

Letter to the Editor

ROR γ t inhibitors suppress T_H17 responses in inflammatory arthritis and inflammatory bowel disease

To the Editor:

Increased frequencies of T-helper cells producing the proinflammatory cytokine IL-17A (T_H17) have been reported in ankylosing spondylitis (AS), rheumatoid arthritis (RA), psoriatic arthritis (PsA), and inflammatory bowel disease (IBD).¹ Genome-wide association studies have linked multiple T_H17-associated genes, including *IL23R*, with AS, psoriasis, and IBD, further supporting a common pathogenic role for the IL-17/IL-23 axis in these diseases.² Neutralizing IL-17A with mAbs has shown efficacy in AS and PsA,³ and some benefit in RA,⁴ but has proved ineffective and possibly deleterious in patients with IBD.⁵ There is therefore a need for new therapies, including small-molecule inhibitors, that target type 17 immune responses.

T_H17 cells specifically express the DNA-binding transcription factor retinoic acid receptor-related orphan receptor γ t (ROR γ t).⁶ Antagonizing ROR γ t is therefore an attractive strategy in diseases associated with the IL-17/IL-23 axis. Indeed, small-molecule ROR γ t antagonists that suppress IL-17A responses in murine inflammatory diseases and human psoriasis have been described.^{7,8} However, ROR γ t inhibitors have not been tested in human inflammatory arthritis or IBD.

We here describe 2 novel indazole small-molecule ROR γ t inhibitors, MRL-248 and MRL-367 (Fig 1, A), which inhibit steroid receptor coactivator 1 peptide binding to ROR γ t with IC₅₀ potency of 1 to 2 nM in a FRET-based assay (see Table E1 in this article's Online Repository at www.jacionline.org). ROR γ /Gal4 cell-based reporter assays showed inhibition of ROR γ t by MRL-248 and MRL-367 with IC₅₀s of 118 nM and 41 nM, respectively (Table E1). In contrast, the ROR γ t inhibitors digoxin, TMP778, and SR1001 have shown IC₅₀s in the micromolar range.⁷ MRL-248 and MRL-367 showed no appreciable activity against a panel of related nuclear hormone

