Received: 30 August 2021 | Revised: 28 February 2022 | Accepted: 13 March 2022

DOI: 10.1111/exd.14566

CONCISE COMMUNICATION

Experimental Dermatology WILEY

Suppression of IL-12/IL-23 p40 subunit in the skin and blood of psoriasis patients by Tofacitinib is dependent on active interferon-γ signaling in dendritic cells: Implications for the treatment of psoriasis and interferon-driven diseases

Nanette L. A. Vincken[1,2] | Paco M. J. Welsing[2] | Sandra C. Silva-Cardoso[1,2] | Cornelis P. J. Bekker[1,2] | Ana P. Lopes[1,2] | Michel Olde Nordkamp[1,2] | Emmerik F. A. Leijten[1,2] | Timothy R. D. J. Radstake[1,2] | Chiara Angiolilli[1,2]

[1]Center for Translational Immunology, Department of Immunology, University Medical Center Utrecht, Utrecht University, Utrecht, The Netherlands

[2]Department of Rheumatology and Clinical Immunology, University Medical Center Utrecht, Utrecht University, Utrecht, The Netherlands

Correspondence
Nanette L. A. Vincken, Center for Translational Immunology (CTI) University Medical Center Utrecht, Utrecht University, Lundlaan 6, 3508 GA, Utrecht, The Netherlands.
Email: N.L.A.Vincken@umcutrecht.nl

Funding information
This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors

Abstract

Interleukin (IL)-12 and IL-23 are pro-inflammatory cytokines produced by dendritic cells (DCs) and associated with Psoriasis (Pso) and Psoriatic Arthritis (PsA) pathogenesis. Tofacitinib, a Janus kinase inhibitor, effectively suppresses inflammatory cascades downstream the IL-12/IL-23 axis in Pso and PsA patients. Here, we investigated whether Tofacitinib directly regulates IL-12/IL-23 production in DCs, and how this regulation reflects responses to Tofacitinib in Pso patients. We treated monocyte-derived dendritic cells and myeloid dendritic cells with Tofacitinib and stimulated cells with either lipopolysaccharide (LPS) or a combination of LPS and IFN-γ. We assessed gene expression by qPCR, obtained skin microarray and blood Olink data and clinical parameters of Pso patients treated with Tofacitinib from public data sets. Our results indicate that in DCs co-stimulated with LPS and IFN-γ, but not with LPS alone, Tofacitinib leads to the decreased expression of IL-23/IL-12 shared subunit *IL12B* (p40). In Tofacitinib-treated Pso patients, IL-12 expression and psoriasis area and severity index (PASI) are significantly reduced in patients with higher IFN-γ at baseline. These findings demonstrate for the first time that Tofacitinib suppresses IL-23/IL-12 shared subunit *IL12B* in DCs upon active IFN-γ signaling, and that Pso patients with higher IFN-γ baseline levels display improved clinical response after Tofacitinib treatment.

KEYWORDS
dendritic cells, interferon, Janus Kinase Inhibitors, p40, psoriasis

1 | BACKGROUND

Dendritic cells (DCs) are professional antigen-presenting cells that form the bridge between innate and adaptive immunity. Myeloid dendritic cells (mDCs) are circulating DCs,[1] while monocyte-derived dendritic cells (moDCs) are tissue-resident DCs that are present at the sites of inflammation.[2] Activated DCs produce pro-inflammatory cytokines that prime naïve T-lymphocytes into specific effector phenotypes[3,4] and, when undergoing inappropriate chronic activation, can drive autoimmunity.[5]

Some of the pathways by which DCs contribute to chronic immune activation are induced by the production of IL-23 and IL-12.[6] The IL-23 cytokine consists of two subunits, *IL23A* (IL-23p19) and *IL12B* (IL-12p40). The p40 subunit is shared with IL-12, while p19 is unique to IL-23. Both IL-12 and IL-23 have an important role in the differentiation of naïve T-lymphocytes into T-helper (Th) interferon (IFN)-γ-producing Th1 or IL17-producing Th17 cells, respectively.[7-10]

Disorders associated with deregulations of the IL-23/IL-17 and IL-12/IFN-γ immune axis include psoriasis (Pso) and psoriatic arthritis (PsA), among others.[6] Pso patients display an increased presence of inflammatory DCs expressing IL-12 and IL-23 in lesional skin.[11] Similarly, in PsA patients, elevated IL-12 expression is observed in serum and synovial fluid.[12-14]

Treatment of these diseases with Janus kinase (JAK) inhibitors has proven to be effective in recent clinical trials.[15] Specifically, Tofacitinib, an oral small molecule inhibitor targeting JAK1, JAK2, JAK3 and, to a lower extent, Tyrosine Kinase 2 (TYK2)[16] significantly reduces psoriatic and arthritic manifestations, while displaying comparable benefit/risk profiles with biologicals.[17,18]

In vitro studies indicate that Tofacitinib suppresses the differentiation of Th1 and Th17 cells and the production of IFN-γ and IL-17.[19,20] A plausible mechanism of action consists in the suppression of JAK2/TYK2 activation mediated by IL-12 and IL-23 binding, which further prevents STAT3 and STAT4 nuclear translocation.[21] However, Tofacitinib was also shown to reduce *IL12B* and *IL23A* mRNA levels in Pso lesional skin and in imiquimod-treated mice, suggesting a direct role in the regulation of the upstream cytokines leading to Th1 and Th17 differentiation.[22,23]

2 | QUESTIONS ADDRESSED

Current literature indicates that Tofacitinib suppresses the inflammatory cascades downstream IL-12/IL-23, key cytokines in Pso/PsA development.[6] However, whether Tofacitinib is able to suppress IL-12/IL-23 production by DCs has not been documented. Here, we aimed to investigate whether Tofacitinib is able to regulate the expression of IL-12 and IL-23 in DCs, and to determine how this regulation can influence responses to Tofacitinib in Pso patients.

