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Inhibition of TYK2 and JAK1 Ameliorates Imiquimod-Induced Psoriasis-like Dermatitis by Inhibiting IL-22 and the IL-23/IL-17 Axis

Melissa G. Works,* Fangfang Yin,* Catherine C. Yin,* Ying Yiu,* Kenneth Shew,* Thanh-Thuy Tran,* Nahoko Dunlap,* Jennifer Lam,* Tim Mitchell, † John Reader, † Paul L. Stein,* and Annalisa D’Andrea*

Psoriasis is a chronic autoimmune disease affecting the skin and characterized by aberrant keratinocyte proliferation and function. Immune cells infiltrate the skin and release proinflammatory cytokines that play important roles in psoriasis. The Th17 network, including IL-23 and IL-22, has recently emerged as a critical component in the pathogenesis of psoriasis. IL-22 and IL-23 signaling is dependent on the JAK family of protein tyrosine kinases, making JAK inhibition an appealing strategy for the treatment of psoriasis. In this study, we report the activity of SAR-20347, a small molecule inhibitor with specificity for JAK1 and tyrosine kinase 2 (TYK2) over other JAK family members. In cellular assays, SAR-20347 dose dependently (1 nM–10 μM) inhibited JAK1- and/or TYK2-dependent signaling from the IL-12/IL-23, IL-22, and IFN-α receptors. In vivo, TYK2 mutant mice or treatment of wild-type mice with SAR-20347 significantly reduced IL-12-induced IFN-γ production and IL-22-dependent serum amyloid A to similar extents, indicating that, in these models, SAR-20347 is probably acting through inhibition of TYK2. In an imiquimod-induced psoriasis model, the administration of SAR-20347 led to a striking decrease in disease pathology, including reduced activation of keratinocytes and proinflammatory cytokine levels compared with both TYK2 mutant mice and wild-type controls. Taken together, these data indicate that targeting both JAK1- and TYK2-mediated cytokine signaling is more effective than TYK2 inhibition alone in reducing psoriasis pathogenesis. The Journal of Immunology, 2014, 193: 000–000.

Psoriasis is a chronic, relapsing autoimmune disorder that affects 2–3% of the population and manifests as lesions of the skin with scaling and redness (1). These lesions show excessive keratinocyte proliferation (acanthosis), retention of nuclei in the stratum corneum due to aberrant keratinocyte differentiation (parakeratosis), and the presence of inflammatory cells, including T cells, B cells, neutrophils, and dendritic cells (DCs) (1). In active psoriatic lesions, the inflammatory environment includes the expression of a host of Th17- and Th1-related cytokines such as IL-23, IL-6, IL-22, IL-17, and IFN-γ (1). Therapeutic approaches directed at blocking some of these inflammatory mediators have proven effective in reducing patients’ symptoms. Administration of IL-12/23 anti-p40 mAbs such as ustekinumab results in an improvement of the disease because it inhibits the IL-23/IL-17 axis (2).

Th17 and γδ T cells that are found in psoriatic skin produce both IL-17 and IL-22 (3). These two cytokines together are responsible for the formation of skin lesions characteristic of psoriasis. Furthermore, IL-22 plays a critical role in the maintenance of normal barrier homeostasis, and psoriatic patients have elevated serum levels of IL-22, contributing to barrier disruption (4). By inhibiting keratinocyte differentiation, IL-22 promotes the normal formation of the stratum corneum of the skin and desquamation (5). IL-22 also induces keratinocytes to produce antimicrobial peptides and IL-20, a cytokine that has similar function as IL-22 and amplifies the inflammatory process (6). Increased levels of IL-20 have been detected in lesional skin and blood of patients with psoriasis (7, 8).

Many of the inflammatory cytokines implicated in the pathogenesis of psoriasis use JAKs for signaling. JAKs are a family of nonreceptor tyrosine kinases, including tyrosine kinase 2 (TYK2), JAK1, JAK2, and JAK3, which are critical for the intracellular signal transduction of multiple cytokines, hormones, and growth factors. JAKs phosphorylate STAT proteins, leading to gene expression changes. TYK2 and JAK1 are critical for signal transduction for many of the cytokines upregulated in psoriatic lesions, such as IL-23, IL-12, IL-6, and IL-22. TYK2 is mainly associated with IL-23 and IL-12 signaling (9, 10). In contrast, IL-22 signaling is mediated by TYK2 and JAK1, although JAK1 is proposed to play the dominant role in signaling from this receptor (9).

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Abbreviations used in this article: DC, dendritic cell; BHC, immunohistochemistry; iTreg, induced regulatory T cell; LC-MS/MS, liquid chromatography–mass spectrometry/mass spectrometry; MSD, meso scale discovery; p.o., per os; qRT-PCR, quantitative real-time PCR; SAA, serum amyloid A; SEAP, secreted embryonic alkaline phosphatase; siRNA, small interfering RNA; SKIL, Sareum Kinase Inhibitor Library; Tb, terbium; TR-FRET, time-resolved fluorescent resonance energy transfer; Treg, T regulatory; TYK2, tyrosine kinase 2; WT, wild-type.
encephalomyelitis (14), and imiquimod-induced psoriasis-like dermatitis (10). Together these observations provide a strong rationale for targeting TYK2 and JAK1 for therapeutic purposes.

Small molecule inhibitors of JAK1 have been developed to target diseases with aberrant production of inflammatory cytokines. In particular, the use of a JAK1-selective inhibitor has been shown to be effective in reducing symptoms in multiple rodent models of arthritis (15), demonstrating the therapeutic potential for inhibiting JAK in treatment of inflammatory diseases. Signaling from many proinflammatory cytokines uses a combination of both JAK1 and TYK2, suggesting that dual inhibition may be more efficacious than targeting a single JAK. Using the imiquimod-induced psoriasis-like dermatitis mouse model, we investigated whether combined inhibition of TYK2 and JAK1 would be effective in reducing the disease severity relative to TYK2 or JAK1 inhibition alone. To that end, we developed small molecule inhibitors against JAK1 and TYK2 and selected SAR-20347 for further studies based on its selectivity in biochemical and cell-based assays. The compound was effective at inhibiting signaling from a number of cytokines that are dependent on JAK1 and TYK2. We also compared TYK2 and JAK1 inhibition by SAR-20347 with TYK2 mutant mice in the imiquimod-induced psoriasis model. These studies demonstrated that blocking both TYK2 and JAK1 was more effective than inhibition of TYK2 alone at reducing psoriasis-like disease severity, keratinocyte proliferation, as well as IL-23, IL-17, IL-6, IL-22, and antimicrobial peptide gene expression. These results indicate that targeting a combination of JAK1 and TYK2 using an orally available inhibitor may be a viable approach for treating psoriasis.

