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Dual Inhibition of TNFR1 and IFNAR1 in Imiquimod-Induced Psoriasiform Skin Inflammation in Mice

Lynda Grine, Lien Dejager, Claude Libert,1 and Roosmarijn E. Vandenbroucke1

Psoriasis is a chronic inflammatory skin disease affecting 2–3% of the world population and is mainly characterized by epidermal hyperplasia, scaling, and erythema. A prominent role for TNF in the pathogenesis of psoriasis has been shown, and consequently various types of TNF antagonists such as etanercept and infliximab have been used successfully. Recently, increasing amounts of data suggest that type I IFNs are also crucial mediators of psoriasis. To investigate whether blocking their respective receptors would be useful, TNFR1- and IFNAR1-deficient mice were challenged with Aldara, which contains imiquimod, and is used as an experimental model to induce psoriasis-like skin lesions in mice. Both transgenic mice showed partial protection toward Aldara-induced inflammation compared with control groups. Additionally, TNFR1 knockout mice showed sustained type I IFN production in response to Aldara. Double knockout mice lacking both receptors showed superior protection to Aldara in comparison with the single knockout mice and displayed reduced levels of IL-12p40, IL-17F, and S100A8, indicating that the TNF and type I IFN pathways contribute significantly to inflammation upon treatment with Aldara. Our findings reveal that dual inhibition of TNFR1 and IFNAR1 may represent a potential novel strategic treatment of psoriasis. The Journal of Immunology, 2015, 194: 5094–5102.
Aldara, which contains imiquimod (IMQ), a TLR7/8 agonist, has been increasingly used as an experimental model in mice because it induces psoriasiform lesions that resemble human psoriasis (7).

Clearly, both TNF and type I IFNs play a role in psoriasis (27). In this study, we show that mice lacking TNFR1 or IFNAR1 are modestly protected from Aldara-induced psoriasiform skin inflammation. Double knockout (DKO) mice deficient in both receptors are significantly better protected from psoriasis-like lesions induced by Aldara. We show that TNFR1 signaling mediates a negative feedback on type I IFN signaling upon TLR7 activation, and that IFNAR1 induces systemic IL-12p40 in response to Aldara. We propose dual inhibition of TNFR1 and IFNAR1 as a novel therapeutic strategy for the treatment of psoriasis.

Materials and Methods

Mice

C57BL/6J mice were purchased from Janvier Labs (Le Genest Saint Isle, France). TNFR1 KO mice generated by M. Rothe were a gift from H. Bleutmann. They were kept on a C57BL/6J background. IFNAR1 KO mice were provided by D. Bonaparte (Galbenik Institute of Science, Oeiras, Portugal). These mice were backcrossed for >10 generations into a homogeneous C57BL/6J background. Homozygous TNFR1/IFNAR1 DKO mice were generated by crossing homozygous TNFR1 KO mice with homozygous IFNAR1 KO mice, followed by intercrossing and selecting DKO mice. All animals were maintained in a specific pathogen-free background. IFNAR1 KO mice were provided by D. Bonaparte (Galbenik Institute of Science, Oeiras, Portugal). These mice were backcrossed for >10 generations into a homogeneous C57BL/6J background. Homozygous TNFR1/IFNAR1 DKO mice were generated by crossing homozygous TNFR1 KO mice with homozygous IFNAR1 KO mice, followed by intercrossing and selecting DKO mice.

IMQ-induced psoriasis-like skin lesions and scoring of symptoms

Mice were briefly sedated with isoflurane and the back skin of mice was shaved. Two days later, a dose of 62.5 mg Aldara cream (5% IMQ, 3M Pharmaceuticals) was applied daily on the back for 4–7 consecutive days. Mice were scored daily on type I IFN signaling upon TLR7 activation, and that IFNAR1 induces systemic IL-12p40 in response to Aldara. We show that TNFR1 signaling mediates a negative feedback on type I IFN signaling upon TLR7 activation, and that IFNAR1 induces systemic IL-12p40 in response to Aldara. We propose dual inhibition of TNFR1 and IFNAR1 as a novel therapeutic strategy for the treatment of psoriasis.