3 | EXPERIMENTAL DESIGN

3.1 | Cell isolation

Peripheral blood mononuclear cells (PBMCs) derived from either healthy control blood or buffy coats were separated with Ficoll gradient (#17-1440-02, GE Healthcare). Blood was collected following institutional ethical approval. CD1c (BDCA-1)+ mDCs and CD14+ monocytes were isolated (Miltenyi Isolation Kit #130-090–506, #130-050–201) and separated on autoMACS Pro Separator according to manufacturer's instructions. Purity was checked by flow cytometry on BD LSRFortessa™ (BD Biosciences). Before culturing, cells were washed with complete medium consisting of RPMI 1640 medium, GlutaMAX™ Supplement (#61870-036, ThermoFisher Scientific), 10% Fetal Bovine Serum (Biowest) and 1% Penicillin-Streptomycin (#15070063, ThermoFisher Scientific). mDCs were stimulated on the day of isolation, after they were rested for 1 h at 37°C.

3.2 | Generation of monocyte-derived dendritic cells

To generate moDCs, monocytes were cultured at 37°C for 6 days at a density of 10^6 cells/ml in the presence of 500 U/ml IL-4 and 800 U/ml GM-CSF (#204-IL-50, #215-GM-500, both from R&D Systems).

TABLE 1 Sequences of human primers used for qPCR analysis

Gene	Sequence (5′ → 3′)
IL12B (IL12p40)	**Forward** TGCCGTTCACAAGCTCAAGT **Reverse** TGGGTCAGGTTTGATGATGTCC
IL23A (IL23p19)	**Forward** CAACAGTCAGTTCTGCTTGC **Reverse** GAAGGCTCCCCTGTGAAA AT
IL12A (IL12p35)	**Forward** AGGGCCGTCAGCAACATG **Reverse** TCTTCAGAAGTGCAAGGGTAAAATTC
CXCL10	**Forward** TGAAATTATTCCTGCAAGCCAA **Reverse** CAGACATCTCTTCTCACCCTTCTTT
TNF	**Forward** TCTTCTCGAACCCCGAGTGA **Reverse** CCTCTGATGGCACCACCAG
RPL35	**Forward** CATCTGGGGAAAAGTAACTCG **Reverse** AGCATCACTCGGATTCTGTG

Cytokines and medium were refreshed on day 3. On day 6, cells were harvested, washed with fresh complete medium and replated at a cell density of 10^6 cells/ml. Cells were left resting overnight at 37°C.

3.3 | Stimulation of moDCs and mDCs

Immature moDCs and mDCs were pre-treated or not with 1 μg/ml Tofacitinib (CP-690550, Selleckchem) or Ruxolitinib (INCB018424, Selleckchem) for 30 min. After, cells were stimulated with either 10 μg/ml Lipoteichoic acid (LTA) (#L2515, Sigma Aldrich), 100 ng/ml LPS, 1 μg/ml R848 (#tlrl-3pelps, #tlrl-r848, Invivogen), 1000 U/μl IFN-α (#CRI003B, Cell sciences) or 1000 U/ml IFN-γ (#14-8319–80, eBioscience) for 4 h.

3.4 | Statistical analyses of in vitro experimental data

Statistical analyses of DCs mRNA data were performed using GraphPad Prism 8.3 Software. Non-parametric Friedman test for paired samples followed by Dunn's multiple comparisons test were computed to compare gene expression levels between 0 and 4 h of stimulation. Values with a $p < 0.05$ were considered significant.

Details are provided in Appendix S1.

4 | RESULTS

4.1 | Treatment with Tofacitinib does not reduce *IL23A* and *IL12B* mRNA expression in TLR4-activated DCs

Consistent with previous findings,[24] we observed that the mRNA expression of IL-23p19 (*IL23A*), IL-12p40 (*IL12B*) and IL-12p35 (*IL12A*) in both mDCs and moDCs is induced by TLR2, TLR4 and TLR7/8 activation(Figure S1A–C). TLR4 was previously found upregulated in Pso PBMCs[25] and skin.[26,27] Additionally, serum and epidermal expression of S100A8 and S100A9, TLR4 ligands contributing to keratinocyte hyperproliferation, were found to be significantly higher in Pso patients in comparison with healthy controls.[28] Therefore, we chose TLR4 activation by LPS as a model to mimic DC activation in Pso blood (Table 1).