Materials and Methods

Small molecule inhibitor

SAR-20347 was developed from the Sareum Kinase Inhibitor Library (SKIL) platform, a chemical template that makes key hydrogen bond interactions with the hinge region of certain kinases and directs substituents toward the selectivity (hydrophobic) pocket, and the solvent-accessible surface. A subset of compounds, with favorable in vitro pharmacokinetic properties, was screened against a 291-member kinase panel that included proinflammatory cytokines and interfering RNAs (siRNAs) (TYK2, JAK1, and negative control) were purchased from Life Technologies. Individual or combined siRNAs were incubated with TF-1 cells for 24 h at 37°C, 5% CO2. TF-1 cells were serum re-

Kinase screens

Radiolabeled ATP. Kinase inhibition was determined by Reaction Biology (Malvern, PA). Kinases were prepared in base reaction buffer (20 mM HEPES [pH 7.5], 10 mM MgCl2, 1 mM EDTA, 0.02% Brij35, 0.02 mg/ml BSA, 0.1 mM Na2VO4, 2 mM DTT, 1% DMSO), and substrate was added with 1.5 mM CaCl2, 16 μg/ml calmodulin, and 2 mM MnCl2. Varying concentrations of SAR-20347 in DMSO were added to the kinase reaction along with 10 μM [32P]-ATP (activity 0.01 μCi/μl final) for IC50 determination. The reaction was incubated for 120 min at room temperature and then spotted on a fib filter paper (Whatman). Filter paper was washed in 0.75% phosphoric acid, and the [32P] signal was determined using Typhoon phosphorimagery (GE Healthcare). The assay was run three times. After subtraction of background (control reactions containing inactive enzyme), IC50 values were determined using the nonlinear regression function in Prism (GraphPad).

Time-resolved fluorescent resonance energy transfer. A LanthaScreen fluorescent resonance energy transfer assay (Invitrogen) was used to determine the inhibitory effect of SAR-20347. Fluorescent resonance energy transfer is measured between the GFP-labeled STAT3 protein and a terbium ( Tb)-labeled Ab bound to the phosphorylated STAT3. The assay is read in a time-specific manner, instituting a delay after excitation, thereby reducing background fluorescence. To determine time-resolved fluorescent resonance energy transfer (TR-FRET) activity, dose-response measurements for small molecules were determined at a final concentration of 1% DMSO in the presence of recombinant kinase (TYK2, JAK1, JAK2, or JAK3), 10 μM ATP, and substrate (GFP-STAT3). The reaction was stopped after 1 h, and Tb-labeled antiphosphorylated STAT3 (pSTAT3) Ab was added and incubated for 1 h. Plates were read using an Analyst HT with LJI Bio-systems software. Activity of TYK2, JAK1, JAK2, or JAK3 was measured as the ratio of the acceptor signal (GFP) to the donor signal (Tb), triplicate values for condition were averaged, and the assay was run three times.

Cell-based assays

Cytokine-induced STAT phosphorylation. TF-1 (ATCC CRL-2003) and NK-92 (ATCC CRL-2407) cells were cultured according to American Type Culture Collection recommendations. TF-1 cells were serum re-

NK-92 cells were cultured overnight in RPMI 1640 with 10% charcoal/dextran-stripped FBS (Sigma-Aldrich), 0.1 mM nonessential amino acids (Life Technologies), and 1 mM sodium pyruvate (Life Technologies). NK-92 cells were cultured overnight in RPMI 1640 with 10% charcoal/dextran-stripped FBS. CD4+ (Rosette Sep CD4 negative selection) or CD4+ (Easy Sep positive selection) cells were isolated following manufacturer’s directions (StemCell Technologies) from fresh human blood (Stanford Blood Center) and plated in complete media. Cells were plated in a 96-well V-bottom plate (Costar) in starvation medium; incubated with SAR-20347 (0.5% DMSO) for 20 min at 37°C, 5% CO2; and stimulated with individual cytokines (Table II). P-STAT levels were measured in duplicate using meso scale discovery (MDS) plates following the manufacturer’s instructions (MSD). The IC50 was determined by subtracting background (no cytokine) and relative to DMSO/cytokine control.

Th cell development and proliferation. Splenic CD4+ cells were isolated from naive mice using CD4+ T Cell Negative Selection Kit (StemCell Technologies). Cells were plated on anti-TCR– and anti-CD28–coated 24-well dishes at 2 × 105 cells/well in assay media (RPMI 1640, 10% FCS, 1% Glutamax, 1% sodium pyruvate, 25 mM HEPES, 0.1% 2-ME) in the presence of DMSO control, 1 mM ATP, and 0.5% DMSO. Cells were stimulated with cytokines for Th1 (5 ng/ml IL-12, 3.3 μg/ml anti-IL-4, 10 U/ml IL-2), Th2 (10 ng/ml IL-4, 5 μg/ml anti–IFN-γ, 10 U/ml IL-2), Th17 (20 ng/ml IL-16, 0.5 mg/ml TGF-β, 5 μg/ml anti–IL-1β, 4 μg/ml anti–IFN-γ), or induced regulatory T cells (iTregs) (0.5 mg/ml TGF-β, 5 μg/ml anti–IL-1β, 4 μg/ml anti–IFN-γ, 10 U/ml IL-2) were added to the cultures, and cells were grown for 4 d. Cells were then fixed/permeabilized, and stained for CD4, IFN-γ, and IL-4 (Th1/Th2) or IL-17 and FoxP3 (Th17) (BD Biosciences). The cells were analyzed via flow cytometry, gating for lymphocytes, live cells (Live/Dead Fixable Violet; Life Technologies), and the CD4+ population. Alternatively, 2 × 105 CD4+ T cells were incubated for 4 d in plates coated with 250 ng anti–CD28 and 25 ng anti-TCR, and then proliferation was measured by measuring incorporation of 3H-thymidine added to the culture for the last 24 h. Each of these experiments was performed three times.

TYK2/JAK1 small interfering RNA. Validated SilencerSelect small interfering RNAs (siRNAs) (TYK2, JAK1, and negative control) were purchased from Life Technologies. Individual or combined siRNAs were incubated with LipofectamineRNAMax (Life Technologies) at room temperature for 20 min on 6-well plates, followed by addition of HT-29 cells at 2 × 105 cells/well for 48 h at 37°C, 5% CO2.

IL-22/pSTAT3. HT-29 cells (a gift of L. Sambucetti, SRI International) were cultured in RPMI 1640 with 10% FBS, 25 mM HEPES (Life Technologies), and 2 mM Glutamax. Cells were serum starved overnight (RPMI 1640). Cells were plated at 2.5 × 105 cells/well in a 96-well plate in starvation medium and incubated with SAR-20347 for 20 min (final concentration 0.5% DMSO) at 37°C, 5% CO2. Cells were stimulated with 10 ng/ml human IL-22 (PeproTech) for 15 min, rinsed, and fixed in 4% paraformaldehyde for 10 min at room temperature. Cells were rinsed with 0.5% BSA in PBS, resuspended in 90% methanol, and incubated for 30 min on ice. Cells were stained with rabbit anti-pSTAT3 (1:200; Invitrogen) for 30 min at room temperature, then analyzed by flow cytometry, and gated on forward/side light scatter. The IC50 was determined by calculating mean fluorescence intensity, then subtracting background (secondary Ab alone), and plotted relative to DMSO/IL-22 control (as 100%).

