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RESEARCH ARTICLE

Unraveling the Challenges in the Retinoid-related Orphan Receptor (ROR)-γ(t) Therapeutic Landscape for Autoimmune Diseases

Daliya Banerjee[1]*

[1]Nirogy Therapeutics

***Corresponding email:** daliya.banerjee@gmail.com

ABSTRACT:
RORγt/γ driven type-17 "pro-inflammatory "gene expression profile has been implicated in the pathogenesis of several human immune-mediated diseases and remains a compelling target for therapeutic intervention. However, several challenges, from both drug discovery and biology standpoint, have made it intractable. While biologics targeting IL-23, IL-17, IL-17R, and other cytokines in the RORγ pathway have demonstrated clinical efficacy in psoriasis, ankylosing spondylitis, etc., it has been challenging to extend the utility of these therapeutics beyond a handful of autoimmune diseases, especially in atopic or rheumatic diseases. This review summarizes the complexities in defining the pathogenicity of the human type-17 pathway and underscores the need for targeting a phenotypically heterogeneous and therapeutically relevant pathogenic cell subset marked by CD161.

Introduction

IL-17-producing lymphocytes include conventional $CD4^+$ T_h (helper)17 cells, γδ T cells, $CD4^-CD8^-$ double negative T cells, $CD8^+$ MAIT (Mucosal-associated invariant T) cells, and LTi (lymphoid tissue inducer cells). They commonly express the nuclear receptor RORγ that controls a "Type-17 (T-17)" pro-inflammatory gene expression program in these lymphocyte population (Ciofani et al., 2012; Cua & Tato, 2010; Korn, Bettelli, Oukka, & Kuchroo, 2009). T-17 cells are regarded as pathogenic in various autoimmune diseases based on both extensive pre-clinical research work and clinical evidence in patients. Genome-wide association studies have linked STAT3 (Signal transducer and activator of transcription 3) and IL23R (interleukin-23 receptor)- key regulators of RORγ-dependent responses- to multiple human autoimmune diseases, including psoriasis, inflammatory bowel disease, rheumatoid arthritis and ankylosing spondylitis (Cenit et al., 2010; Duerr et al., 2006; Nair et al., 2009; Stahl et al., 2010). In addition, there is strong preclinical validation in mouse models where disruption of the RORγ pathway in mice reduces or eliminates disease in multiple models of autoimmune pathology. More importantly, biologics targeting IL-17A/F, IL-23, and p40 (a component of IL-12/IL-23) that promote T-17/T_h1 mediated inflammation are approved in pathologies like psoriasis, psoriatic arthritis, ankylosing spondylitis and Crohn's disease (Gara et al., 2023; Hueber et al., 2010; Leonardi et al., 2008; Schinocca et al., 2021). However, it has been challenging to extend the utility of these therapeutics beyond a handful of autoimmune diseases, especially in atopic or rheumatic diseases like allergies, asthma, rheumatoid arthritis (RA), primary Sjogren's syndrome (pSS), lupus, etc. Thus, pathogenic T-17 cells have a clinically validated role in autoimmunity, but there are limitations. It is anticipated that therapies that block "all T-17" function would be beneficial in treating a larger array of autoimmune and inflammatory pathologies than targeting individual cytokines alone. While attempts to therapeutically target RORγ- the master T-17 regulator - have proven to be futile, there is a need to understand the complexity of the T-17 pathway and identify alternative approaches. This review provides some insight into the challenges and complexities in determining the pathogenicity of the T-17 pathway in autoimmunity and warrants an evaluation of CD161-expressing lymphocytes as targets since CD161-marked cells embody a diverse subset of pathogenic T cells enriched in various autoimmune and inflammatory disorders.

