Ror-gamma T inhibition as a Pharmacological Approach for Inflammatory Bowel Disease

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ABSTRACT

Retinoic Acid Related Orphan Nuclear Receptor gamma T (ROR-γT) is the lineage specifying transcription factor for IL-17 expressing cells. ROR-γT expression is up-regulated in various animal models of colitis, as well as in certain patients with Inflammatory Bowel Disease (IBD). Transfer of ROR-gamma null T cells to RAG1 deficient mice did not induce colitis in these animals. Recently, data with a specific small molecule inhibitor (VPR-7) of ROR-γT showed efficacy in a murine model of IBD. In principle, ROR-γT inhibitors would specifically inhibit Th17 cell differentiation and expansion. ROR-γT, which is functioning in innate intestinal lymphoid cells, may also be a target for ROR-γT inhibitors. These drugs would block the transcription of possible pro- inflammatory receptors (IL-23R) cytokines (IL-17, IL-21) and chemokines (CCL20) putatively involved in the pathogenesis of IBD. Off-target effects of ROR-γT inhibitors have been reported, including the inhibition of colonic IL-23 in a mouse model of colitis. Several pharmaceutical companies are actively involved in the development of specific inhibitors targeting ROR-γT. It is possible that ROR-γT inhibitors will enter clinical trials for the treatment of IBD in the near future.

Keywords – Ror-Gama T, Colitis, Mice, Inflammatory Bowel Disease

INTRODUCTION

Retinoic Acid Related Orphan Nuclear Receptor gamma T (ROR-γT) is the lineage specifying transcription factor for IL-17 expressing cells such as T-helper cell 17 (Th17), and gamma-delta (γδ) T cells (Zhou and Littman 2009, Kanai et al. 2012 and Fitzpatrick et al. 2015). Within the context of Inflammatory Bowel Disease (IBD), ROR- γT-dependent gene transcription in Th17 cells, as well as in innate intestinal lymphoid cells, may also contribute to the pathogenesis of intestinal inflammation (Buonocore et al. 2010, Eken et al. 2014 and Kanai et al. 2012). Generally, ROR-γT expression is up- regulated in the intestinal tract of patients with IBD (Dambacher et al. 2009 and Martin et al. 2015). These data are reviewed in more detail in section 1 of this manuscript.

Of note, the results from several pre-clinical studies, using animal models of colitis, have suggested that ROR-γT inhibition may be a useful pharmacological approach for IBD (Leppkes et al. 2009, Buonocore et al. 2010 and Fitzpatrick et al. 2015). These data, in addition to contradictory experimental evidence, are presented in section 2 of this paper. In this manuscript, the pharmacological rationale for ROR-γT inhibition as a therapeutic approach for Crohn's Disease (CD) and/or Ulcerative Colitis (UC) [i.e., IBD] will be depicted in a succinct fashion (see section 3). Interestingly, several pharmaceutical and biotech companies are developing specific inhibitors of ROR-γT (section 5), including selective inverse agonists, for the treatment of various autoimmune diseases (Xiao et al. 2014, Wang et al 2014 and Fitzpatrick et al. 2015). One of these prototype drugs (VPR-7) recently showed evidence of efficacy in a murine model of IBD (Fitzpatrick et al. 2015).

1. ROR-γT Expression in Human IBD

The up-regulation of key transcription factors in patients with IBD identifies these proteins as potential therapeutic targets for the disease [Fitzpatrick 2012, 2013 and Galvez 2014]. Table 1 contains several citations that reported increased intestinal ROR-γT expression in IBD patients.

Table1
Intestinal Ror-γT expression in Patients with IBD

Disease	Results	Reference
Crohn's Disease	Demonstrated the presence of Th17 cells, some of which produce both IL-17 and IFNγ in the gut of patients with CD. Both Th17 and Th17/Th1 clones showed selective expression of the transcription factor Ror-γT.	(Annuziato et al. 2007)
Crohn's Disease	Suggested an increased number of Th17 ROR-γT cells in active CD	(Dambacher et al. 2009)
Ulcerative Colitis	Increased ROR-γT expressing Th17 cells within the colonic tissue.	(Martin et al. 2015)

Crohn's Disease Ulcerative Colitis	Reported no increase in ROR-γT expression in IBD patients.	(Punkenburg et al. 2015)