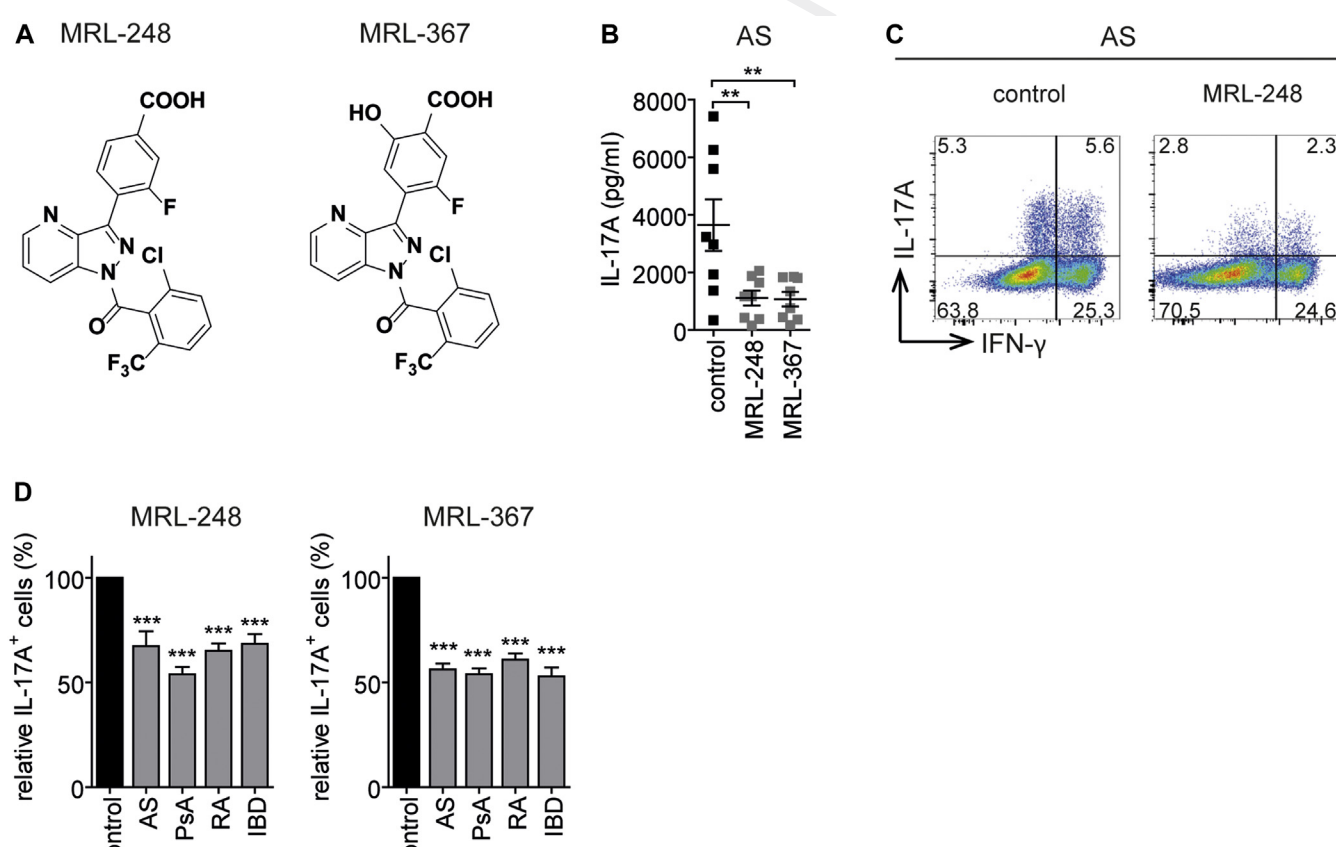


FIG 1. Structure of 2 novel ROR γ t inhibitors and their suppression of T_H17 polarization in patients with AS, PsA, RA, and IBD. **A**, Chemical structure of small-molecule ROR γ t inhibitors MRL-248 and MRL-367. **B**, PBMCs derived from patients with AS (n = 8) were cultured for 6 days, and 200,000 cells were subsequently restimulated in the presence of ROR γ t inhibitors (MRL-248 or MRL-367; 1 μ M). Data are mean \pm SEM of n = 8; **P < .01 by Friedman test. IL-17A secretion after 48 hours was measured by ELISA. **C** and **D**, PBMCs derived from patients with AS, PsA, RA, or IBD were cultured for 6 days in the presence of ROR γ t inhibitor MRL-248 or MRL-367. **C**, Representative fluorescence-activated cell sorting plot of 1 patient with AS. **D**, Relative suppression of IL-17A⁺ T-helper cells by MRL-248 or MRL-367 from patients with AS (n = 29), PsA (n = 11), RA (n = 13), and IBD (n = 16). Data are mean \pm SEM; ***P < .005 by Friedman test.