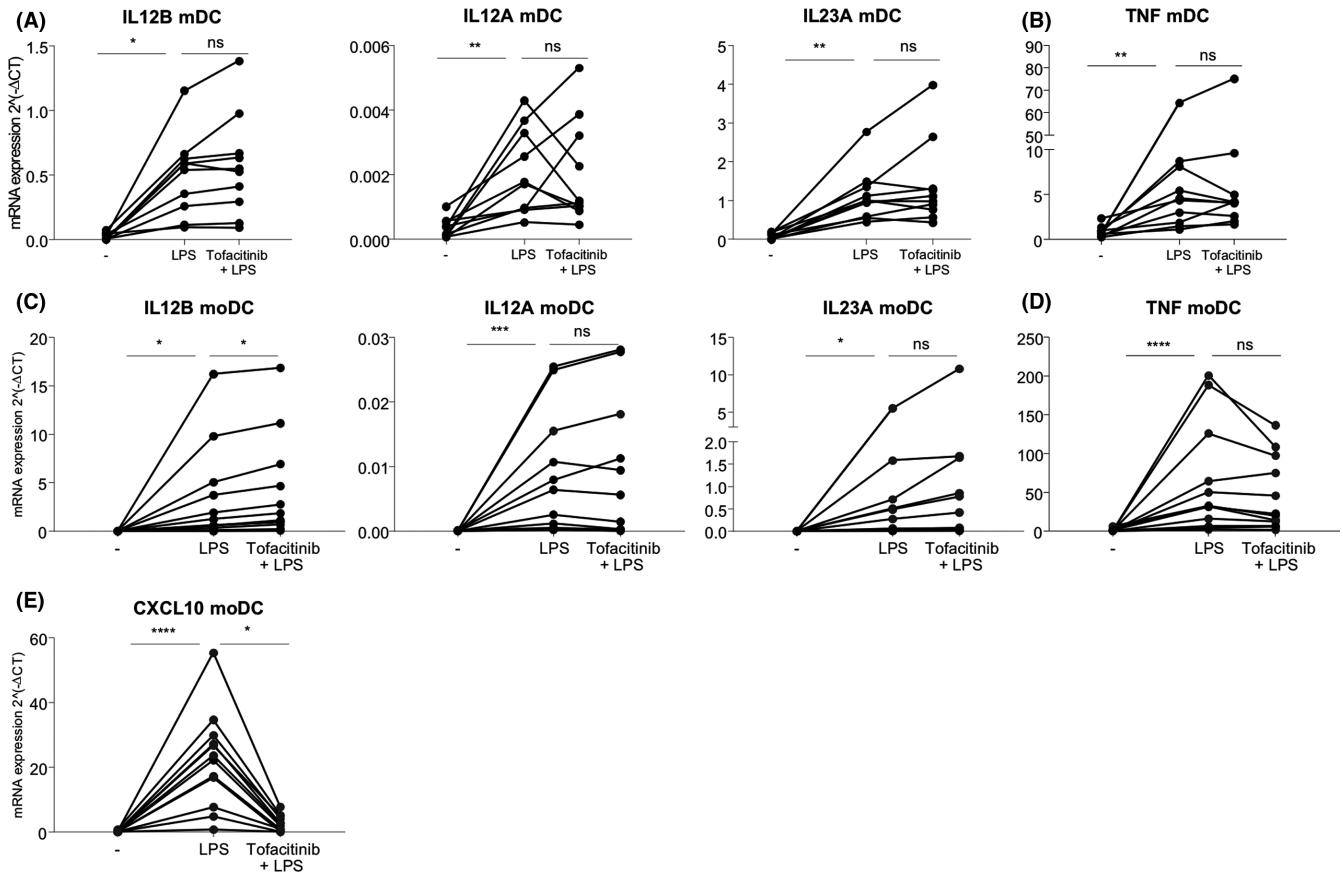

FIGURE 1 Cytokine gene expression in LPS-stimulated DCs treated with Tofacitinib. (A-B) Gene expression of *IL12B*, *IL12A* and *IL23A* subunits (A) and *TNF* (B) in mDCs ($n = 10$). (C-E) *IL12B*, *IL12A* and *IL23A* subunits (C), *TNF* (D) and CXCL10 (E) in moDCs ($n = 12$). mDCs and moDCs were pre-treated or not with Tofacitinib for 30 min and stimulated with LPS for 4 h. Gene expression was measured by real-time qPCR and represented as relative expression ($2^{-\Delta CT}$). Lines connect individual donors. Significance was determined by Friedman's test followed by Dunn's multiple comparisons test