IFN-α function. HEK-BLUE IFN-α/β reporter cells (Invivogen) were cultured according to manufacturer’s directions. Triplicate wells of a 96-well plate were seeded with 50,000 cells in complete media containing inhibitor (final DMSO concentration of 0.5%) and incubated with 200 U IFN-α for 24 h at 37°C, 5% CO2. IFN-α signaling was detected by measuring secreted embryonic alkaline phosphatase (SEAP) levels, according to manufacturer’s recommendations (Invivogen). P-STAT analysis with IFN-α/DMSO control was calculated.

IL-12 function. PBMcs (Stanford Blood Center) were obtained from six donors and seeded onto 96-well plates precoated with anti-CD3 Ab (BD Biosciences). SAR-20347 was diluted in complete medium (0.5% DMSO)
and added to 1 x 10^6 cells for 5 min prior to addition of 10 ng/ml human IL-12 to the culture. After 24 h, cell supernatant was transferred to a new plate and stored at −20°C until analysis using a human IFN-γ ELISA kit (R&D Systems), following the manufacturer’s directions.

**In vitro pharmacokinetics**

All in vitro pharmacokinetic assays were performed by Apteum (Verona, Italy). To measure permeability, the epithelial cell line Caco-2 was plated on multwell filter plates (ReadyCell) and were handled according to manufacturer’s directions. The passive permeability of 5 μM SAR-20347 was determined at pH 7.4 in HBSS (0.5% DMSO). Briefly, Caco-2 cell monolayers were conditioned for 30 min in HBSS at 37°C, prior to addition of test items or reference controls in either the apical or basal compartment. Cells were then incubated (37°C, with shaking) for 90 min, and concentrations in the apical and basal compartments were measured by liquid chromatography–mass spectrometry/mass spectrometry (LC-MS/MS) analysis.

Male mouse liver microsomes (Xenotech) were thawed rapidly at 37°C and then diluted with 50 mM potassium phosphate buffer (pH 7.4) to a protein concentration of 0.56 mg/ml. SAR-20347 was added at a final concentration of 0.5 μM. At 0, 3, 6, 9, 15, and 30 min, 50-μl aliquots were taken from the incubation mixture, mixed with quenching solution, and subjected to LC-MS/MS analysis.

**In vivo pharmacokinetics**

In vivo pharmacokinetics assays were performed by WuXi AppTec (Shanghai, China). Male CD-1 mice at 7–9 wk were individually housed and provided with water ad libitum. Animals were fasted 12 h prior to administration of SAR-20347, and, 4 h postadministration, food was returned. SAR-20347 was formulated in 10% DMSO/90% PEG400. Compound purity was >99%, as measured by HPLC. Animals were dosed with 1 mg/kg (0.2 mg/ml, bolus) i.v. or 10 mg/kg (1 mg/ml) oral gavage, and blood was collected into EDTA-K2 tubes at 5, 15, and 30 min and 1, 2, 4, 8, and 24 h postadministration. Plasma samples were quick frozen over dry ice and kept at −70°C until LC-MS/MS analysis, with a lower limit of quantification of 1 ng/ml.

**In vivo pharmacology**

**Mice.** All mice were housed at least 1 wk prior to the study with ad libitum access to food and purified water. All studies were performed in accordance with American Association for the Accreditation of Lab Animal Care animal welfare standards and under Internal Animal Care and Use Committee approval.

IL-22–induced serum amyloid A. Female 7– to 9-wk-old C57Bl/6 mice (Taconic Laboratories) were administered 50 mg/kg SAR-20347 in 10% DMSO/90% PEG400 by oral gavage 30 min prior to injection of 10 μg IL-22 (i.p.). Blood was sampled from the retro-orbital sinus at 6 h post–IL-22 injection. Serum was run on a serum amyloid A (SAA) ELISA (Kamiya).

Imiquimod-induced dermatitis. The backs of female B10.Q/Ai (wild type [WT]; Taconic Laboratories) or B10.D1-H2b/SgJ (TYK2 mutant; Jackson Laboratories) were shaved with an electric clipper 1 d prior to treatment. Mice were administered vehicle or 50 mg/kg SAR-20347 by oral gavage 30 min prior to application of 62.5 μg 5% imiquimod cream (Butcher Schein) or control cream (Curel). Another dose of vehicle or 50 mg/kg SAR-20347 was given 5.5 h following the first dose. This treatment was repeated for 5 d, and, on days 3 and 4, animals were injected with 100 μl saline to prevent dehydration. Each day, the mice were assessed by the same researcher for redness and scaling on a 0–4 scale, ranging from the least to the most severe. Skin from imiquimod-treated animals was processed by IDEXX Laboratories (Sacramento, CA). Briefly, skin was paraffin embedded, sliced at 6 μm, and stained with hematoxylin and eosin or immunohistochemistry (IHC), sections were deparaffinized, rehydrated prior to Ag retrieval (Dako Target Retrieval Solution) for 30 min at 97°C, blocked for 2 h at room temperature in 20% normal donkey serum (Jackson Immunoresearch Laboratories) wash buffer (0.1% Triton X-100 in PBS), and incubated with biotinylated hamster anti-mouse Vv3-TCR (1:400; Leinco) and rabbit anti-mouse IL-17 (1:1400; Abcam), or Ki67 (1:100) Ab overnight at 4°C. After washing, slides were incubated with donkey anti-rabbit Alexa488 (1:400; Invitrogen) or streptavidin-Cy3 (1:200; Jackson Immunoresearch Laboratories) secondary Ab for 1 h at room temperature, then washed and stained with Hoechst (1:20,000; a gift of L. Gambucci, SRI International), and coverslipped.

**Histopathological and immunohistochemical analysis.** H&E slides were blinded, and an overall score of severity was assessed (0–4) by a trained researcher. The number of keratinocytes comprising the epidermal layer was counted in four randomly selected areas of the slide and represented as epidermal thickness. For IHC quantification, representative pictures were taken (original magnification x10) and the number of IL-17+ cells was manually counted. In addition, overall signal intensity for Vv3 or IL-17 was measured by taking the single-channel image (Alexa488 or Cy3) and measured the mean signal intensity in Image J. For Ki67 staining, the image was set to a constant threshold across all images. In each animal, the number of pixels above the threshold was calculated in the epidermal layer in a rectangle set to a length of 350 pixels.