In 2007, Annunziato and colleagues reported the presence of CD4+/Th17 cell populations in the intestines of patients with CD. Some of these cells also produced IFNγ and were identified as Th17/Th1 cells. Both Th17 and TH17/Th1 cells showed selective expression of ROR-γT, as well as its dependent genes [IL-23 receptor and CCR6] (Annunziato et al. 2007). More recently, other investigators suggested an increased number of Th17 cells containing ROR-γT and IL-26 in the colons of patients with active CD (Dambacher et al., 2009). A very recent manuscript found (by immunohistochemistry) a significantly increased number of ROR-γT expressing cells in the colons of patients with UC, or colitis-associated cancer (CAC), compared to a control cohort of patients (Martin et al. 2015). Interestingly, other investigators recently reported increased ROR-γT mRNA expression in PBMC's from patients with IBD. Particularly, there was a large increase in ROR-γT expression in patients with active UC (Dong et al., 2013).

In contrast to these results, another research group found no enhancement in colonic ROR-γT mRNA expression (by quantitative PCR) in patients with active IBD (CD or UC), as well as in those patients with colorectal cancer (Punkenberg et al. 2015). The reason(s) for these divergent results are currently unknown. However, different patient populations, as well as methods of ROR-γT quantification, were used in these studies (Martin et al. 2015 and Punkenberg et al. 2015).

1.1 ROR-γT Expression in Animal Models of IBD

Acute Dextran Sulfate Sodium (DSS) and Trinitrobenzenesulfonic Acid (TNBS) models of colitis are often used for testing of potential IBD drugs, including ROR-γT inhibitors (Fitzpatrick et al. 2015). Shen et al. reported that ROR-γT expression is up-regulated in conjunction with acute DSS-Induced colitis in mice (Shen et al. 2011). Other investigators found up-regulation of ROR-γT in association with a murine TNBS colitis model (Chen et al. 2013). More specifically, Almeida and colleagues found increased ROR-γT expression in both CD4+ T cells and innate lymphoid cells, with TNBS-induced colitis in mice (Almeida et al. 2015).

Innate models of colitis have been developed, and colonic ROR-γT expression has been evaluated by various investigators. Using a *Helicobacter hepaticus* colitis model, IL-23 was shown to mediate up-regulation of ROR-γT expression specifically in innate immune cells (Buonocore et al. 2010). Another group of investigators reported increased percentages of IL-23 responsive innate colonic lymphoid cells, when using the TRUC (T-bet-/- RAG2-/- Ulcerative Colitis) model of IBD (Ermann et al. 2014).

2. Inhibition of ROR-γT in Animal Models of IBD (Direct Evidence)

Table 2 summarizes some of the data from animal models of IBD, which tested the effectiveness of ROR-γT inhibition on various parameters of colitis.

Table 2

Ror-γT Inhibition in Animal Models of IBD

Model	Results	Reference
Mice/T Cell Transfer	Transfer of ROR-gamma null T cells to RAG1 deficient mice did not induce colitis.	(Leppkes et al. 2009)
Mice/alpha-CD40 AB	This innate colitis was decreased in ROR-/Rag- mice.	(Buonocore et al. 2010)
Mice/Acute TNBS	TNBS-induced colitis was improved by treatment with VPR-7 (a specific ROR-γT inverse agonist).	(Fitzpatrick et al. 2015)
Mice/Chronic DSS	ROR-γT deficient mice showed the same level of chronic DSS colitis as wild type (control) mice.	(Martin et al. 2015)
Mice/Chronic DSS	ROR-γT deficient mice showed worsened colitis.	(Lochner et al. 2011)
Mice/Acute DSS	In RAG 2/ ROR-γT double deficient mice, acute DSS colitis was lethal.	(Sawa et al. 2011)

A well designed study, using the T cell transfer model of colitis, showed a protective role against colitis with ROR-γT deficient mice (Leppkes et al. 2009). Specifically, the authors found that adoptive transfer of IL-17A, IL-17F and IL-22 deficient lymphocytes into RAG null mice caused colitis. In contrast, transfer of ROR-γT-deficient T cells failed to increase colonic IL-17 and did not cause colitis. The authors concluded that ROR-γT played a crucial role in this chronic murine colitis model. Moreover, based on their results, it was suggested that IL-17A and IL-17F play a redundant role in chronic gut inflammation. These investigators suggested that agents targeting ROR-γT could be developed for the treatment of CD (Leppkes et al. 2009).