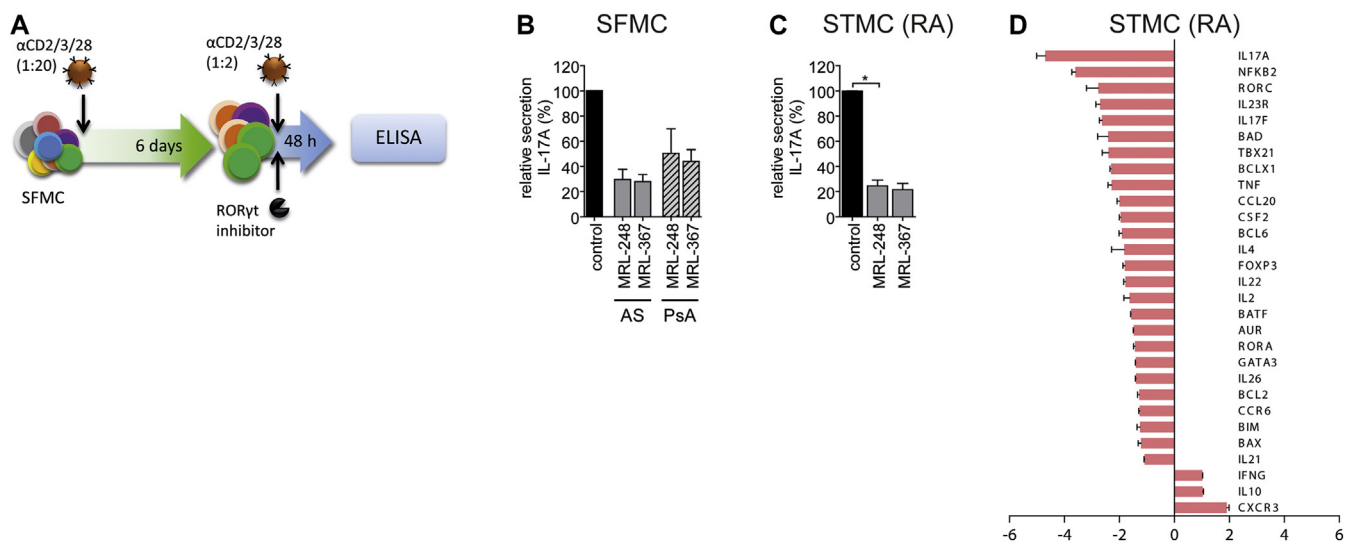


FIG 2. ROR γ t inhibition suppresses IL-17A secretion in synovial tissue-derived cells. **A**, Mononuclear cells from the synovial fluid (SFMC) of patients with AS and PsA were cultured in T_H17-promoting conditions for 6 days and subsequently restimulated for 48 hours with anti-CD2/anti-CD3/anti-CD28 beads in the presence of ROR γ t inhibitors. **B**, Secreted IL-17A (ELISA) measured from patients with AS (n = 3) or PsA (n = 3). Data are mean \pm SEM and relative to control conditions. **C**, Mononuclear cells were obtained from a 14-day explant culture of synovial tissue (STMC) derived from patients with RA and subsequently cultured in the presence of anti-CD2/anti-CD3/anti-CD28 beads and ROR γ t inhibitors (see cartoon Fig E10). IL-17A secretion was measured after 48 hours. Data are mean \pm SEM from 4 patients with RA; *P < .05 by Friedman test. **D**, RT-PCR was performed on mRNA isolated from synovial tissue cells of patients with RA after 48-hour stimulation in the presence of MRL-367. Three technical replicates of n = 1, mean \pm SD, are shown.

receptors (see Table E2 in this article's Online Repository at www.jacionline.org).

Next, we tested the suppression of the T_H17 function of human total CD4⁺ T cells from healthy donors by MRL-248 and MRL-367. Both ROR γ t inhibitors reduced IL-17A⁺ T-cell numbers in fluorescence-activated cell sorting–based assays in a comparable and dose-dependent manner (see Fig E1 in this article's Online Repository at www.jacionline.org). Both T_H17 skewing from naive CD4 T cells (see Fig E2, A, in this article's Online Repository at www.jacionline.org) and polarization of memory CD4 T cells (Fig E2, B) was inhibited by MRL-248 and MRL-367, without global effects on cellular proliferation (Fig E2, C). Secretion of IL-17A by established CCR6⁺CD161⁺ T_H17 cells was also reduced by both inhibitors (see Fig E3 in this article's Online Repository at www.jacionline.org).