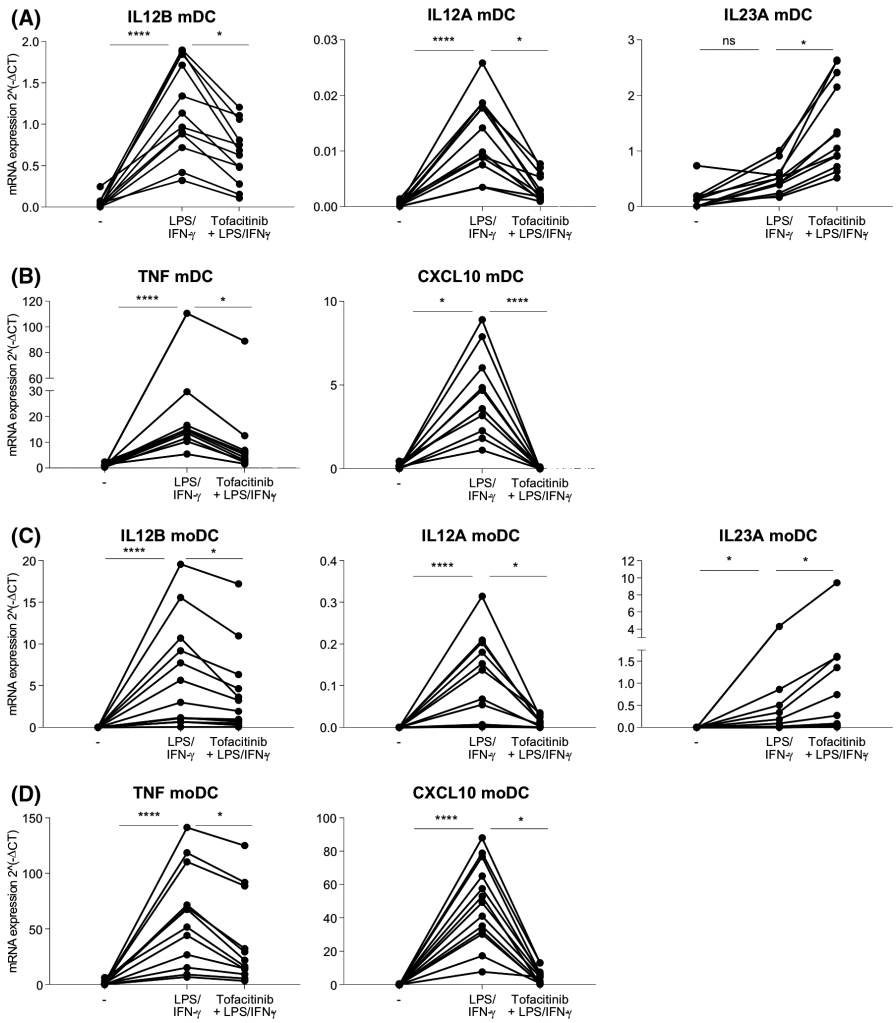

FIGURE 2 *IL12B* gene expression is suppressed by Tofacitinib in the presence of IFN-γ in LPS-stimulated mDCs and moDCs. (A, B) Gene expression of *IL12B*, *IL12A*, *IL23A* (A) and *TNF* and *CXCL10* (B) in mDCs ($n = 12$). (C-D) Gene expression of *IL12B*, *IL12A* and *IL23A* (C) and *TNF* and *CXCL10* (D) in moDCs ($n = 13$). mDCs and moDCs were pre-treated or not with Tofacitinib for 30 min and stimulated with a combination of LPS and IFN-γ for 4 h. Gene expression was measured by real-time qPCR and represented as relative expression ($2^{-\Delta CT}$). Lines connect individual donors. Significance was determined by Friedman's test followed by Dunn's multiple comparisons test

In LPS-stimulated mDCs and moDCs pre-treated with Tofacitinib, we could not observe a reduction in the expression of *IL23A*, *IL12B* and *IL12A* (Figure 1A,C). Surprisingly, the expression of *TNF*, another key cytokine playing a role in Pso pathogenesis and suppressed by Tofacitinib *in vivo*,[22] was also not suppressed by Tofacitinib in LPS-stimulated DCs (Figure 1B,D). We could confirm that Tofacitinib efficiently blocks the JAK/STAT signaling in these cells, as determined by the suppression of control *CXCL10* expression in paired samples (Figure 1E). We further demonstrated that lack of downregulation of IL-12/IL-23 cytokines in LPS-stimulated DCs also occurs in the presence of the JAK1/JAK2 inhibitor Ruxolitinib (Figure S2), thus it is not solely attributed to Tofacitinibs mode of action.

4.2 | Co-stimulation with LPS and IFN-γ leads to reduced expression of *IL12B* mRNA in DCs treated with Tofacitinib

Previously, Bechera et al. reported that JAK1 inhibition in moDCs suppresses IL-23 and IL-12 expression when cells are stimulated with a combination of nickel sulphate, a TLR4 agonist, and IFN-γ.[29] We thus investigated the effects of LPS and IFN-γ stimulation on mDCs and moDCs pre-treated with Tofacitinib. Co-stimulation of DCs with LPS and type II IFN-γ led to a significant reduction of *IL12B*, *IL12A*, *TNF* and *CXCL10* mRNA expression upon Tofacitinib treatment (Figure 2A–D). Conversely, co-stimulation with type I IFN-α did not lead to similar modulatory effects in these cells (Figure S3). These data indicate that an active type II, but not type I, IFN signaling in DCs is required for the suppression of *IL12B* and other pro-inflammatory cytokines by Tofacitinib.

4.3 | Psoriasis patients with higher IFN-γ levels display reduced IL-12 expression and skin severity score after Tofacitinib treatment

To test the relevance of IFN-γ stimulation in the suppression of IL-12 by Tofacitinib, we analysed publicly available data of Pso patients before and after Tofacitinib treatment to assess whether higher baseline levels of IFN-γ could predict lower IL-12 production and improved Psoriasis Area and Severity Index (PASI) score after Tofacitinib treatment.

From blood protein Olink data (GSE136435) obtained from 266 psoriasis patients, we found that IL-12 levels were significantly