H&E staining

Tissue samples were fixed in 4% paraformaldehyde and embedded in paraffin. Deparaffinized 5-μm sections were stained with H&E. Images were taken with an Olympus BX51 light microscope (×40 magnification) and epidermal thickness was quantified with Volocity software (PerkinElmer).

Detection of TNFR1, TNF, IFN ligands, IL-17A/F, IL-12p40, IL-23p19, and IL-2p19

Mice were treated with IMQ and euthanized for blood, spleen, and skin collection at the indicated times. Blood was collected through cardiac puncture and allowed to clot at 4˚C overnight. Serum was collected after removal of the clot and centrifugation and stored at −80˚C until assayed. Whole-skin samples were homogenized in ice-cold buffer (PBS, 0.5% CHAPS, Roche Complete protease inhibitor). Homogenates were centrifuged for 30 min at 20,000 × g and 4˚C, after which the supernatant was collected and stored at −20˚C. Protein concentration was determined by the Bradford method (Bio-Rad). For the detection of TNFR1 levels, the mouse sTNF RI/TNFRSF1A duoset ELISA (R&D Systems) was used. Luminex technology was used to detect TNF, IL-12p40, IL-12p70, IL-23p19, IL-17A/F (Bio-Rad), and IFN-α and IFN-β (mouse IFN-α/β platinum; Procarta). All above-mentioned techniques were performed according to the manufacturers’ instructions.

Quantitative real-time PCR

Skin samples were collected in RNAlater (Ambion). RNA was isolated with the RNeasy fibrous tissue kit (Qiagen) according to the manufacturer’s instructions. RNA concentration was measured with the NanoDrop 1000 (Thermo Scientific) and quality was checked using the Agilent 2100 Bioanalyzer, and 1 μg RNA was used to prepare cDNA with SuperScript II (Invitrogen). Quantitative real-time PCR (qRT-PCR) was performed using SensiFAST SYBR No-ROX kit (Bioline) and the LightCycler 480 (Roche). Expression levels were normalized to the expression of the two most stable reference genes, which were determined for each condition using geNorm (qBase, Biogazelle). Values are represented as relative expression normalized to the geometric mean of the two selected most stable reference genes. Primer sequences and references genes can be found in Tables I and II, respectively. Table III lists the fold inductions of the transcript levels in IFNAR1 KO, TNFR1 KO, and DKO skin for the indicated genes from day 0 to 6 after IMQ.

Statistical analysis

Results (mean ± SD or SEM) were compared with a two-tailed Student t test. A p value < 0.05 was considered significant. In vivo experiments were repeated four times. Transcriptional data has been performed twice. Serum markers were measured three times.

<table>
<thead>
<tr>
<th>Condition</th>
<th>Samples</th>
<th>Reference Genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>0, 2, 4, or 6 d + IMQ</td>
<td>TNFR1 + IFNAR1 KO + control groups</td>
<td>Rpl + Hprt</td>
</tr>
<tr>
<td>0, 2, 4, or 6 d + IMQ</td>
<td>DKO</td>
<td>Rpl + Ubc</td>
</tr>
<tr>
<td>0, 2, or 6 h + IMQ</td>
<td>TNFR1 KO + control group</td>
<td>Rpl + Hprt</td>
</tr>
</tbody>
</table>

Table II. Reference genes per condition
Results

**TNFR1 signaling partially mediates IMQ-induced psoriasiform skin inflammation**

In 2009, Van der Fits et al. (7) proposed the topical application of the TLR7/8 agonist IMQ in the form of Aldara cream as a model for psoriasis. To investigate the role of TNFR1 in the TNF-mediated disease, we treated wild-type mice daily with Aldara, hereafter termed IMQ, on the back for 4 consecutive days and looked at the kinetics of TNF and TNFR1 in serum and whole-skin lysates, respectively. Upon treatment with IMQ, TNFR1 and TNF were induced on days 2 and 4, respectively (Fig. 1A). We then questioned whether inhibiting TNFR1 signaling is sufficient to ameliorate the Aldara-induced symptoms. Therefore, we treated wild-type and TNFR1 KO mice for 7 consecutive days with IMQ. Redness and scaling were scored daily as described previously (6).