**Statistical analysis**

The IC_{50} for kinase screen and pSTAT cell-based assays was calculated by plotting log concentration by percent activity compared with DMSO control with kinase or cytokine. A variable slope, sigmoidal dose-response curve was generated while constraining the top and bottom values to 100 and 0%, respectively, and the value to achieve 50% inhibition was determined (GraphPad Prism). Immunofluorescent data were analyzed using Phoenix WinNonlin software and linear/log trapezoidal calculation method. Cell proliferation and differentiation data were analyzed and compared with control media by two-way ANOVA (concentration, treatment) with Tukey post hoc tests. For the psoriasis study, clinical scores, histopathology score, keratinocyte count, and IHC measures for TYK2 mutant mice or SAR-20347–treated mice were analyzed by one-tailed t test compared with vehicle/imiquimod-treated, WT mice.

**Results**

SAR-20347 inhibits TYK2 > JAK1 > JAK2 > JAK3 in enzymatic assays

As TYK2 and JAK1 play a critical role in the signaling of cytokines driving many autoimmune diseases, we used the SKIL platform to screen for small molecule inhibitors of TYK2 and JAK1. The SKIL platform is a chemical template that makes key hydrogen bond interactions with the hinge region of certain kinases and directs substituents toward the selectivity (hydrophobic) pocket and the solvent-accessible surface. The screening efforts provided us with early leads with good potency against TYK2 and a favorable selectivity profile against the other JAKs. A subsequent medicinal chemistry campaign led to the identification of SAR-20347 as a lead compound in the series (Supplemental Fig. 1A). Characterization of SAR-20347 was achieved using two distinct enzymatic assays to determine its selectivity for JAK family members, as follows: an ATP competitive binding assay and TR-FRET analysis to measure inhibition of STAT3 phosphorylation. Using a 33P-ATP competitive binding assay, the IC_{50} for TYK2 was calculated to be 0.6 nM (Table I). Selectivity for TYK2 was 41–73 times higher than other JAK family members (Table I). A kinase analysis was performed to determine whether SAR-20347 inhibited additional kinases. These experiments were performed using 100 nM SAR-20347 and compared with previous literature for the JAK inhibitors tofacitinib and ruxolitinib at 100 nM (Supplemental Table II). SAR-20347 inhibitory activity toward non-JAK was comparable to the previously established values for tofacitinib and ruxolitinib.

The TR-FRET–based method was used to confirm the selectivity of SAR-20347 for JAK family members and measures the ability of the inhibitor to block phosphorylation of rGFP-labeled
Inhibition of JAK family members was assessed by ATP assay (33P-ATP) and TR-FRET-based STAT3 phosphorylation assay. For pSTAT-based cell assays, cells were incubated with SAR-20347 or DMSO for 20 min and then stimulated with cytokines indicated in Table II for 30 min (see Materials and Methods). Values represent the IC50 (nM). For the biochemical assays, the variation between three individual experiments is indicated. The fold selectivity for TYK2 is calculated by comparing the IC50 for TYK2 and JAK2 against each JAK family member.

STAT3 by each JAK family member. The TR-FRET approach yielded a TYK2 IC50 of 13 nM (Fig. 1A, Table I). By comparison, tofacitinib and ruxolitinib showed IC50 values for TYK2 of 78 and 6 nM, respectively. In both assays, SAR-20347 demonstrated a selectivity of TYK2 > JAK1 > JAK2 > JAK3 (Table I).

**Table I.** SAR-20347 selectivity for JAK in biochemical and cellular assays

<table>
<thead>
<tr>
<th>IC50 (nM)</th>
<th>IC50 JAK2 (nM) (TYK2 &gt; JAK2)</th>
<th>IC50 JAK3 (nM) (TYK2 &gt; JAK3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>33P-ATP</td>
<td>0.6 ± 0.1</td>
<td>23 ± 19 (41)</td>
</tr>
<tr>
<td>TR-FRET</td>
<td>13 ± 3</td>
<td>59 ± 43 (4.7)</td>
</tr>
<tr>
<td>TR-FRET ruxolitinib</td>
<td>6.5</td>
<td>1.1</td>
</tr>
<tr>
<td>TR-FRET tofacitinib</td>
<td>78</td>
<td>1.6</td>
</tr>
<tr>
<td>pSTAT (cell lines)</td>
<td>126</td>
<td>345 (2.7)</td>
</tr>
<tr>
<td>pSTAT (primary cells)</td>
<td>107</td>
<td>423 (3.9)</td>
</tr>
</tbody>
</table>

**FIGURE 1.** SAR-20347 inhibits TYK2- and JAK1-mediated IL-12 and IFN-α signaling. (A) Representative curve from the biochemical TR-FRET assay comparing TYK2, JAK1, JAK2, and JAK3 specificity. (B) PBMCs were incubated with the indicated concentration of SAR-20347 or DMSO and stimulated with 10 ng/ml human IL-12 for 24 h. IFN-γ concentration in the supernatant was compared with control cells incubated with DMSO and IL-12. (C) HEK-BLUE IFN-αβ reporter cells were incubated with SAR-20347 and stimulated with 200 U IFN-α for 24 h. SEAP levels were compared with control cells incubated with DMSO and IFN-α. The x-axes indicate concentration of SAR-20347. Graphs represent mean ± SD for five individual experiments.

Inhibition of TYK2- and JAK1-mediated signaling by SAR-20347 in cell-based assays

The ability of SAR-20347 to inhibit TYK2-, JAK1-, and JAK2-mediated phosphorylation of STAT proteins also was measured in cell lines and in human PBMCs, CD4+, and CD14+ cells (Tables I, II). When NK-92 cells were stimulated with IL-12, SAR-20347 potently inhibited IL-12–mediated STAT4 phosphorylation, a TYK2-dependent event, with an IC50 of 126 nM (17). Similarly, in assays using CD4+ cells stimulated with IL-12, SAR-20347 had an IC50 of 107 nM (Table I). In addition, SAR-20347 inhibited JAK1-mediated IL-6 pSTAT3 signaling at an IC50 of 345 nM in TF-1 and 407 nM in CD4+ cells. These data indicate that SAR-20347 is 2.7- to 3.9-fold more selective for TYK2 relative to JAK1 (Table I). Importantly, SAR-20347 poorly inhibited JAK2-mediated STAT5 phosphorylation from IL-3 (in cell lines) and GM-CSF (CD14+ cells) with an IC50 of 1.06 and 2.22 μM, respectively, and JAK3 with an IC50 of 1.608 μM (Table I). These data indicate a preferred selectivity for SAR-20347 (8.4- to 20.8-fold) for TYK2 over JAK2 and JAK3. Although in these cell-based studies the IC50 is higher compared with the biochemical assays, these findings are not surprising as compound potency in whole-cell assays is usually lower than in biochemical assays (15, 18). Overall, the relative inhibition in these cell-based assays confirmed the previous biochemical data and indicated a selectivity of SAR-20347 for TYK2 > JAK1 > JAK2 > JAK3.