Buonocore et al. reported that ROR-γT plays an important functional role in the pathogenesis of innate (α-

CD40 antibody-induced) colitis in mice. Specifically, they found that Ror¯/¯Rag¯/¯ mice did not develop prominent colitis. Interestingly, these mice also had reduced colonic expression of the IL-23 receptor (IL-23R). The authors suggested that ROR-γT was important in the transcriptional control of the IL23-R, as well as pro-inflammatory cytokine expression in innate intestinal lymphoid cells (Buonocore et al. 2010).

Recently, we found that the intraperitoneal (i.p.) administration of a specific ROR- γT inverse agonist (VPR-7, Visionary Pharmaceuticals, San Diego, CA) improved various macroscopic, morphometric (colonic length and weight), biochemical (IL-17) and histological parameters of TNBS-induced colitis in mice. Our data seems to coincide with previous results from this model, which showed that TNBS-induced colitis was attenuated in IL-17R deficient mice, or mice treated with an IL-17 receptor (IL-17R) fusion protein (Zhang et al. 2006).

In contrast to these results, suggesting that ROR-γT inhibition is beneficial in murine IBD models, other data (table 2) suggest that targeting this transcription factor was either ineffective, or worsened colitis. Interestingly, these negative results were all obtained with the DSS-colitis model. Of note, with immunocompromised RAG 2 deficient mice, a deficiency in ROR-γT was lethal following acute exposure to DSS (Sawa et al., 2011). Overall, these results are a potential warning that strong ROR-γT inhibition in severely immunocompromised patients would not be a wise approach, due to the potential for bacterial infection. In support of this suggestion, it was reported that the worsened chronic DSS-induced colitis in ROR-γT mice was completely abrogated by treatment with antibiotics (Lochner et al. 2011 and Ohnmacht et al. 2011)

2.1. Inhibition of ROR-yT in animal models of IBD (Indirect Evidence)

Several other indirect lines of evidence suggest that inhibition of ROR-γT is effective in animal models of IBD. It was reported that treatment with a Pim-1 kinase inhibitor attenuated acute DSS-induced colitis in mice. This effect occurred in conjunction with a reduction in colonic ROR-γT expression (Shen et al. 2011). Treatment of mice with mesenchymal stem cells or Ocotillol (a majonoside metabolite) improved murine TNBS-induced colitis and attenuated colonic ROR-γT (Chen et al.
2011 and Lee et al. 2015). Finally, using a T cell transfer model, investigators demonstrated that mice treated with carbonic anhydrase 1 attenuated the colitis and concomitantly reduced colonic ROR-γT expression (Mori et al. 2013).

3 Pharmacological Rationale for ROR-yT Inhibition

The pharmacological rationale for developing drugs that would specifically inhibit ROR-yT, for use in patients with IBD, is illustrated in Figure 1.