Initially, all mice scored 0 on erythema, scaling, and thickening. During IMQ treatment, wild-type mice showed increased erythema, scaling, and epidermal thickening (Fig. 1B). TNFR1-deficient mice were moderately protected from both scaling and erythema during IMQ treatment. There was no difference in the IMQ-induced epidermal hyperplasia, suggesting that TNFR1 is not involved in the hyperproliferation of keratinocytes in the Aldara model (Fig. 1C, 1D). Parakeratosis, the retention of nuclei in the corneal layer, was not blocked by the absence of TNFR1 (data not shown). IMQ has been reported to cause splenomegaly (28), which we also observed in the wild-type mice (Fig. 1E). In contrast, TNFR1 KO mice showed significantly less splenomegaly (Fig. 1E).

To assess transcriptional differences between wild-type and TNFR1-deficient skin, markers that are generally upregulated in psoriasis were measured by qRT-PCR. On day 6 of IMQ treatment, expression of various genes was increased in wild-type mice (Fig. 1F). Remarkably, markers such as *Cornifelin* (*Cfn*) and *Involucrin* (*Ivl*) were significantly stronger induced in TNFR1 KO skin, whereas others, including *Keratin6b* (*Krt6b*) and *S100a8*, were less induced in the TNFR1 KO mice. The induction of *Loricrin* (*Lor*) expression in the absence of TNFR1 was higher than in wild-type mice.

### Table III. Transcriptional fold induction of markers in skin

<table>
<thead>
<tr>
<th>Markers</th>
<th>IFNAR1 KO</th>
<th>TNFR1 KO</th>
<th>DKO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cornifelin (<em>Cfn</em>)</td>
<td>3.1 ± 1.0</td>
<td>16.2 ± 3.7</td>
<td>2.3 ± 1.3</td>
</tr>
<tr>
<td>Involucrin (<em>Ivl</em>)</td>
<td>0.3 ± 0.1</td>
<td>7.9 ± 0.9</td>
<td>3.9 ± 1.8</td>
</tr>
<tr>
<td>Loricrin (<em>Lor</em>)</td>
<td>0.5 ± 0.1</td>
<td>15.2 ± 5.9</td>
<td>6.5 ± 5.3</td>
</tr>
<tr>
<td>Keratin6b (<em>Krt6b</em>)</td>
<td>2.1 ± 2.5</td>
<td>316.0 ± 167.5</td>
<td>12.2 ± 1.9</td>
</tr>
<tr>
<td><em>S100a8</em></td>
<td>1048.6 ± 787.4</td>
<td>235.5 ± 82.8</td>
<td>1.8 ± 0.8</td>
</tr>
</tbody>
</table>

**Sustained type I IFN signaling in TNFR1 KO mice**

IMQ induces large amounts of type I IFNs by stimulating pDCs, and these cytokines can initiate psoriasis (18). We wondered whether TNFR1 is modulating this process in the Aldara model. We treated wild-type and TNFR1 KO mice with IMQ and isolated...
skin and serum early after the first IMQ treatment and every other day during the following treatment days. After repeated treatment with IMQ, levels of IFN-α in the skin showed a tendency to be decreased in wild-type mice, but this difference did not reach statistical significance (Fig. 3A). This is in line with the observations of Walters et al. (29) and is presumably due to exhaustion of myeloid precursor cells and reduced pDC numbers. To measure the potential induction of IFN-α, we measured its levels in serum at different time points based on the observations of Walters et al., who reported that IFN-α was induced 3 h after application of Aldara. Indeed, both IFN-α and IFN-β were induced in wild-type mice 3 h after IMQ application, but decreased rapidly (Fig. 2B). Analysis of gene expression in skin lysates revealed transient upregulation of ISRE genes such as Cxcl10, Ifit2, and Usp18, which reflect IFN-α/β activity, as early as 2 h after topical application of IMQ (Fig. 2C). In contrast, TNFR1 KO mice did not display this transient expression of both type I IFN ligands and ISRE genes: IFN-α and IFN-β expression remained strong in the sera 4.5 h after IMQ application and in the skin 4 d later (Fig. 2A, 2B). Likewise, abrogation of TNFR1 signaling leads to sustained transcriptional expression of Cxcl10, Ifit2, and Usp18 six hours after IMQ challenge (Fig. 2C). These data clearly indicate that TNFR1 signaling is involved in silencing the type I IFN response in the Aldara model.