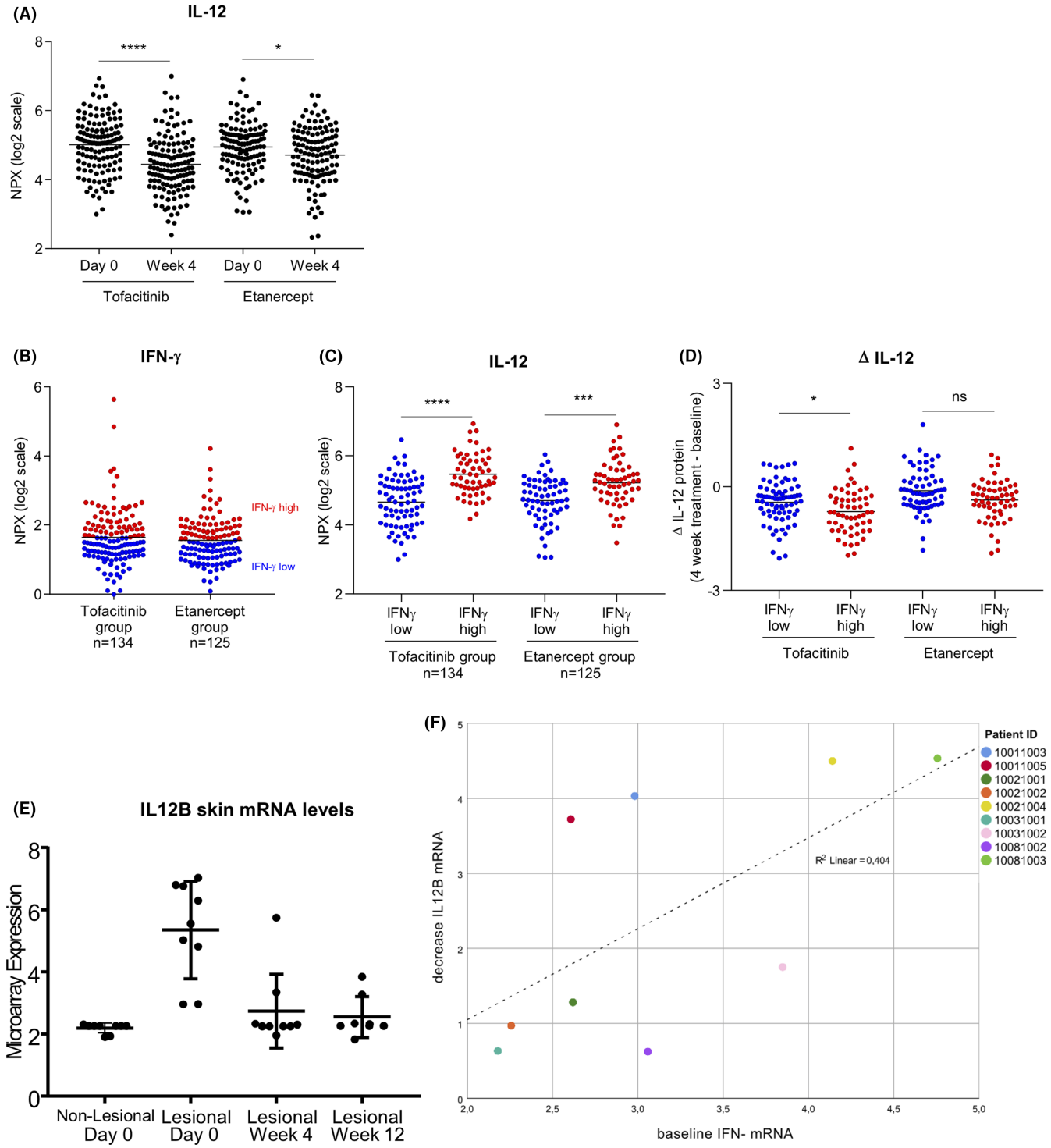

FIGURE 3 Patients with higher basal IFN-γ display higher basal IL-12 levels and have a significant reduction in IL-12 levels when treated with Tofacitinib (A) Normalized Protein eXpression (NPX) of IL-12 protein levels in the blood of Pso patients before and after 4 weeks treatment with either Tofacitinib ($n = 134$) or Etanercept ($n = 125$). (B) NPX of baseline blood IFN-γ protein levels for patients treated with Tofacitinib or Etanercept. Treatment groups were divided by the mean IFN-γ expression to define IFN-γ high or low patients. (C) NPX of baseline IL-12 protein levels per treatment group for IFN-γ high or low patients. (D) Delta decrease of IL-12 (ΔIL-12) after 4 weeks of treatment with either Tofacitinib or Etanercept for IFN-γ high or low patients. Significance was determined by the Kruskal-Wallis test followed by Dunn's multiple comparisons test. Series matrix data derived from public database GSE136435. (E) Gene expression of *IL12B* after 0, 4 and 12 weeks of Tofacitinib treatment in whole lesional psoriatic skin ($n = 9$) compared to paired non-lesional skin. (F) Delta decrease of *IL12B* mRNA in relation to baseline *IFN-γ* mRNA in lesional psoriatic skin after 12 weeks of Tofacitinib treatment ($n = 9$). Data from patient 10021001 was only available until 4 weeks of treatment. Series matrix data derived from public database GSE69967

reduced after 4 weeks of Tofacitinib treatment, while more modestly reduced after 4 weeks of Etanercept treatment (Figure 3A). IL-23 was not determined in this assay.[30] We further assessed the baseline IFN-γ levels in the two treatment groups and defined IFN-γ-high and IFN-γ-low patients (Figure 3B). We found that patients with higher IFN-γ levels at baseline also displayed higher IL-12 levels (Figure 3C). By calculating the difference in IL-12 levels between 4 week treatment and baseline (ΔIL-12), we identified that patients with higher IFN-γ levels better benefited from Tofacitinib, but not Etanercept, treatment in terms of suppression of IL-12 levels (Figure 3D). To account for the low expression of circulating IFN-γ, we performed a principal component analysis (PCA) to identify IFN-γ-clustering proteins (Table S1). We found that a higher IFN-signature at baseline was related with a better reduction in PASI scores after 12 weeks of treatment with Tofacitinib (unstandardized $B = -2.701$, $p = 0.033$, 95%CI [−5.177, −0.226]). Conversely, in Pso patients treated for 12 weeks with Etanercept, an improved PASI outcome related to higher IFN-signature was not observed (unstandardized $B = 0.331$, $p = 0.735$, 95%CI [−1.606, 2.268]) (Figure S4).