The current T-17 pathway therapeutic landscape and its challenges

Neutralizing antibodies for IL-17 (e.g., secukinumab and ixekizumab) and IL-17R blocking antibody (brodalumab), are effective in treating psoriasis, psoriatic arthritis, and ankylosing spondylitis. Biologics blocking T_h1/T_h17 differentiation axis that includes the anti-IL-12/IL-23p40 antibody (Ustekinumab), and anti-IL-23p19 antibodies (guselkumab, risankizumab, and tildrakizumab) have been very effective beyond psoriasis, e.g., in Crohn's disease. While effective and safe in treating a handful of pathologies, these biologics are very costly and require frequent injection. This prompted a search for an orally bioavailable, safe inhibitor to broadly target T-17 function. Oral Janus kinase (JAK) inhibitors (e.g., tofacitinib, baricitinib, upadacitinib), targeting cytokine signaling including the T-17 axis, work in various inflammatory settings but fatal complications with toxicity led to a black box warning by U.S. Food and Drug Administration (US-FDA) (Kragstrup et al., 2022). RORγ, being the master regulator of pathogenic T-17 differentiation and function, has been a highly sought-after target by several pharmaceutical companies for more than a decade. However, there are several challenges both from a drug discovery and biology standpoint (Zeng et al., 2023). The highly lipophilic ligand binding pocket of RORγ, together with incredibly high exposures needed in the blood to suppress robust cytokine responses from innate IL-17 producing cells (with pre-existing RORγ transcriptional complexes), made it challenging to identify a potent molecule with good drug-like properties. Additionally, thymic toxicity was observed by various companies in their pre-clinical studies due to the role of RORγ in thymocyte maturation and viability (Haggerty et al., 2021). This led to rapid attrition of molecules from Phase I/II clinical trials, including a recent one – cedirogant (AbbVie/Inventiva), thereby rendering RORγ largely intractable. Therefore, there is an urgent need to identify alternative targets and

approaches to abrogate the T-17 pathway for the treatment of other autoimmune and inflammatory disorders.

Complexities in defining pathogenicity of T-17 cells in different autoimmune diseases- a perspective on RA.

Several lines of evidence in experimental mouse models of RA, like genetic deficiency of IL-17A and IL-23p19 or antibody-mediated IL-17 blockade and neutralizing of IL-23p19, protected the animals from the development of collagen-induced arthritis (CIA) (Zeng et al., 2023), thereby implying T_h17 associated inflammatory mediators to play a major role in the pathogenesis of RA. However, clinical trials conducted in RA patients with IL-17 blocking antibodies improved clinical signs, but the effects were marginal compared to other therapeutic strategies like blocking TNF-α (Tumor Necrosis Factor-α) or IL-6 signaling pathways (Kunwar, Dahal, & Sharma, 2016). Similarly, treatment with IL-23p19 or IL-12/23p40 antibodies had no clear beneficial clinical effect in RA patients (Smolen et al., 2017) akin to the lack of efficacy observed in mice when dosed therapeutically after the onset of disease in experimental arthritis models (Cornelissen et al., 2013; Kotake et al., 2016). While the discrepancy between efficacy in pre-clinical animal studies and its lack thereof in patients is not entirely clear, there are several possibilities: 1) IL-17 is not the only mediator of T-17 mediated pathogenicity and there are other players as well. 2) Inhibiting T_h17 differentiation may not be enough in certain diseases. It's important to block all responses that may arise from either memory T_h17 or innate-like T-17 lymphocytes that can respond directly to cytokines like IL-1/IL-18 etc. While murine T_h17 cells primarily develop via *de novo* differentiation from naïve precursors in response to IL-6, IL-1, and transforming growth factor (TGF)-β, all human T-17 cells originate from $CD161^+$ precursors present in umbilical cord blood and newborn thymus. In adults, the majority of circulating human $CD161^+$ T-17 cells belong to the memory T cell subset (Cosmi et al., 2008; Maggi et al., 2010). Thus, it is possible that certain human T-17 driven pathologies may rely less on *de novo* differentiation and more on innate-like T-17 responses that are much more rapid and robust (Annunziato & Romagnani, 2009). 4) T-17 cells show plasticity and studies in both mice and humans have shown that more pathogenic T-17 cells tend to shift to T_h1 like phenotype (Annunziato, Cosmi, Liotta, Maggi, & Romagnani, 2012; Nistala et al., 2010). IL-17-producing T_{fh} (follicular helper) cells are better inducers of antibodies from B cells and their levels are elevated in autoimmunity (J. Zhao et al., 2018). Additionally, increased frequency of IL-17–producing T_H2 cells has been detected in patients with atopic asthma (Cosmi et al., 2010). Thus, the cytokine milieu of the diseased tissues may facilitate the transition of T-17 cells to other T helper cell phenotypes including T_H1, T_{fh}, T_H2, or T_{reg}-like. Unless T-17 cells stably maintain their identity upon migration into inflamed tissues, like the joints (RA) or salivary glands (primary Sjogren's syndrome), IL-17 or IL-23 blockade alone may not be enough to ameliorate disease pathology.