**Type I IFNs and IFNAR1 contribute to epidermal thickening during IMQ-induced psoriasiform skin inflammation**

To further investigate the role of type I IFNs signaling in the Aldara model, we examined the response to IMQ in mice deficient in the receptor 1 of type I IFNs (IFNAR1 KO mice). We treated wild-type mice and IFNAR1 KO mice for 7 consecutive days with IMQ and scored the responses as described above. Before any IMQ was applied, all mice scored 0 for each parameter. IFNAR1 KO mice exhibited significantly less erythema on day 4 but this protection was lost on day 7. Both mouse strains had similar scaling at each time point (Fig. 3A). Analysis of H&E-stained sections from affected back skin showed that epidermal hyperproliferation on day 7 was markedly less in IFNAR1 KO mice compared with WT mice, as depicted in Fig. 3B (quantification) and Fig. 3C (representative H&E images).

Similarly to the TNFR1 KO mice, IFNAR1 KO mice developed significantly less splenomegaly in comparison with IFNAR1 wild-type mice after IMQ (Fig. 3D). Additionally, IFNAR1 abrogation led to reduced expression of Cnfn, Ivl, Lor, and Ker6b, but it did not affect the gene expression levels of S100a8 (Fig. 3E).

It has been reported that treatment with IFN-α or IFN-β can affect TNFR1 and TNFR2 levels (30, 31). To test whether IFNAR1 is involved in the upregulation of TNFR1 observed in Fig. 1A, we measured TNFR1 protein levels in wild-type and IFNAR1-deficient skin treated with IMQ. As shown in Fig. 3F, the increase in TNFR1 protein level in response to IMQ was comparable in IFNAR1 KO mice and wild-type mice. Collectively, these results suggest that deletion of either TNFR1 or IFNAR1 can ameliorate IMQ-induced psoriasis-like skin lesions. Moreover, our data indicate that TNFR1 signaling is involved early in the Aldara model, in addition to type I IFNs, which have already been described as the initiating cytokines of psoriasis (18). **Deficiency in both TNFR1 and IFNAR1 leads to superior protection against IMQ-induced psoriasiform skin inflammation**

Next, we wondered whether abrogation of both receptors simultaneously could enhance protection. Therefore, we crossed TNFR1 KO mice with IFNAR1 KO mice to generate DKO mice deficient for both receptors. The same parameters were measured to assess the response of the DKO mice to IMQ-induced psoriasiform skin inflammation. In comparison with the single KO and wild-type mice, DKO mice had significantly reduced erythema and scaling on day 4 and no psoriatic lesions by day 7 (Fig. 4A). Abrogation of both receptors did not result in less splenomegaly compared with the single KOs (Fig. 4B). DKO mice also displayed significantly less IMQ-induced epidermal thickening compared with the single KOs and wild-type mice on day 7, but not in comparison with control cream (Fig. 4C, 4D). Overall, DKO

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**FIGURE 2.** Deficient TNFR1 signaling leads to increased type I IFNs and ISRE signature. Wild-type and TNFR1 KO mice were treated with IMQ for 4 consecutive days and skin and blood were isolated at the indicated time points (mean ± SD; n = 4). (A) Kinetics of IFN-α protein levels in whole skin lysates during 4 d. (B) Kinetics of IFN-α (left) and IFN-β (right) protein levels in serum. (C) qRT-PCR analysis of typical ISRE genes (Cxcl10, Ifit2, and Usp18) in whole-skin lysates. Bars represent relative transcript levels (mean ± SD; n = 4). Black indicates wild-type mice; light gray indicates TNFR1 KO mice. *p < 0.05, **p < 0.01, ***p < 0.001, ****p > 0.0001.
mice displayed reduced or intermediate fold induction of several markers in comparison with TNFR1 KO and IFNAR1 KO mice (Table III). Altogether, our data suggest that simultaneous deletion of TNFR1 and IFNAR1 leads to better control of IMQ-induced psoriasis-like skin lesions.

