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Review

Retinoid-related orphan receptor γ (RORγ) adult induced knockout mice develop lymphoblastic lymphoma

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ABSTRACT

RORγ is a nuclear hormone receptor which controls polarization of naive CD4$^+$ T-cells into proinflammatory Th17 cells. Pharmacological antagonism of RORγ has therapeutic potential for autoimmune diseases; however, this mechanism may potentially carry target-related safety risks, as mice deficient in *Rorc*, the gene encoding RORγ, develop T-cell lymphoma with 50% frequency. Due to the requirement of RORγ during development, the *Rorc* knockout (KO) animals lack secondary lymphoid organs and have a dysregulation in the generation of CD4+ and CD8+ T cells. We wanted to extend the evaluation of RORγ deficiency to address the question whether lymphomas, similar to those observed in the *Rorc* KO, would develop in an animal with an otherwise intact adult immune system. Accordingly, we designed a conditional RORγ knockout mouse (*Rorc* CKO) where the *Rorc* locus could be deleted in adult animals. Based on these studies we can confirm that these animals also develop lymphoma in a similar time frame as embryonic *Rorc* knockouts. This study also suggests that in animals where the gene deletion is incomplete, the thymus undergoes a rapid selection process replacing *Rorc* deficient cells with remnant thymocytes carrying a functional *Rorc* locus and that subsequently, these animals do not develop lymphoblastic lymphoma.

Contents

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1. Introduction

1.1. T helper cell 17 and its regulator RAR-related orphan receptor γ

T helper cells (Th) have traditionally been divided into Th1 and Th2 [1] but a succession of papers subsequently demonstrated that a third major Th cell class existed [2]. This Th cell subset was shown to be dependent upon interleukin 23 (IL-23) stimulation [3] and produced IL-17 in response to antigen recognition, hence it was named Th17. The differentiation of this cell type is dependent upon the transcription factor RAR-related orphan receptor gamma (RORγ) [4]. RORγ is a member of the large nuclear hormone receptor (NHR) family, many of which are controlled by endogenous ligands, and as such the NHR family has attracted considerable attention as drug targets [5,6]. RORγ, together with its close neighbors RORα and RORβ, are considered orphan receptors where no single endogenous ligand has yet been identified. Several oxy-sterols have been shown to bind and modulate RORγ transcription [7–10] by recruiting co-activators and co-repressors. However, RORγ is considered to be constitutively active in vivo, perhaps due to the ubiquitous presence of endogenous ligands such as the oxy-sterols. The ROR family has a DNA binding domain which directs the specificity of the transcription through binding to retinoic-related receptor enhancer sequences (RORE) and thereby takes part in regulating important processes such as embryonic development, cellular metabolism, circadian rhythm, and immunity [11]. RORα is predominately expressed in the brain, whereas RORβ is expressed mainly in the brain and the liver. The RAR-related orphan receptor C (*RORC*) locus encodes two major splice forms both in mouse and man, RORγ and RORγt. Both isoforms share the same ligand and DNA binding domains, only differing from each other in the N terminal region where RORγ is 21 amino acids longer. No known functional difference has been identified between these two isoforms. However, the transcription of each splice form is regulated through a unique promoter sequence, resulting in the expression of RORγ in several tissues, such as muscle, kidney, and liver while RORγt expression is restricted to cells of the immune system, including the thymus [12].

RORγt is essential for the development of T cells in the thymus [13,14] where it is expressed at the double-positive ($CD4^+CD8^+$) stage as part of the positive selection mechanism. RORγt has also been suggested to be involved in the regulation of negative selection before mature naive T cells finally leave the thymus [12,15]. When these naive T cells first encounter their specific antigen, presented to them by a dendritic cell, they become activated and simultaneously receive signals directing them to polarize into a specific class of T helper cells. IL-23 together with other cytokines such as TGFβ and IL6 [16–18] promotes Th17 polarization. Th17 cells mainly up-regulate RORγt but also RORγ [19] and will thereby gain the ability to produce IL-17A, IL-17F, IL-21, IL-22, and other proinflammatory effector cytokines [4,20].

1.2. RORγ/γt as a potential therapeutic target in autoimmune disease

Genetic evidence suggests a causative involvement of the IL-23 immune axis in autoimmune diseases [21] such as psoriasis [22], ankylosing spondylitis, psoriatic arthritis, Crohn's disease, and ulcerative colitis. Other diseases such as multiple sclerosis, rheumatoid arthritis, and celiac disease are also genetically associated with the IL-23 axis but to a lesser degree. For some diseases, such a genetic link has not been established to date but a correlation between Th17 cytokines and markers of disease progression has been identified for example in lupus nephritis [23], graft versus host disease [24], and Sjögren's syndrome [25]. RORγ/γt might therefore be expected to have a central role in driving these pathologies, thus making it an attractive target for small molecule pharmaceutical intervention [26]. It is worth noting that since the ligand-binding domains are identical in RORγ and RORγt, a synthetic small-molecule ligand is unlikely to differentiate between the isoforms.

Clinical validation of this pathway comes from the therapeutic efficacy of antibodies that neutralize Th17-associated cytokines and receptors (Secukinumab, Ixekizumab-IL-17, Ustekinumab-IL12/23p40, Guselkumab, Tildrakizumab, BI655066-IL23p19, and Brodalumab-IL-17RA) [27,28]. A RORγ/γt small molecule inhibitor could have multiple benefits over neutralizing antibodies for the patient. Oral administration is generally preferred over the parenteral routes of administration required for antibodies and also offers the possibility to adjust dose in response to the therapeutic effect. In addition, the more rapid clearance of oral small molecules allows for treatment to be discontinued in the event of an infection requiring an effective Th17 response, for example, *Mycobacterium tuberculosis* or *Candida albicans*.

1.3. The Rorc knockout

Abnormal expression of molecules involved in T cell development have the potential to alter immune homeostasis and have been suggested to lead to the appearance of hematological malignancies in genetically susceptible hosts [30]. *Rorc* KO mice have been shown to develop T-cell lymphoma at a high incidence [31] with a concomitant dramatic drop in survival after 4–6 months of age. *Rorc* KO mice have a complete lack of RORγ/γt from conception onwards, resulting in a compromised development of the immune system. For example, lymphoid tissue inducer cells (LTi) are dependent upon RORγ/γt for their role [32] in directing the formation of lymph nodes and Peyer's patches [13] which are absent in the *Rorc* KO mice. T cell development is also dysregulated in these KO mice resulting in lymphopenic animals. The effect is evident on both helper T cells ($CD4^+$) and cytotoxic T cells ($CD8^+$) [13].

However, the *Rorc* KO animal may display a different phenotype than what would be observed using pharmacological intervention, particularly since RORγ/γt is active during fetal development. To this end, we wanted to extend the evaluation of RORγ/γt deficiency to address the question whether lymphomas, similar to those observed in the *Rorc* KO [31], would develop in an animal with an intact, fully developed immune system. Accordingly, we designed a conditional RORγ/γt knockout mouse (*Rorc* CKO) where the *Rorc* locus can be deleted in adult animals. Based on these studies, we can confirm that *Rorc* CKO animals also develop lymphoma in a similar time frame as embryonic *Rorc* knockouts. This study also indicates that in animals where the gene deletion is incomplete, the thymus undergoes a rapid selection process