Thinking beyond RORγ - CD161 embodies a diverse subset of pathogenic T cells enriched in various autoimmune and inflammatory disorders

A large body of published work has unequivocally identified CD161/KLRB1, originally a marker of NK cells, to specifically define all human T-17 cells, including $CD8^+$, $CD4^+$, and TCRγδ that co-express RORγ, CCR6 and IL-23R and belong to memory phenotype (Fergusson et al., 2014). However, CD161 marker is not exclusive to RORγ expressing cells and encompasses other $ROR\gamma^-$ T cell phenotypes. For example, only 30% of circulating $CD161^+$ $CD4^+$ T express RORγ, whereas 80% of $CD161^{hi}$ $CD8^+$T (MAIT) cells are $ROR\gamma^+$ (Banerjee et al., 2016). All CD161 expressing lymphocytes possess a core transcriptional signature comprising of RORc, CCR6/CXCR6 (C-C chemokine receptor type 6/ CXC motif chemokine receptor 6), IL23R, IL12Rβ2 and IL18R1, and uniquely display a shared innate response to IL-12 and IL-18 (Fergusson et al., 2014). Thus, $CD161^+$ T cells are phenotypically heterogeneous and display diverse functions.

What makes $CD161^+$ T cell subsets therapeutically interesting?

In healthy normal individuals, $CD161^+$ T cells are present in the circulation and enriched in the gut but under inflammatory conditions, $CD161^+$ T cells

become pathogenic (polyfunctional) and migrate to the inflamed tissue to promote inflammation (Basdeo et al., 2015). $CD161^+$ T cells are enriched in RA synovial fluid and tissue and in the skin of psoriasis patients (Aguilar-Flores et al., 2020; Basdeo et al., 2015). Increased frequency of activated HLA-DR^+ $CD161^+CD4^+$T cells in the salivary glands and blood from primary Sjogren's Syndrome patients (L. Zhao et al., 2017). $CD161^+$ $CD4^+$ T cells are also abundant in the gut of Crohn's disease patients compared to control subjects (Jaeger et al., 2021; Yokoi et al., 2023). It will be interesting to understand the differential impact of $ROR\gamma^+$ and $ROR\gamma^-$ subsets of $CD161^+$ T cells in contributing to pathogenicity of diseases outlined above to better predict the efficacy of the current T-17 targeted therapies in this context. In 2017, Wambre et.al. and others have identified a subset of human pathogenic allergen specific memory T_H2A cells, enriched and confined to allergic individuals compared to nonatopic individuals that co-express chemoattractant receptor CRT_H2 and CD161 (Blom et al., 2017; Wambre et al., 2017). These $CD161^+$ CRT_H2^+- T_H2A cells express T_h2 cytokines instead of IL-17/IFNγ and represents a suitable therapeutic target. A direct correlation is observed between decrease in allergen-specific $CD161^+$ CRT_H2^+-T_H2A cell frequency and achievement of allergen desensitization induced by immunotherapy.

Conclusion

These studies underscore the importance of the pathogenicity of CD161 expressing heterogeneous T cell subsets in diverse inflammatory and autoimmune diseases and warrant the shifting of focus from RORγ/T-17 to $CD161^+$ T cells as a therapeutic target.

Conflict of interest: The author has no conflicts of interest to declare

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