**TNFR1 and IFNAR1 signaling and the IL-23/IL-17 axis in IMQ-induced psoriasiform skin inflammation**

Van der Fits et al. (7) described the importance of the IL-23/IL-17 axis in IMQ-induced psoriasis-like skin lesions using mice deficient in either IL-23p19 or IL-17RA (7). To assess the role of TNFR1 and IFNAR1 in the IL-23/IL-17 axis, serum was isolated at different time points during IMQ application. IMQ induced IL-12p40 expression in serum of wild-type mice with a peak 48 h after application (Fig. 5A). IL-12p40 is a subunit in both IL-12 and IL-23 and is a novel drug target for the treatment of psoriasis (32–34). The induction was also observed in serum of TNFR1 KO mice (Fig. 5A), indicating that the induction of IL-12p40 does not require TNFR1 activation. However, the increase of IL-12p40 was significantly less pronounced in serum of IMQ-treated IFNAR1 KO mice and DKO mice (Fig. 5A). As IL-12p40 is a common subunit of IL-23 and IL-12, we measured total IL-12 (IL-12p70) and IL-23p19 levels in serum. We did not observe a change in circulating IL-12 levels and, surprisingly, IL-23p19 levels were not detectable in serum (data not shown) in the Aldara model.

Next, we measured IL-17A and IL-17F separately in serum. Pantelyushin et al. (35) found that IL-17F has a more important role in the IMQ-induced psoriasiform skin inflammation model than does IL-17A by comparing the response of IL-17A and IL-17F KO mice. IL-17F KO mice were better protected against challenge with repeated IMQ treatments compared with the IL-17A KO mice. In blood, we detected an increase of IL-17F levels (Fig. 5B) but not of IL-17A levels (data not shown). This induction was significantly less outspoken in TNFR1 KO mice, whereas IFNAR1 KO mice had significantly reduced IL-17F serum levels compared with wild-type and TNFR1 KO mice. The DKO mice showed no significant difference in serum IL-17F levels after IMQ treatment, and levels were significantly lower compared with wild-type and TNFR1 KO mice, but not in comparison with IFNAR1 KO mice. These data provide evidence that TNFR1 and IFNAR1 signaling affects the systemic levels of IL-23/IL-17 axis–associated cytokines in the Aldara model.

**Discussion**

IMQ is a TLR7/8 agonist used to treat basal cell carcinoma, squamous cell carcinoma, actinic keratosis, and genital and perianal warts and is used in the form of a topically applied cream called Aldara. Its medicinal use has been associated with de novo induction or exacerbation of psoriasis (36–38). This led to the use of a cream containing IMQ on mice to mimic the inflammatory skin disease (7, 29, 35, 39).

The Aldara model has been increasingly used as a model in mice to induce psoriasis-like lesions in mice. It is an easy model, as it does not require any surgical expertise such as xenograft models,
and it induces skin inflammation in all murine backgrounds, although severity can differ. Although its active ingredient is IMQ, other ingredients of Aldara may also affect the skin (29). Its main disadvantage is the lack of chronicity to closely resemble psoriasis, but over the years it has been shown that most relevant pathways in human psoriasis are also implicated in the pathology of IMQ-induced skin inflammation, justifying its use as an experimental in vivo model (40). In the present study, we studied the response of TNFR1, IFNAR1, and double-deficient mice upon daily treatment of the skin with Aldara.