SAR-20347 inhibits IL-12 and IFN-α signaling in primary cells

We determined the ability of SAR-20347 to inhibit cellular functions downstream of STAT activity, by measuring inhibition of IL-12 and IFN-α signaling, two cytokines requiring TYK2 and JAK1, respectively, for their function. Human PBMCs were treated with both IL-12 (10 ng/ml) and increasing concentrations of SAR-20347, and then IFN-γ production was determined. Cells without IL-12 in the culture media had no measurable IFN-γ, whereas cells incubated with IL-12 and SAR-20347 demonstrated dose-dependent inhibition of IFN-γ production. The greatest inhibition observed in our experiments was achieved at 10 μM (Fig. 1B). Importantly, this inhibition was not due to differences in cell death, as vehicle-treated cells showed similar viability as SAR-20347-treated cells (data not shown).

Both TYK2 and JAK1 have been shown to be critical mediators of IFN-α signaling, with JAK1 potentially playing a more dominant role (9, 19). To determine the ability of SAR-20347 to inhibit IFN-α signaling, HEK-BLUE IFN-αβ SEAP reporter cells were incubated 24 h with 200 U IFN-α and increasing amounts of SAR-20347, and then production of SEAP was measured. SAR-20347 dose dependently inhibited the production of SEAP, with greatest inhibition occurring with 5 μM SAR-20347 in these experiments (Fig. 1C). These data indicate that SAR-20347 is capable of inhibiting TYK2- and JAK1-mediated cytokine signaling.
The pharmacokinetic profile of SAR-20347 was investigated in vitro and in vivo to determine whether dosing with the small molecule would provide sufficient bioavailability to affect cytokine signaling. In mouse liver microsomes assays, SAR-20347 showed a \( t_{1/2} \) of 52.7 min and a clearance rate of 26 \( \mu \)L/min/mg. In addition, SAR-20347 showed good permeability and favorable efflux parameters in the Caco-2 assay with an apical to basolateral (AB:BA) ratio of 1.3, indicating the drug is unlikely to undergo active efflux (data not shown). For in vivo evaluations, male CD-1 mice were dosed with 1 mg/kg (i.v.), 10 mg/kg per os (p.o.), or 50 mg/kg (p.o.), and blood was sampled at seven time points for pharmacokinetics assessment. Plasma samples demonstrated high bioavailability (F) of 69.8% at 10 mg/kg to 100% at 50 mg/kg (Table III). The volume of distribution was >1 L/kg, indicating distribution to tissues (Table III). SAR-20347 also showed a favorable in vivo \( t_{1/2} \) of 2.4–2.8 h and clearance rate of 52 ml/min/kg (Table III).

**Pharmacokinetics evaluation of SAR-20347**

The pharmacokinetic profile of SAR-20347 was investigated in vitro and in vivo to determine whether dosing with the small molecule would provide sufficient bioavailability to affect cytokine signaling. In mouse liver microsomes assays, SAR-20347 showed a \( t_{1/2} \) of 52.7 min and a clearance rate of 26 \( \mu \)L/min/mg. In addition, SAR-20347 showed good permeability and favorable efflux parameters in the Caco-2 assay with an apical to basolateral (AB:BA) ratio of 1.3, indicating the drug is unlikely to undergo active efflux (data not shown). For in vivo evaluations, male CD-1 mice were dosed with 1 mg/kg (i.v.), 10 mg/kg per os (p.o.), or 50 mg/kg (p.o.), and blood was sampled at seven time points for pharmacokinetics assessment. Plasma samples demonstrated high bioavailability (F) of 69.8% at 10 mg/kg to 100% at 50 mg/kg (Table III). The volume of distribution was >1 L/kg, indicating distribution to tissues (Table III). SAR-20347 also showed a favorable in vivo \( t_{1/2} \) of 2.4–2.8 h and clearance rate of 52 ml/min/kg (Table III).

**TKY2 and SAR-20347 inhibit IL-12 signaling in vivo**

As previously shown, mice injected with IL-12 (with added IL-18 for optimal stimulation) require TKY2 function in vivo for the production of IFN-\( \gamma \) (21). We compared the degree of inhibition found in TKY2 mutant mice versus WT mice dosed with SAR-20347. TKY2 mutant mice show a 95% reduction in serum concentrations of IFN-\( \gamma \) compared with WT mice 3 h post-IL-12/18 injection (Supplemental Fig. 1B). To assess whether SAR-20347 was capable of inhibiting IL-12 signaling in vivo, mice were pretreated with 60 mg/kg SAR-20347 30 min prior to injection of IL-12/IL-18. The 30-min pretreatment time point was selected based on pharmacokinetic data indicating a maximum serum concentration (\( T_{max} \)) at 30 min postinjection (Table III). Strikingly, 60 mg/kg SAR-20347 inhibited the production of...

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**Table II. Comparison of cytokines and induction of relevant pSTAT protein**

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Cells per Well</th>
<th>Concentration Cytokine</th>
<th>Measurement</th>
</tr>
</thead>
<tbody>
<tr>
<td>TF-1</td>
<td>250,000</td>
<td>30 ng/ml IL-6</td>
<td>pSTAT3</td>
</tr>
<tr>
<td>TF-1</td>
<td>250,000</td>
<td>30 ng/ml IL-3</td>
<td>pSTAT5</td>
</tr>
<tr>
<td>NK-92</td>
<td>75,000</td>
<td>30 ng/ml IL-12</td>
<td>pSTAT4</td>
</tr>
<tr>
<td>CD4+</td>
<td>750,000</td>
<td>10 ng/ml IL-6</td>
<td>pSTAT3</td>
</tr>
<tr>
<td>CD4+</td>
<td>750,000</td>
<td>10 ng/ml IL-12</td>
<td>pSTAT4</td>
</tr>
<tr>
<td>CD14+</td>
<td>400,000</td>
<td>10 ng/ml GM-CSF</td>
<td>pSTAT5</td>
</tr>
<tr>
<td>PBMCs</td>
<td>750,000</td>
<td>10 ng/ml IL-2</td>
<td>pSTAT5</td>
</tr>
</tbody>
</table>
Table III. Mouse pharmacokinetics for SAR-20347

<table>
<thead>
<tr>
<th>Parameter (Unit)</th>
<th>1 mg/kg (i.v.)</th>
<th>10 mg/kg (p.o.)</th>
<th>50 mg/kg (p.o.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cmax or C0 (ng/ml)</td>
<td>433</td>
<td>1,343</td>
<td>6,273</td>
</tr>
<tr>
<td>t1/2 (h)</td>
<td>0.4</td>
<td>0.4</td>
<td>0.5</td>
</tr>
<tr>
<td>AUC C0–8h (ng/ml)</td>
<td>305</td>
<td>2,131</td>
<td>18,740</td>
</tr>
<tr>
<td>Vdss (L/kg)</td>
<td>5.6</td>
<td>5.6</td>
<td>5.6</td>
</tr>
<tr>
<td>F (%)</td>
<td>69.8</td>
<td>69.8</td>
<td>69.8</td>
</tr>
</tbody>
</table>

Values represent mean for n = 3 mice per dose after single administration. AUC, area under the curve (time 0–8 h); Cl, clearance rate; Cmax, peak plasma concentration; T max, time at maximum plasma concentration; Vdss, volume of distribution at steady state.