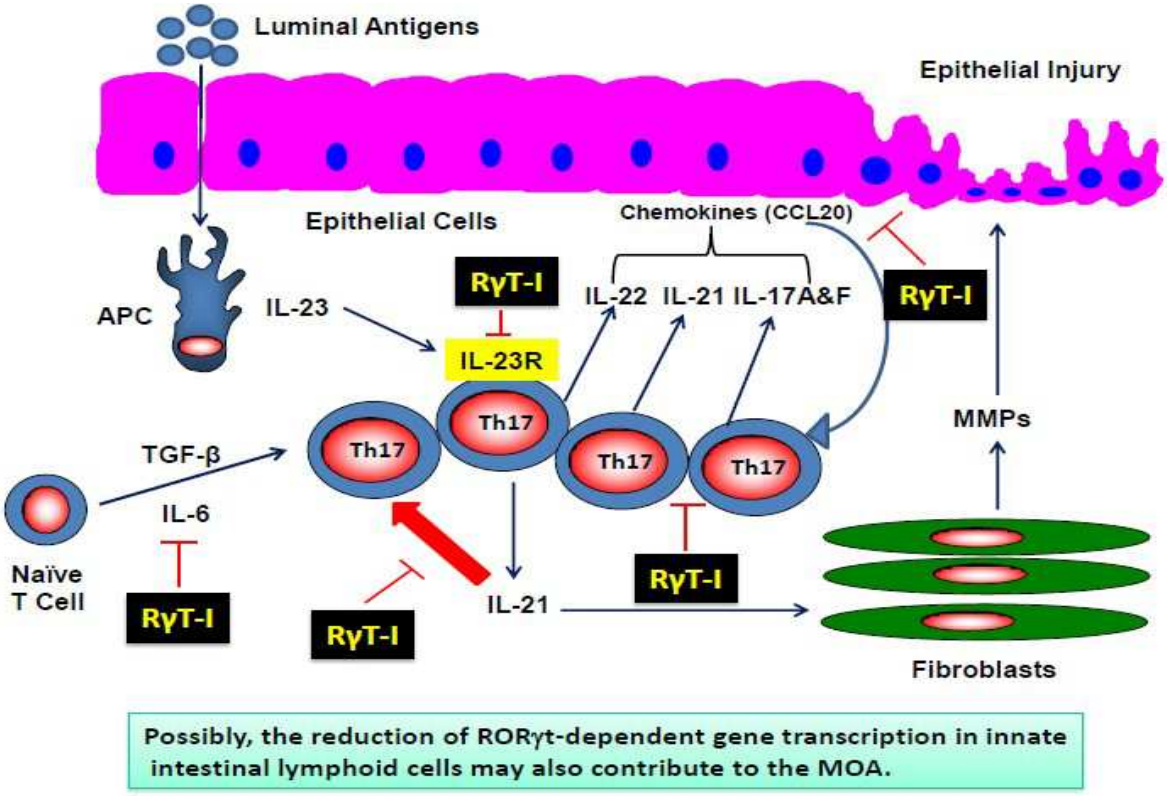

Figure 1: Pharmacological rationale for developing ROR-γT inhibitors (RγT-I) to treat IBD. RγT-I would inhibit Th17 cell differentiation and IL-23 mediated Th17 expansion. These drugs would also block by various pathways the transcription of possible pro- inflammatory receptors (IL-23R) cytokines (IL-17, IL-21) and chemokines (CCL20). The reduction of ROR-γT gene transcription in innate intestinal lymphoid cells could also contribute to the mechanism of action (MOA).

As shown in figure 1, RγT-I would theoretically inhibit Th17 cell differentiation, as well as IL-23 mediated Th17 cell expansion. In this regard, several review papers have discussed the IL-23/ Th17 axis as playing a prominent role in the pathogenesis of IBD (McGovern and Powrie 2007 and Fitzpatrick 2012). Specifically, Th17 cells have been implicated in the etiology of the disease. Recent review papers have been written that detail the role(s) of this CD4+ T cell population in IBD (Fitzpatrick 2013 and Galvez 2014). As suggested in these papers, there are potential pros and cons to developing drugs like RγT-I for IBD. One important consideration is the idea of critically evaluating RγT-I, in order to utilize drug doses that would result in significant (but not complete) inhibition of key downstream cytokines like IL-17 and IL-22 (Fitzpatrick 2013).

The IL-23R, which is under the transcriptional control of ROR-γT, has been implicated in human IBD. This concept is based on genetic studies demonstrating polymorphisms in patients with disease (Sheikh et al. 2008 and Cravo et al. 2014). Data from animal models of IBD suggests divergent roles for the IL23-R. A pathogenic role for this receptor has been proposed in the anti-CD40 innate colitis model of IBD, as well as in the acute DSS colitis model (Cox et al. 2012 and Eken et

al. 2014). Interestingly, Eken and colleagues proposed that IL-23R signaling promoted innate colitis via IL-22 (Eken et al. 2014).

IL-22 Is also under the transcriptional control of ROR-γT (Skepner et al. 2014). Initially, IL-22 was proposed to play a protective role in various IBD models (Zenewicz et al. 2008). However, more recent results suggest that IL-22 can have a pathogenic role in certain colitis models, including a $CD45RB^{low}$ $Foxp3^{-}$ T cell transfer model (Kamanaka et al. 2011). As a whole, developing a better understanding for the divergent roles of IL-22 within the context of intestinal inflammation may help to better determine the best niche for RγT-I in patients with IBD.

