correspondence: jfzhu@niaid.nih.gov.

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alternative to antibody-mediated cytokine and/or receptor neutralization in treating Th17-related autoimmune diseases.

In addition to Th17 cells, many other mature lymphocytes, including type 3 innate lymphoid cells (ILC3s), CD8⁺ IL-17-producing (Tc17) cells, and subsets of $\gamma\delta$ T, natural killer T (NKT), regulatory T (Treg) cells, both in human and in mice, express ROR γ t (Figure 1). Furthermore, during T cell development, CD4⁺CD8⁺ double positive (DP) thymocytes express ROR γ t, and ROR γ t promotes the survival of DP thymocytes partly through promoting the expression of anti-apoptotic molecule Bcl-x_L.

Several ROR γ t antagonists or inverse agonists, such as digoxin, SR1001 (targeting both ROR γ t and ROR α), TMP778, GSK805, and MRL-871, have been identified through independent efforts of many groups in drug screening and testing^{3, 4, 5, 6, 7}. MRL-871 binds to a non-canonical site at the LBD of ROR γ t and induces an aberrant conformational change at the LBD, which blocks the binding of co-activators to ROR γ t. Therefore, even without altering ROR γ t DNA binding activity, an allosteric antagonist can still inhibit the transcriptional activity of ROR γ t. While the impact of ROR γ t inhibitors on Th17 cells has been extensively studied, their effects on other ROR γ t-expressing cells are still elusive.

Cua and colleagues recently reported that pharmacological inhibition of ROR γ t by allosteric ROR γ t antagonists, MRL-871 or MRL-248 (structurally similar to MRL-871 but with improved *in vivo* pharmacodynamics) resulted in rapid DP thymocyte apoptosis within 3 days and immediate changes in gene expression within 2 hours of drug treatment⁸. Through RNA-Seq analysis of changes in gene expression in DP cells shortly after ROR γ t inhibition (2 hr) or with ROR γ t-overexpression, the authors identified 33 genes, including *Bcl-x_L*, *Plxnd1* and *Sla*, that are known to be involved in cell apoptosis and survival, cell migration and positive selection, respectively. Therefore, besides maintaining the survival of DP thymocytes, ROR γ t has a broad role in regulating many other pathways during thymopoiesis. MRL-248 was also very effective in inhibiting IL-23-mediated skin inflammation, although it is not clear whether the induction of ROR γ t-expressing CD3⁺ T cells was blocked or these ROR γ t-expressing T cells failed to produce Th17-related effector cytokines with MRL-248 treatment in this model. Nevertheless, small-molecule ROR γ t inhibitors not only suppress the differentiation and/or functions of IL-17-producing T cells, but also regulate thymopoiesis (Figure 1).

Consistent with the previous reports using ROR γ t-deficient DP cells⁹, ROR γ t inhibition also led to a reduced expression of anti-apoptotic factor Bcl-x_L and shortened survival, which is presumably responsible for skewed TCR α -chain rearrangement with J region usage towards the proximal regions. In the absence of functional ROR γ t, many sequential productive rearrangement events using the distal J regions may not have enough time to occur before the DP thymocytes with functional TCRs can be positively selected by the major histocompatibility complex (MHC). Therefore, ROR γ t inhibition results in a limited T cell repertoire. These effects of ROR γ t antagonists on DP thymocytes appear ROR γ t-specific, since inducible deletion of *Rorc* (gene encoding ROR γ t) also led to shortened lifespan of DP thymocytes, reduced expression of Bcl-x_L, skewed TCR α V-J usage towards the proximal regions and reduced T cell repertoire. Skewing of TCR α usage by ROR γ t

inhibition may also interfere the development of other lymphocytes including regulatory T cells and cells with an invariant TCR, including NKT cells and mucosal associated invariant T (MAIT) cells.