TNF is upregulated in human psoriatic lesional skin and is thought to maintain the inflammatory loop in these lesions (5). The clinical benefits of TNF antagonists have confirmed its proinflammatory role in psoriasis and other autoimmune diseases, such as rheumatoid arthritis and Crohn’s disease. However, these drugs have side effects ranging from increased susceptibility to infections to de novo induction of psoriasis, as mentioned above. Although TNF is mainly known as a very potent proinflammatory cytokine, it is essential for eliciting an appropriate immune response. We think that the current strategy of TNF inhibition is not optimal because of the different axes involved in TNF signaling (12). Indeed, TNF can bind to two different receptors, TNFR1 and TNFR2. Using the current TNF antagonists, the actions of TNF via both TNFR1 and TNFR2 are abolished. Although both receptors bind TNF, they differ in biological actions. Inhibition of soluble TNF or TNFR1 increases the specificity of the treatment by targeting the proinflammatory axis of TNF signaling while leaving the regulatory TNFR2 pathway intact. Consequently, several studies suggest that specific inhibition of soluble TNF or TNFR1 in autoimmune diseases might be sufficient to achieve benefits similar to those of TNF antagonists, but without causing side effects (12, 41, 42). Although we did not address this issue, we do show that genetic ablation of TNFR1 is sufficient to partially protect mice from the full-blown skin inflammation upon IMQ treatment. Furthermore, we report specific upregulation of TNFR1 in the skin treated with IMQ, drawing another parallel to human psoriasis, in which TNFR1 expression is also increased (43, 44). This finding shows another similarity between the IMQ-induced psoriasis-like skin inflammation mouse model and human psoriasis.

To elucidate the role of type I IFNs in IMQ-induced psoriasis-like skin lesions, we investigated the induction of type I IFN-responsive genes. We found elevated levels of IFN-α, IFN-β, and a significant ISRE signature early after topical application of IMQ, which rapidly decreased in wild-type mice after their induction peak. We hereby confirm the very early and transient actions of type I IFNs in psoriasis proposed by Nestle et al. (18). However, a study showed that treatment with an mAb raised against IFN-α, MEDI-545, failed to reveal efficacy in reducing disease activity (45). Possibly, blocking IFN-α might not be sufficient, because IFN-β also present in psoriatic plaques and signals through the same heterodimeric complex IFNAR1/IFNAR2. Additionally, type I IFNs are thought to initiate psoriasis, whereas MEDI-545 was used in patients with established psoriatic plaques, which could also explain the inefficacy of MEDI-545. Remarkably, in the absence of TNFR1 signaling, both IFN production and ISRE gene expression were sustained in serum and skin, respectively. This might be due to inefficient maturation of pDCs and enhanced release of IFN-α in response to blockade of TNF signaling, as previously reported by Palucka et al. (26). This correlates with the observations that patients on anti-TNF therapy to treat other autoimmune diseases can have increased type I IFN levels and develop cutaneous lesions with psoriasiform morphology (46, 47). It has been suggested that a balance between TNF and type I IFNs is lost upon treatment with TNF antagonists, leading to unopposed increases in IFN levels. We hypothesize that patients with rheumatoid arthritis or Crohn’s disease on anti-TNF drugs are more vulnerable to developing psoriasis upon, for example, TLR7 activation and will produce excessive and sustained amounts of type I IFNs. Moreover, Ueyama et al. (39) described how the effects of IMQ are enhanced in the presence of IFN-α, leading to production of IL-1β, IL-6, IL-23, inducible NO synthase/NO, and TNF. Although TNF can be inhibited by anti-TNF treatment, the afore-
mentioned cytokines can lead to increased inflammation in these patients, especially when type I IFNs are already increased in these patients. It is therefore recommended to evaluate whether these patients would benefit from anti-IFNAR1 treatment to avoid psoriasis as a side effect. In human psoriatic lesions, a set of 57 genes was identified that were typically associated with antiviral defense and termed STAT1−57 and were repressed following treatment with biologics (48). These genes were enriched for ISRE sequences and thus inducible by type I IFNs, but also by, for example, IFN-γ and TNF. This implies that the interaction between TNFR1 and IFNAR1 is much more complex than anticipated, as it depends on the genetic background, environment, and other parameters that contribute to the heterogeneity. Although we did not provide evidence for this, TNFR1 and IFNAR1 signaling might come together at the level of transcription factors, such as PI3K and STAT.

In our study, we evaluated skin-specific markers to assess the severity of psoriasiform lesions. Remarkably, when both IFNAR1 and TNFR1 signaling are impaired, each marker follows one of the single KO phenotypes, except for S100a8, which is less induced in DKO mice compared with the single KO mice, indicating that its induction is dependent on both pathways. However, TNF and IL-17 have been reported to induce S100a8 synergistically (49, 50). Because IL-17F is reduced in both TNFR1 KO and IFNAR1 KO mice, this raises the question whether the reduced IL-17F levels affect the expression of S100a8 in DKO mice as well. This remains to be investigated in more depth.