IFN-γ in the serum by 91% compared with vehicle-treated animals (Supplemental Fig. 1C), demonstrating that SAR-20347 can inhibit TYK2 signaling in vivo.

**SAR-20347 inhibits IL-22 signaling via TYK2 and JAK1**

The ability of SAR-20347 to inhibit IL-22 signaling (mediated by JAK1 and TYK2) was evaluated in the human colonic cell line, HT-29. We used siRNA-mediated knockdown to further investigate the relative contribution of TYK2 and JAK1 in IL-22 signaling. Using pSTAT3 levels as a readout of IL-22 signaling, HT-29 cells treated with siRNA against TYK2, JAK1, or both were stimulated by IL-22 (10 ng/ml for 15 min), and then pSTAT3 was evaluated by flow cytometry. Knockdown of TYK2 alone showed 55% reduction in pSTAT3, whereas JAK1 knockdown or dual TYK2/JAK1 knockdown resulted in 99% inhibition (Fig. 3A). Importantly, knockdown of TYK2 and JAK1 protein was specific for the target (Supplemental Fig. 1D, 1E), and scrambled siRNA showed no inhibition of IL-22 signaling.

We then quantified SAR-20347 inhibition of IL-22 signaling in HT-29 cells. SAR-20347 potently inhibited IL-22–induced pSTAT3 in a dose-dependent manner with an IC50 of 148 nM (Fig. 3B). At 10 µM SAR-20347, STAT3 phosphorylation could be inhibited 94%, a similar potency as observed using a MSD-based assay for IL-12/pSTAT4 and IL-6/pSTAT3 in cell-based models (Table I). These data indicate that TYK2 contributes to IL-22 signaling in this colon cell line, and the effect of SAR-20347 is most likely mediated by inhibition of both TYK2 and JAK1.

Injection of IL-22 induces a rapid accumulation of SAA in the serum, but the mechanism of this signaling pathway has not been elucidated (22). To determine whether TYK2, JAK1, or both kinases are responsible for IL-22–mediated SAA production, we first treated TYK2 mutant mice (B10 background) and WT controls (B10) with 10 µg IL-22 or PBS. SAA levels were reduced 37% in TYK2 mutant mice relative to WT counterparts (Fig. 3C). These results suggested that JAK1 may also be involved in the IL-22 response. To further investigate the role of TYK2 and JAK1 inhibition on IL-22 signaling, C57BL/6 mice were pretreated with 50 mg/kg SAR-20347 or vehicle and 30 min later injected with 10 µg IL-22 or PBS. Treatment with SAR-20347 decreased SAA levels by 45% compared with vehicle-treated mice (Fig. 3D). In contrast with HT-29 cells, in which JAK1 appeared to play the dominant role in IL-22 signaling, these data suggest that TYK2 may be the primary JAK needed to induce SAA. Although comparing SAA production across strains (B10 and TYK2 mutant versus C57BL/6) may not be representative of a universal IL-22–induced SAA response, these results suggest that additional pathways may contribute to SAA production.

**SAR-20347 significantly improves imiquimod-induced dermatitis**

As JAK1 and TYK2 are critical for the signaling of several of the cytokines playing pathogenic roles in psoriasis, we explored the activity of SAR-20347 in imiquimod-induced dermatitis, a mouse model for psoriasis (16). Mice received imiquimod cream and two daily doses of 50 mg/kg SAR-20347. To establish the relative contribution of JAK1 and TYK2 in this disease, we also compared WT (B10) mice receiving vehicle or SAR-20347 and TYK2 mutant mice (B10 background) receiving vehicle treatment. Al-
though TYK2 mutant mice had lower clinical scores, this difference was not statistically significant compared with vehicle-treated WT mice. In contrast, SAR-20347 markedly reduced scaling and redness compared with TYK2 mutant mice or vehicle-treated, WT mice (Fig. 4A, Supplemental Fig. 2A), and mice treated with SAR-20347 showed no gross toxicity (data not shown). In addition, histopathological assessment of the back skin indicated that SAR-20347–treated mice showed significantly reduced histological score, keratinocyte cell numbers, and Ki67 staining, demonstrating that the compound can substantially reduce disease severity (Fig. 4B–F). Although TYK2 mutant mice also showed a significant reduction in Ki67 staining, there was no change in overall histopathology score (Fig. 4D, 4F). Neither WT nor TYK2 mutant mice showed signs of inflammation or keratinocyte hyperproliferation in response to treatment with control cream (data not shown). These results suggest that inhibition of both TYK2 and JAK1 reduces disease severity more than TYK2 inhibition alone.

Proinflammatory cytokines and antimicrobial peptides are up-regulated in psoriatic skin in both humans and mice. Analysis of mRNA expression in the skin of SAR-20347–treated mice showed significantly reduced expression of TNF, IL-17, IL-22, IL-23, IL-6, and IL-20 compared with both WT and TYK2 mutant mice treated with imiquimod (Fig. 5). SAR-20347 did not affect the expression levels of IL-2 and IL-10 (Fig. 5). In addition, genes associated with activated keratinocytes, such as S100A8, S100A9, and defensin β1, were also significantly decreased in SAR-20347–treated mice (Fig. 5). In contrast, TYK2 mutant mice showed only a significant reduction of IL-22, IL-6, and S100A9. Follow-up repeat studies confirmed these results (data not shown). Overall, not only did SAR-20347 inhibit a greater number of proinflammatory cytokines and antimicrobial genes, but it also reduced expression of these factors to a greater extent than TYK2 mutants alone.

Finally, by immunohistochemical analysis of the skin, we observed that, compared with control cream treatment, imiquimod treatment increases the number of γδ T cells (data not shown) that are thought to contribute to psoriasis (3). Among animals treated with imiquimod, Vγ3 expression was significantly lower (as measured by average signal intensity) in SAR-20347–treated and TYK2 mutant mice, potentially indicating reduced activity of these cells (Fig. 6A–G). In addition, SAR-20347 treatment significantly reduced IL-17 production as measured by average signal intensity (Fig. 6H), consistent with the gene expression analysis. TYK2 mutant mice showed significantly reduced IL-17 production as well (Fig. 6H). The number of CD4+ cells was not affected (Supplemental Fig. 2B) in either SAR-20347–treated or TYK2-deficient mice, potentially indicating a noncritical role for CD4+ T cells in this psoriasis model, but a more important role for IL-17–secreting γδ T cells (Fig. 7).