From skin microarray data (GSE69967) obtained from 9 psoriasis patients, *IL12B* mRNA levels were reduced in lesional skin of Tofacitinib-treated Pso patients (Figure 3E), as previously described.[22] Given the low number of patients in this study, we could only observe a trend for lower ΔIL-12 expression in individuals with higher *IFN-γ* at baseline (Figure S5). However, higher basal *IFN-γ* levels correlated with a greater reduction of *IL12B* mRNA (decrease of 0.015 *IL12B* units per higher *IFN-γ* unit on average, $p = 0.039$, 95%CI [−0.029, −0.001]) (Figure 3F). Baseline characteristics are provided in Table S2.

5 | CONCLUSIONS AND PERSPECTIVES

The accumulating knowledge that IL-23 and IL-12 play a key role in the initiation and maintenance of several inflammatory diseases, such as Pso and PsA,[6] led to the development of multiple selective therapeutic agents that target either specific cytokines or their downstream inflammatory events.[31] Tofacitinib is the first JAK-inhibitor registered for the indication of Pso and has shown promising results in clinical trials.[32] Studies have shown that multiple immunoregulatory pathways, such as STAT1/STAT3 in Pso skin and NF-κB in PsA synovial fibroblasts, are targeted by Tofacitinib.[22,33,34] However, whether Tofacitinib suppresses IL-12 and IL-23 directly, or rather indirectly through general immune suppression, has not yet thoroughly been studied.[35]

To answer this research question, we made use of mDCs and moDCs as representative models for systemic and localized inflammation.[36] We observed that both cell types increased the expression of *IL23A* and *IL12B* after TLR4 stimulation, in line with previous studies.[24,35,37] The stimulation with IFN-γ, a direct activator of the JAK/STAT-pathway, did not induce the expression of these cytokines, while potently inducing *CXCL10*, a known IFN-inducible gene (Figure S1B).

Upon LPS activation, we found that Tofacitinib was not able to suppress *IL23A* and *IL12B* mRNA expression in neither mDCs nor moDCs. A similar observation was reported earlier in a model of allergic contact dermatitis by Bechara et al.[29] These results indicate that the presence of IFN-γ is needed for Tofacitinib to efficiently reduce *IL12B* expression in DCs.

Unlike *IL12B*, *IL23A* expression was not potentiated by LPS+IFN-γ stimulation, nor reduced by Tofacitinib. In fact, *IL23A* expression was decreased upon stimulation with LPS+IFN-γ, as compared with LPS alone (Figure S6). Combined LPS+IFN-γ stimulation in DCs was previously shown to promote *IL23A* mRNA degradation, while enhancing *IL12A* and *IL12B* mRNA stabilization.[38] Thus, these findings indicate that IL-12 and IL-23 could be differentially regulated by Tofacitinib in DCs.[38,39] However, as previously documented by Bechara et al., the overall IL-23 protein production is reduced by JAK inhibition as a result of the significant suppression of the IL-23 subunit IL12p40.[29] As such, Tofacitinib could still play a significant role in reducing IL-23 production, despite transiently inducing IL23A mRNA expression.

A differential response to Tofacitinib was observed in DCs stimulated with type I or type II IFN. Type II IFN-γ signals through JAK1/JAK2, which in turn promote STAT1 homodimers and interferon regulatory factor (IRF)1/IRF8 complexes.[40,41] Conversely, type I IFN-α and IFN-β signal through JAK1/TYK2, which lead to STAT1/STAT2 heterodimers associating with IRF9.[42] Indeed, IRF1 and IRF8 are potently induced upon LPS+IFN-γ co-stimulation, in comparison with LPS stimulation alone.[40,43] Thus, it is plausible that Tofacitinib-dependent suppression of *IL12B* expression requires inflammatory events triggered by type II, but not type I IFN signaling, such as modulation of IRF1/IRF8 transcription factors. From the skin data set, we could identify a distinct expression of IRF1/IRF8 in paired lesional and non-lesional samples of Pso patients and a reduced expression in lesional skin after Tofacitinib treatment (Figure S7).

In whole lesional Pso skin, we found that higher basal levels of IFN-γ correlated with a greater reduction in *IL12B* after 12 weeks of Tofacitinib treatment. Additionally, from whole blood data, we observed that Pso patients with higher IFN-signature at basal levels displayed a better decrease in IL-12 after Tofacitinib, but not Etanercept, treatment which was accompanied by a significant reduction in PASI score.

Overall, our findings imply a novel role for IFN-γ in eliciting *IL12B* suppression by Tofacitinib in DCs. These results can be relevant for diseases characterized by IFN-γ involvement and aid to predict therapeutic responses to Tofacitinib in Pso patients.

ACKNOWLEDGEMENTS

The reviewers are thanked for critically reading the manuscript and suggesting substantial improvements.

CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

AUTHOR CONTRIBUTIONS

Performed the research: NV, CA, MON, CB and EL. Designed the research study: NV, CA, SSC and AL. Contributed essential reagents or tools: NV, CA, SSC, AL and EL. Analysed the data: NV, CA and PW. Wrote the paper: NV, CA, TR and PW. All authors listed qualify for authorship according to the criteria as mentioned by the journal.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Nanette L. A. Vincken https://orcid.org/0000-0003-2892-4814

REFERENCES

1. Merad M, Sathe P, Helft J, Miller J, Mortha A. The dendritic cell lineage: ontogeny and function of dendritic cells and their subsets in the steady state and the inflamed setting. *Annu Rev Immunol*. 2013;31(9):563-604. doi:10.1146/annurev-immunol-020711-074950
2. Solano-Gálvez S, Tovar-Torres S, Tron-Gómez M, et al. Human dendritic cells: ontogeny and their subsets in health and disease. *Med Sci*. 2018;6(4):88. doi:10.3390/medsci6040088
3. Steinman RM, Hawiger D, Liu K, et al. Dendritic cell function in vivo during the steady state: a role in peripheral tolerance. *Ann N Y Acad Sci*. 2003;987:15-25. doi:10.1111/j.1749-6632.2003.tb06029.x
4. O'Keeffe M, Mok WH, Radford KJ. Human dendritic cell subsets and function in health and disease. *Cell Mol Life Sci*. 2015;72(22):4309-4325. doi:10.1007/s00018-015-2005-0
5. Ganguly D, Haak S, Sisirak V, Reizis B. The role of dendritic cells in autoimmunity. *Nat Rev Immunol*. 2013;13(8):566-577. doi:10.1038/nri3477
6. Croxford AL, Kulig P, Becher B. IL-12-and IL-23 in health and disease. *Cytokine Growth Factor Rev*. 2014;25(4):415-421. doi:10.1016/j.cytogfr.2014.07.017
7. Floss DM, Schröder J, Franke M, Scheller J. Insights into IL-23 biology: from structure to function. *Cytokine Growth Factor Rev*. 2015;26(5):569-578. doi:10.1016/j.cytogfr.2015.07.005
8. Yawalkar N, Tscharner GG, Hunger RE, Hassan AS. Increased expression of IL-12p70 and IL-23 by multiple dendritic cell and macrophage subsets in plaque psoriasis. *J Dermatol Sci*. 2009;54(2):99-105. doi:10.1016/j.jdermsci.2009.01.003
9. Sun M, He C, Cong Y, Liu Z. Regulatory immune cells in regulation of intestinal inflammatory response to microbiota. *Mucosal Immunol*. 2015;8(5):969-978. doi:10.1038/mi.2015.49
10. Fujino S, Andoh A, Bamba S, et al. Increased expression of interleukin 17 in inflammatory bowel disease. *Gut*. 2003;52(1):65-70. doi:10.1136/gut.52.1.65
11. Zaba LC, Fuentes-Duculan J, Eungdamrong NJ, et al. Psoriasis is characterized by accumulation of immunostimulatory and Th1/Th17 cell-polarizing myeloid dendritic cells. *J Invest Dermatol*. 2009;129(1):79-88. doi:10.1038/jid.2008.194
12. Nograles KE, Brasington RD, Bowcock AM. New insights into the pathogenesis and genetics of psoriatic arthritis. *Nat Clin Pract Rheumatol*. 2009;5(2):83-91. doi:10.1038/ncprheum0987
13. Wendling D, Cedoz JP, Racadot E. Serum and synovial fluid levels of p40 IL12/23 in spondyloarthropathy patients. *Clin Rheumatol*. 2009;28(2):187-190. doi:10.1007/s10067-008-1011-0
14. Schurich A, Raine C, Morris V, Ciurtin C. The role of IL-12/23 in T cell-related chronic inflammation: implications of immunodeficiency and therapeutic blockade. *Rheumatol*. 2018;57(2):246-254. doi:10.1093/rheumatology/kex186
15. Jamilloux Y, El Jammal T, Vuitton L, Gerfaud-Valentin M, Kerever S, Sève P. JAK inhibitors for the treatment of autoimmune and inflammatory diseases. *Autoimmun Rev*. 2019;18(11):102390. doi:10.1016/j.autrev.2019.102390
16. Hodge JA, Kawabata TT, Krishnaswami S, et al. The mechanism of action of tofacitinib - an oral Janus kinase inhibitor for the treatment of rheumatoid arthritis. *Clin Exp Rheumatol*. 2016;34(2):318-328. doi:10.1200/JCO.1987.5.2.266
17. Strober BE, Gottlieb AB, Kerkhof P, et al. Benefit-risk profile of tofacitinib in patients with moderate-to-severe chronic plaque psoriasis: pooled analysis across six clinical trials. *Br J Dermatol*. 2019;180(1):67-75. doi:10.1111/bjd.17149
18. Mease P, Hall S, FitzGerald O, et al. Tofacitinib or Adalimumab versus placebo for psoriatic arthritis. *N Engl J Med*. 2017;377(16):1537-1550. doi:10.1056/NEJMoa1615975
19. Ghoreschi K, Jesson MI, Li X, et al. Modulation of innate and adaptive immune responses by Tofacitinib (CP-690,550). *J Immunol*. 2011;186(7):4234-4243. doi:10.4049/jimmunol.1003668
20. Maeshima K, Yamaoka K, Kubo S, et al. The JAK inhibitor tofacitinib regulates synovitis through inhibition of interferon-γ and interleukin-17 production by human CD4+ T cells. *Arthritis Rheum*. 2012;64(6):1790-1798. doi:10.1002/art.34329
21. O'Sullivan LA, Liongue C, Lewis RS, Stephenson SEM, Ward AC. Cytokine receptor signaling through the Jak-Stat-Socs pathway in disease. *Mol Immunol*. 2007;44(10):2497-2506. doi:10.1016/j.molimm.2006.11.025
22. Krueger J, Clark JD, Suárez-Fariñas M, et al. Tofacitinib attenuates pathologic immune pathways in patients with psoriasis: a randomized phase 2 study. *J Allergy Clin Immunol*. 2016;137(4):1079-1090. doi:10.1016/j.jaci.2015.12.1318
23. Hashimoto T, Sakai K, Sanders KM, Yosipovitch G, Akiyama T. Antipruritic effects of Janus kinase inhibitor Tofacitinib in a mouse model of psoriasis. *Acta Derm Venereol*. 2019;99(3):298-303. doi:10.2340/00015555-3086
24. Roses RE, Xu S, Xu M, Koldovsky U, Koski G, Czerniecki BJ. Differential production of IL-23 and IL-12 by myeloid-derived dendritic cells in response to TLR agonists. *J Immunol*. 2008;181(7):5120-5127. doi:10.4049/jimmunol.181.7.5120
25. Garcia-Rodriguez S, Arias-Santiago S, Perandrés-López R, et al. Increased gene expression of Toll-like receptor 4 on peripheral blood mononuclear cells in patients with psoriasis. *J Eur Acad Dermatol Venereol*. 2013;27(2):242-250. doi:10.1111/j.1468-3083.2011.04372.x
26. Seung NR, Park EJ, Kim CW, et al. Comparison of expression of heat-shock protein 60, Toll-like receptors 2 and 4, and T-cell receptor gammadelta in plaque and guttate psoriasis. *J Cutan Pathol*. 2007;34(12):903-911. doi:10.1111/j.1600-0560.2007.00756.x
27. Hari A, Flach TL, Shi Y, Mydlarski PR. Toll-like receptors: role in dermatological disease. *Mediators Inflamm*. 2010;2010:437246. doi:10.1155/2010/437246
28. Benoit S, Toksoy A, Ahlmann M, et al. Elevated serum levels of calcium-binding S100 proteins A8 and A9 reflect disease activity and abnormal differentiation of keratinocytes in psoriasis. *Br J Dermatol*. 2006;155(1):62-66. doi:10.1111/j.1365-2133.2006.07198.x
29. Bechara R, Antonios D, Azouri H, Pallardy M. Nickel sulfate promotes IL-17A producing CD4+ T cells by an IL-23-dependent mechanism regulated by TLR4 and Jak-STAT pathways. *J Invest Dermatol*. 2017;137(10):2140-2148. doi:10.1016/j.jid.2017.05.025
30. Tomalin LE, Kim J, Correa da Rosa J, et al. Early quantification of systemic inflammatory proteins predicts long-term treatment response to Tofacitinib and Etanercept. *J Invest Dermatol*. 2020;140(5):1026-1034. doi:10.1016/j.jid.2019.09.023
31. Benson JM, Sachs CW, Treacy G, et al. Therapeutic targeting of the IL-12/23 pathways: generation and characterization of