The exact roles of IL-17A and IL-17F in IBD models, as well as in human IBD, are controversial and have been the subject of previous papers (Fitzpatrick 2013, Galvez 2014 and Wedebye Schmidt et al. 2014). Initial clinical results with antibodies targeting IL-17A or the IL-17 receptor have been disappointing (Fitzpatrick 2013). Nevertheless, the potential use of RγT-I for IBD represents a different multi-factorial pharmacological approach (see Figure 1) rather than targeting IL-17 alone (Fitzpatrick et al. 2015).

As shown in Figure 1, IL-21 is also produced by differentiated Th17 cells. This cytokine has been implicated in the pathogenesis of IBD. Specifically, by a positive feedback loop (large red arrow in figure 1) this cytokine is involved in the differentiation of TH17 cells (Liu et al. 2009). Previous results from IL-21 transgenic mice showed more severe colitis, as well as increased ROR-γT in the colon of these animals (Araki et al. 2013). Moreover, IL-21 reportedly played a pathogenic role in the murine T cell transfer model of colitis (Fantini et al., 2007). These data suggest that targeting the ROR-γT/IL-21 pathway (Figure 1), which is involved in intestinal inflammation, could be beneficial for patients with IBD.

Chemokine (C-C motif) ligand 20 (CCL20) or Macrophage Inflammatory Protein-3 alpha (MIP3A) is a cytokine belonging to the CC chemokine family. It is strongly chemotactic for T-lymphocytes. Both CCL20 and its receptor CCR6 (Chemokine receptor 6) are under the transcriptional control of ROR-γT (Skepner et al. 2014). As shown in figure 1, the inhibition of CCL20-mediated chemotaxis (using a specific RγT-I) would limit further Th17 cell influx into the colon, and could be of benefit for IBD patients. Preclinical and clinical data support this contention. Specifically, CCL20 is involved in the development of chronic DSS and acute TNBS-induced colitis in mice (Teramoto et al. 2005 and Katchar et al. 2007). Moreover, the expression of CCL20 in UC patients was significantly higher than in a disease free control group (Zhang et al.2012).

4. Off-Target Effects of ROR-Yt Inhibitors

Recent data with a small molecule ROR-γT inverse agonist (TMP 778) found that it inhibited Th17 cell development *in vivo*, in conjunction with a murine model of multiple sclerosis. Interestingly, this compound inhibited the expression of more than 150 genes outside of the canonical Th17 transcriptional pathway (Skepner et al. 2015). In this regard, we recently reported that treatment of mice with the specific ROR-γT inverse agonist (VPR-7) inhibited colonic IL-23, which is not under direct transcriptional control of the intended drug target (Fitzpatrick et al., 2015). Therefore, it is important to do future gene-profiling studies in colitis models, in order to better understand the potential efficacy and safety profiles of

RγT-I in patients with IBD (Fitzpatrick et al. 2015 and Skepner et al. 2015).

5. ROR-yT Inhibitors under Development

Several recent papers suggest that pharmaceutical companies are actively involved in the development of RγT-I (Wang et al. 2014, Skepner et al. 2014, Xiao et al2014 and Fitzpatrick et al. 2015). Some investigators have described a series of potent RγT-I (including a lead compound) that was effective in mouse models of experimental autoimmune encephalomyelitis and collagen-induced arthritis (Wang et al. 2014). Another potent and selective RγT-I reduced imiquimod-induced psoriasis like cutaneous inflammation in mice (Skepner et al. 2014). Recently, we described the efficacy of a ROR-γT inverse agonist (VPR-7) in a murine TNBS model of IBD (Fitzpatrick et al.2015). Based on positive results in various animal models of autoimmune-type diseases, undoubtedly there will be further development of RγT-I. Likely, one of these compounds will ultimately be tested in IBD patients.

CONCLUSIONS

There is a solid (but evolving) pharmacological rationale supporting the development of RγT-I for the treatment of IBD. Data from animal models of colitis, as well as patients with IBD, suggest enhanced intestinal expression of ROR-γT. Moreover, using both T cells from ROR-γT deficient mice, as well as a small-molecule RγT-I approach; evidence of reduced colonic inflammation has been obtained in murine models of IBD. Currently, pharmaceutical companies are actively involved in the development of RγT-I. It is likely that a prototype compound will ultimately enter controlled clinical trials in patients with IBD.

ACKNOWLEDGEMENTS

The author would like to acknowledge the collaboration of Dr. Jim Zapf and Dr. Gordon Alton (Visionary Pharmaceuticals, San Diego, CA), who shared literature references, which were pertinent to this manuscript.

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