We next investigated the ability of ROR γ t inhibitors to suppress IL-17A secretion by T_H17 cells derived from patients with inflammatory arthritis, patients with IBD, and healthy controls. We cultured total PBMCs (as more representative of the *in vivo* situation) for 6 days with suboptimal T-cell stimulation and IL-2, resulting in T_H17 expansion while preserving T_H1 frequencies (see Fig E4 in this article's Online Repository at www.jacionline.org and data not shown). ROR γ t inhibitors suppressed the secretion of IL-17A by 70% in cells derived from patients with AS (Fig 1, B). Healthy control PBMC cultures produced less IL-17A, but were also profoundly inhibited (see Fig E5 in this article's Online Repository at www.jacionline.org). The number of IL-17A⁺ cells within the CD4⁺ T-cell compartment was reduced by up to 50% by both MRL-248 and MRL-367 in all 4 inflammatory diseases: AS, PsA, RA, and IBD (Fig 1, C and D; see Fig E6 in this article's Online Repository at www.jacionline.org). A significant fraction of T_H17 cells coexpressed

other cytokines, including IFN- γ , IL-22, and GM-CSF. For AS, PsA, RA, and IBD, both ROR γ t inhibitors suppressed polyfunctional (and potentially pathogenic) IL-17A⁺ cells coproducing IFN- γ , IL-17F, IL-22, GM-CSF, or TNF, but not cells expressing these cytokines independently of IL-17A (see Fig E7 in this article's Online Repository at www.jacionline.org), although conversion of double to single producers cannot be excluded. Although most of the IL-17A-producing cells were CD4⁺ T cells, ROR γ t inhibition also suppressed the IL-17A⁺ population of AS, PsA, and IBD CD8⁺ T cells (see Fig E8 in this article's Online Repository at www.jacionline.org).

We next determined the effects of ROR γ t inhibition on the transcriptional profile of T_H17 cells from patients with AS and RA. MRL-367 and MRL-248 significantly reduced the transcription levels of T_H17-associated genes, including *IL17A*, *CCL20*, *CCR6*, *IL17F*, *IL23R*, and *IL22* (see Fig E9 in this article's Online Repository at www.jacionline.org), concurring with previous reports.^{8,9} Interestingly, MRL-248 and MRL-367 markedly reduced *IL26* transcription in T_H17 from both patients with AS and RA. This reduction was not reported for patients with psoriasis treated with TMP778.⁹ The proinflammatory cytokine IL-26 has previously been associated with RA and IBD, but to our knowledge not with AS. We propose that IL-26 may constitute a pathogenic T_H17-associated cytokine in AS and merits further study.

Transcription of other T_H subset-associated genes (such as *IFN- γ* and *IL4*) or transcription factors (*TBX21*, *GATA3*, *FOXP3*, and *BCL6*) was not reduced by ROR γ t inhibitors, confirming a specific effect on type 17 cells. *IL10* transcription was modestly enhanced, supporting the inverse correlation between ROR γ t and Foxp3, although this finding requires confirmation due to the small study size. Overall, these transcriptional

data confirm the cytokine inhibitory effects observed by ELISA and intracellular cytokine staining, and also identify new candidates meriting further study.

The effects of ROR γ t inhibition were tested on CD4⁺ T cells derived from the inflamed tissue of patients with inflammatory arthritis. Mononuclear cells were isolated from the synovial fluid of 3 patients with AS and 3 patients with PsA and cultured for [F2-4/C] 6 days in T_H17-promoting conditions (Fig 2, A). Restimulation of these cells in the presence of ROR γ t inhibitors suppressed IL-17A secretion by 75% (Fig 2, B). Last, we analyzed the efficacy of the inhibitors on T cells obtained after 2-week culture of synovial tissue explants from patients with RA (see Fig E10 in this article's Online Repository at www.jacionline.org). Once again, restimulation of inflamed tissue-derived cells in the presence of ROR γ t inhibitors resulted in a reduction in IL-17A secretion (Fig 2, C). Transcriptional analysis of T_H17 cells derived from the synovial tissue of patients with RA confirmed downregulation of T_H17 signature genes by ROR γ t inhibition (Fig 2, D; see Fig E11 in this article's Online Repository at www.jacionline.org).

The novel ROR γ t inhibitors described here consistently and specifically inhibit the production of IL-17A and coproduced pathogenic cytokines by T_H17 cells *ex vivo* from patients with active AS, PsA, RA, and IBD, while sparing other T-cell immune pathways. Our findings provide a strong rationale for therapeutic trials of ROR γ t inhibition in inflammatory arthritis and IBD.

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