Van der Fits et al. (7) and many others have provided solid evidence for a prominent role for the IL-23/IL-17 axis in psoriasis (35, 51, 52). Although the IL-17 cytokine family consists of multiple ligands, IL-17A is the most characterized ligand and is often referred to as IL-17. However, many studies have revealed an important role for IL-17F as well, including a study performed by Pantelyushin et al. (35) where they reported more IL-17F–producing cells in IMQ-inflamed skin than IL-17A– secreting cells. Similarly, we observed no changes in IL-17A serum levels during IMQ treatment, whereas IL-17F increased significantly. In both single and DKO mice, IL-17F was less increased than in wild-type control mice. Although type I IFNs have been reported in some models to inhibit IL-17A/F through the induction of IL-10 or IL-27 (53, 54), we show in the present study reduced IL-17F in the absence of IFNAR1 signaling. Similarly, TNFR1 deficiency resulted in decreased IL-17F blood levels. There is, however, no additive effect of both signaling pathways, as dual deletion of both TNFR1 and IFNAR1 resulted in IL-17F levels in response to IMQ treatment comparable to those in IFNAR1 KO mice. Moreover, IL-12p40 levels were affected as well. IL-12p40 is a subunit shared by IL-12 and IL-23 and has recently been validated as a therapeutic target for psoriasis (32–34). Although IL-12 and IL-23 are not strictly known as typical type I IFN–induced genes, the presence of ISRE elements in the promoter regions for units p35, p40, and p19 have been described (55–59). It is remarkable that the p40 subunit was detected, but complete IL-12 or IL-23 was not found in circulation. The subunits of IL-12 and IL-23 do not share similar promoters, but are only formed when the respective subunits are coexpressed in the same environment (60, 61). This should allow the immune system to steer the immune response in a delicate and subtle way depending on the situation. However, the role of systemic IL-12p40 in psoriasis remains unclear.

Our results demonstrate the complexity of the interactions of two cytokines, TNF and type I IFNs, and their receptors, TNFR1 and IFNAR1, respectively, and their respective effects on other cytokines and antimicrobial proteins as depicted in a schematic overview in Fig. 6. We show that they both mediate parts of the inflammatory pathogenesis of IMQ-induced psoriasiform skin inflammation and that simultaneous inhibition of both pathways...
améliorates IMQ-induced skin inflammation. Although we have not tested the hypothesis of dual inhibition in other psoriasis models and we lack information from clinical studies, our results show that it might be of interest to evaluate whether blocking both TNFR1 and IFNAR1 in psoriasis patients may achieve clinically superior results compared with TNF antagonists. Interestingly, therapeutic blocking tools for both IFNAR1 and TNFR1 are available.

First, by simultaneously inhibiting both TNFR1 and IFNAR1, one would prevent sustained type I IFN production during blockade of the TNF/TNFRI axis. Consequently, the paradoxical side effects associated with the current TNF antagonists, namely de novo induction of psoriasis, could possibly be resolved by additionally targeting the IFNAR1 pathway. Second, TNFR1 and IFNAR1 have different targets, promoting inflammation through different pathways in psoriasis. As a result, dual inhibition results in additive and synergistic reduction of psoriatic symptoms by silencing various pathways. This is very interesting, because the complex biology underlying a disease is often multifactorial and requires a multifaceted approach, which can be achieved by combination therapies. Blocking both TNFR1 and IFNAR1 reduces activation of the IL-23/IL-17 axis during IMQ-mediated skin inflammation through reduction of IL-12p40 and IL-17F levels, two novel drug targets in psoriasis, Crohn’s disease, and rheumatoid arthritis (34, 64–69).

In conclusion, our findings warrant study of the potential strategic advantages of combined inhibition of the cytokine receptors TNFR1 and IFNAR1 for the treatment of psoriasis.

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Disclosures

The authors have no financial conflicts of interest.

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