Interestingly, ROR γ t inhibition resulted in reduced frequency of myelin oligodendrocyte glycoprotein (MOG)-specific “self-reactive” CD4⁺ T cells in approximately half of the ROR γ t antagonist-treated mice⁸. Consequently, ROR γ t antagonist pre-treatment delayed the autoimmune progression in the experimental autoimmune encephalomyelitis (EAE) model induced by MOG immunization, even though the ROR γ t antagonist was no longer present during the course of disease induction. Therefore, in addition to suppressing the differentiation and function of Th17 cells, ROR γ t antagonists can also ameliorate autoimmunity by restricting the number and repertoire of self-reactive thymic emigrants. However, whether this is true for other self-reactive CD4⁺ T cells requires further investigation. Furthermore, although the contraction of T cell repertoire may reduce the risk of some autoimmune diseases, it could potentially increase susceptibility to cancer and some infections.

ILC3s, which also express ROR γ t, play important roles in host defense and tissue homeostasis. Another recent report indicates that transient inhibition of ROR γ t does not cause reduction in IL-22 production by ILC3s or impairment of ILC3-mediated protective immunity, suggesting that ROR γ t inhibition can effectively target Th17-induced inflammatory bowel diseases (IBD) or other chronic inflammations in the gut¹⁰. The effects of ROR γ t antagonists on ROR γ t-expressing $\gamma\delta$ T, NKT and Treg subsets still require further investigation. Th17 cells and peripherally induced Tregs (pTregs) share a developmental program triggered by TGF β , and there is an intermediate phase of Th17/pTreg differentiation when both Foxp3, the master regulator of Tregs, and ROR γ t are co-expressed in the same cell. Because of the antagonism between Foxp3 and ROR γ t, inhibition of ROR γ t activity by small molecules may divert the differentiation of some newly activated auto-reactive CD4⁺ cells from Th17 cell fate to pTreg fate, which may lead to a permanent cure of autoimmune diseases without a continuous drug treatment. This possibility should be further explored in the future.

In conclusion, the study of Cua and colleagues has shed light on an important effect of ROR γ t antagonists on thymopoiesis. ROR γ t inhibition by small molecule inhibitors recapitulates the effect of *Rorc* deficiency in many aspects, including skewed TCR α rearrangement and reduced T cell repertoire diversity. They also found several previously unknown functions of ROR γ t in DP thymocytes. In addition, their interesting results provide a novel view of explaining the complex mechanism through which ROR γ t antagonists may modulate autoimmune diseases. However, it also raises several outstanding issues that need to be addressed in the future. To avoid the complexity of multiple effects of ROR γ t antagonists on different cell types some of which may be deleterious, one could possibly take advantage of the rapidly developing nanomedicine to deliver ROR γ t inhibitors by nanoparticles to specific cell types such as Th17 cells in treating autoimmune diseases.

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Figure 1.

Small-molecule ROR γ t Antagonists not Only Inhibit the Differentiation and Functions of Th17 cells, but also Regulate Thymopoiesis. Besides Th17 cells, CD8⁺ IL-17-producing (Tc17) cells, type 3 innate lymphoid cells (ILC3s), and subsets of $\gamma\delta$ T, NKT, regulatory T (Treg) cells also express ROR γ t. In the thymus, ROR γ t is expressed by CD4⁺CD8⁺ double positive (DP) thymocytes. Some other lymphoid progenitors such as ILC progenitors may also express ROR γ t during development. It is well known that pharmacological inhibition of ROR γ t by allosteric ROR γ t antagonists suppresses Th17/Tc17 differentiation and function. Small-molecule ROR γ t antagonists also inhibit IL-17 production by ROR γ t-expressing $\gamma\delta$ T cells. In DP thymocytes, these small molecules suppress the expression of ROR γ t target genes that are involved in cell apoptosis and survival, cell migration and positive selection, which leads to shortened lifespan of DP thymocytes, reduced expression of Bcl-x_L, skewed TCR α V-J usage towards the proximal regions and reduced T cell repertoire. As a result, ROR γ t inhibition reduces the frequency of “self-reactive” CD4⁺ T cells and renders the host resistant to autoimmune disease induction. However, transient inhibition of ROR γ t leaves

the functions of ILC3s intact. The effect of small-molecule ROR γ t antagonists on other ROR γ t-expressing cells requires further investigation. In particular, it is intriguing to know whether ROR γ t inhibitors can regulate the balance between self-reactive Th17 cells and pTregs.