Discussion

JAK play a key role in mediating signaling from cytokine receptors. Because of the importance of cytokines in promoting autoimmune and inflammatory diseases, the JAK have received much attention.
attention as possible therapeutic targets for modulating the impact of cytokines on these diseases. TYK2 and JAK1 mediate signaling for a number of cytokines involved in psoriasis pathogenesis, including IL-12, IL-23, IL-6, IL-22, and IFN-α (20). In this study, we identified SAR-20347 as a small molecule inhibitor of TYK2/JAK1-dependent signaling, with nanomolar potency for TYK2/JAK1.

**FIGURE 5.** SAR-20347 reduces imiquimod-induced inflammation and antimicrobial peptide production. All animals were treated with imiquimod. WT animals received either 50 mg/kg SAR-20347 (n = 5) or vehicle (n = 6), and TYK2 mutant mice were treated with vehicle only (n = 6). Skin RNA was extracted after 6 d of imiquimod treatment, and expression of IL-22 (A), IL-23 (B), IL-17 (C), S100A9 (D), IL-6 (E), defensin β1 (F), S100A8 (G), IL-20 (H), IL-10 (I), IL-2 (J), and TNF (K) was determined by qPCR. Graphs represent mean 2^−ΔΔCt ± SD. *p < 0.05, for Δ cycle threshold (Ct) values compared with WT, t test. #p < 0.05, for ΔCt values compared with TYK2 mutant, t test.

**FIGURE 6.** SAR-20347 treatment and TYK2 mutant mice show reduced IL-17 and Vγ3. Back skin samples from mice treated with imiquimod [(A)–(C) at original magnification ×10; (D)–(F) at original magnification ×20]. (A and D) WT/vehicle mice, (B and E) WT/SAR-20347-treated mice, and (C and F) TYK2 mutant mice treated with vehicle. Micrographs of IL-17 (green), Vγ3 (red), and Hoechst (blue) staining. (G and H) Signal intensity for each animal was calculated in Image J and averaged. Graphs represent mean ± SD. *p < 0.05, compared with WT, t test.
of cytokines such as IL-6 (JAK1), IL-23 (TYK2), and IL-22 (JAK1 and TYK2) is predicted to significantly ameliorate psoriasis by modifying the inflammatory environment and the recruitment of pathogenic cells (Fig. 7).

Genome-wide association studies analysis has shown a strong association between psoriasis and TYK2 as well as Th17 cytokines in humans (13). Although the imiquimod model shares many similarities with human psoriasis, the model is not a perfect reflection of the human disease (16). With this in mind, we investigated which aspects of psoriasis were directly linked to TYK2 (using mutant mice) versus a combination of TYK2 and JAK1 (treatment with SAR-20347). Although both SAR-20347-treated animals and TYK2 mutant mice showed significantly reduced IL-6 and IL-17 in skin, mice treated with SAR-20347 also showed reduced IL-23 levels, decreased keratinocyte proliferation, and improved clinical score. The reduction of IL-23 levels in TYK2 mutant animals has been previously postulated to be the consequence of a feedback mechanism (29). Interestingly, the clinical score in TYK2 mutant mice was not significantly different from the WT mice treated with imiquimod, indicating that, although TYK2 mutants have lower overall Th17 and Th1 cell numbers (10), reduction of these cell populations alone is not sufficient for significant disease amelioration in the imiquimod model. Inhibition of both JAK1 and TYK2 by SAR-20347 during in vitro mouse Th cell development was effective in reducing the proliferation of CD4+ T cells and the development of Th1, Th2, and Th17 cells, while increasing the development of Tregs under Th17 conditions and not affecting iTreg development. Tipping the balance toward Tregs over Th17 cells in vivo would promote tolerance for self-Ag and reduce disease severity (30).

IL-17–producing γδ T cells have also been implicated in the pathogenesis of psoriasis. In the imiquimod mouse model, Cai et al. (31) showed that dermal γδ T cells are increased in both skin and lymph nodes of treated mice, and they secrete large amounts of IL-17. In our studies, IL-17 gene expression was lower in SAR-20347–treated mice compared with TYK2 mutants, and there was a reduction in Vγ3 staining in the skin consistent with the concept that dermal resident γδ T cells are a significant source of IL-17 in psoriasis. Therefore, the inability of IL-23 to signal through TYK2 may result in reduced activation and recruitment of both Th17 and γδ T cells that in turn result in diminished production of downstream effector cytokines.

Overexpression of IL-22 leads to psoriasis-like symptoms in mice (6), and IL-22 knockout mice or anti–IL-22 Ab administration protects mice from imiquimod-induced psoriasis-like dermatitis (32, 33). In the imiquimod model, SAR-20347–dosed mice show almost complete abolishment of IL-22 gene expression in affected skin, suggesting that the drug affects the ability of Th17 and γδ to induce IL-22. Not only does SAR-20347 prevent IL-22 production, but it also impairs IL-22 signaling, because treating cells in vitro with SAR-20347 completely blocked IL-22–dependent phosphorylation of STAT3. Moreover, IL-22 induces the production of IL-20, a cytokine that is elevated in both blood and skin lesions of psoriatic patients (6, 7). Our studies show that IL-20 is highly reduced in imiquimod-treated mice receiving SAR-20347, but minimally affected in TYK2 mutant mice, suggesting a dependency on JAK1 signaling. The striking difference in IL-20 gene expression between TYK2 mutant mice and SAR-20347–treated mice supports a role for SAR-20347 treatment in blocking the activity of the remaining IL-22 present in skin lesions, possibly by inhibiting the signaling through pSTAT3, further reducing the proinflammatory signaling cascade.

Previous studies have shown a dominant role for JAK1 in mediating IL-22 signaling in hepatic cell lines (9), but the specific contribution of TYK2 in IL-22 signaling has not been characterized. Consistent with what was observed with hepatic cell lines,

**FIGURE 7.** Proposed mechanism of action of SAR-20347 in ameliorating psoriasis-like symptoms. Activated DCs produce IL-23 and other proinflammatory cytokines, including IL-6. IL-23 induces γδ T cell activation, causing them to secrete IL-17 and IL-22. IL-6 contributes to the differentiation and, along with IL-23, proliferation of Th17 cells, which in turn also produce IL-22 and IL-17. Together with γδ T cells, Th17 cells activate keratinocytes to secrete antimicrobial peptides and proinflammatory cytokines such as IL-20, enhancing the inflammatory reactions in the skin. Treatment with SAR-20347 acts at multiple levels by blocking activity of IL-23, IL-22, and IL-6 and blocking their pathogenic effects on keratinocytes.

JAK1 and selectivity over JAK2 and JAK3 in both biochemical and cell-based assays. The pharmacokinetic profile of SAR-20347 was also favorable, demonstrating a moderate t½ of ~3 h, with excellent bioavailability and distribution to tissues. In assays using human primary cells, SAR-20347 inhibited TYK2 and JAK1 activity, as assessed by complete inhibition of IL-12–induced IFN-γ production in PBMCs, reporter expression in an IFN-α–responsive cell line, and pSTAT inhibition after stimulation with single cytokines (e.g., IL-12/pSTAT4, IL-6/pSTAT3). The in vitro profile of SAR-20347 suggests that it has the potential to be beneficial in diseases in which cytokines utilizing TYK2 and JAK1 play a critical role. The marked preference for TYK2/JAK1 offers distinct advantages over other JAK inhibitors that can interfere with JAK2, because inhibition of JAK2 can cause some degree of unwanted myelosuppression, particularly anemia and thrombocytopenia.

A number of cytokines associated with the Th17 pathway contribute to the pathogenesis of psoriasis in humans and in the imiquimod-induced psoriasis mouse model. Notably, neutralization of IL-23 and IL-17 has shown promising results in the clinic (16, 23, 24). IL-23 may contribute to psoriasis by supporting the proliferation of Th17 cells, which produce proinflammatory cytokines, including IL-17 and IL-22 (25). IL-6 also plays a role in Th17 development and is upregulated in psoriatic skin and serum (26, 27). Upon imiquimod application, DCs produce IL-6 and IL-23 (Fig. 6) (28), causing Th17 and γδ T cells to produce proinflammatory cytokines such as IL-17 and IL-22. The proinflammatory environment in the skin causes keratinocyte hyperproliferation and production of proinflammatory cytokines, including IL-20, antimicrobial peptides, and defensins. Acting as a feedback loop, these proinflammatory conditions cause further production of IL-23 and IL-6 from activated DCs and contribute to psoriasis. Therefore, an inhibitor of TYK2 and JAK1 that blocks the activity

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JAK1 knockout in the colonic cell line HT-29 resulted in almost complete elimination of IL-22–dependent STAT3 phosphorylation. In contrast, the knockdown of TYK2 resulted in ~50% reduction in pSTAT3. Although TYK2 may not be the main kinase used in IL-22 signaling, these results suggest that inhibition of TYK2 can limit or reduce IL-22 signaling functions. This concept was further strengthened by in vivo studies examining IL-22–dependent SAA production by the liver. When TYK2 mutant mice were injected with IL-22, we observed a significant reduction in SAA production compared with WT mice. Unexpectedly, inhibition of both TYK2 and JAK1 by SAR-20347 pretreatment in C57BL/6 mice did not completely abolish SAA production. Although interpretation of these data should take into account potential differences across strains (B10 and TYK2 mutant compared with C57BL/6 treated with SAR-20347), these data suggest that, in addition to TYK2 and JAK1, alternate pathways are capable of supporting IL-22 signaling in the absence of TYK2 and JAK1. Possible pathways may include p38 kinase, JNK, and ERK1/2, which have been previously shown to be activated by IL-22 (34). Further studies are ongoing to fully understand the contribution of JAK1 and TYK2 in IL-22 signaling.

Inhibition of JAK family members, resulting in a broad inhibition of inflammation, has been a very appealing approach in recent years, holding promise in treating a variety of inflammatory diseases. Previously developed JAK inhibitors, although very efficacious, show some side effects, including anemia and neutropenia, potentially due to the inhibition of JAK3–JAK2–mediated signaling of cytokines such as erythropoietin and GM-CSF. Xeloxitinib (INC018424; Jakafi) was originally developed as a JAK1/2 inhibitor, whereas tofacitinib (CP-690,550; Xeljanz), developed as a JAK3 inhibitor, has shown excellent efficacy in cytokine-mediated signaling of cytokines such as erythropoietin and thrombopoietin, potentially due to the inhibition of JAK3- and JAK2-mediated signaling in mice. Although TYK2 may not be the main kinase used in IL-22 signaling, these results suggest that inhibition of TYK2 and JAK1 by SAR-20347 pretreatment in C57BL/6 mice did not completely abolish SAA production. Although inhibition of both TYK2 and JAK1 by SAR-20347 pretreatment in C57BL/6 mice did not completely abolish SAA production. Although inhibition of both TYK2 and JAK1 by SAR-20347 pretreatment in C57BL/6 mice did not completely abolish SAA production. Although inhibition of both TYK2 and JAK1 by SAR-20347 pretreatment in C57BL/6 mice did not completely abolish SAA production. Although inhibition of both TYK2 and JAK1 by SAR-20347 pretreatment in C57BL/6 mice did not completely abolish SAA production. Although inhibition of both TYK2 and JAK1 by SAR-20347 pretreatment in C57BL/6 mice did not completely abolish SAA production. Although inhibition of both TYK2 and JAK1 by SAR-20347 pretreatment in C57BL/6 mice did not completely abolish SAA production. Although inhibition of both TYK2 and JAK1 by SAR-20347 pretreatment in C57BL/6 mice did not completely abolish SAA production. Although inhibition of both TYK2 and JAK1 by SAR-20347 pretreatment in C57BL/6 mice did not completely abolish SAA production. Although inhibition of both TYK2 and JAK1 by SAR-20347 pretreatment in C57BL/6 mice did not completely abolish SAA production. Although inhibition of both TYK2 and JAK1 by SAR-20347 pretreatment in C57BL/6 mice did not completely abolish SAA production. Although inhibition of both TYK2 and JAK1 by SAR-20347 pretreatment in C57BL/6 mice did not completely abolish SAA production. Although inhibition of both TYK2 and JAK1 by SAR-20347 pretreatment in C57BL/6 mice did not completely abolish SAA production. Although inhibition of both TYK2 and JAK1 by SAR-20347 pretreatment in C57BL/6 mice did not completely abolish SAA production. Although inhibition of both TYK2 and JAK1 by SAR-20347 pretreatment in C57BL/6 mice did not completely abolish SAA production. Although inhibition of both TYK2 and JAK1 by SAR-20347 pretreatment in C57BL/6 mice did not completely abolish SAA production. Although inhibition of both TYK2 and JAK1 by SAR-20347 pretreatment in C57BL/6 mice did not completely abolish SAA production. Although inhibition of both TYK2 and JAK1 by SAR-20347 pretreatment in C57BL/6 mice did not completely abolish SAA production. Although inhibition of both TYK2 and JAK1 by SAR-20347 pretreatment in C57BL/6 mice did not completely abolish SAA production. Although inhibition of both TYK2 and JAK1 by SAR-20347 pretreatment in C57BL/6 mice did not completely abolish SAA production. Although inhibition of both TYK2 and JAK1 by SAR-20347 pretreatment in C57BL/6 mice did not completely abolish SAA production. Although inhibition of both TYK2 and JAK1 by SAR-20347 pretreatment in C57BL/6 mice did not completely abolish SAA production. Although inhibition of both TYK2 and JAK1 by SAR-20347 pretreatment in C57BL/6 mice did not completely abolish SAA production. Although inhibition of both TYK2 and JAK1 by SAR-20347 pretreatment in C57BL/6 mice did not completely abolish SAA production. Although inhibition of both TYK2 and JAK1 by SAR-20347 pretreatment in C57BL/6 mice did not completely abol