ustekinumab. *Nat Biotechnol*. 2011;29(7):615-624. doi:10.1038/nbt.1903
32. Fragoulis GE, McInnes IB, Siebert S. JAK-inhibitors. New players in the field of immune-mediated diseases, beyond rheumatoid arthritis. *Rheumatology*. 2019;58(Suppl 1):i43-i54. doi:10.1093/rheumatology/key276
33. Gao W, McGarry T, Orr C, McCormick J, Veale DJ, Fearon U. Tofacitinib regulates synovial inflammation in psoriatic arthritis, inhibiting STAT activation and induction of negative feedback inhibitors. *Ann Rheum Dis*. 2016;75(1):311-315. doi:10.1136/annrheumdis-2014-207201
34. Raychaudhuri SK, Abria C, Raychaudhuri SP. Regulatory role of the JAK STAT kinase signalling system on the IL-23/IL-17 cytokine axis in psoriatic arthritis. *Ann Rheum Dis*. 2017;76(10):e36. doi:10.1136/annrheumdis-2016-211046
35. Lyakh L, Trinchieri G, Provezza L, Carra G, Gerosa F. Regulation of interleukin-12/interleukin-23 production and the T-helper 17 response in humans. *Immunol Rev*. 2008;226(1):112-131. doi:10.1111/j.1600-065X.2008.00700.x
36. Collin M, Bigley V. Human dendritic cell subsets: an update. *Immunology*. 2018;154(1):3-20. doi:10.1111/imm.12888
37. Silva-Cardoso SC, Affandi AJ, Spel L, et al. CXCL4 exposure potentiates TLR-driven polarization of human monocyte-derived dendritic cells and increases stimulation of T cells. *J Immunol*. 2017;199(1):253-262. doi:10.4049/jimmunol.1602020
38. Qian X, Ning H, Zhang J, et al. Posttranscriptional regulation of IL-23 expression by IFN-gamma through tristetraprolin. *J Immunol*. 2011;186(11):6454-6464. doi:10.4049/jimmunol.1002672
39. Liu J, Cao S, Herman LM, Ma X. Differential regulation of interleukin (IL)-12 p35 and p40 gene expression and interferon (IFN)-gamma-primed IL-12 production by IFN regulatory factor 1. *J Exp Med*. 2003;198(8):1265-1276. doi:10.1084/jem.20030026
40. Sikorski K, Chmielewski S, Olejnik A, et al. STAT1 as a central mediator of IFNγ and TLR4 signal integration in vascular dysfunction. *JAK-STAT*. 2012;1(4):241-249. doi:10.4161/jkst.22469
41. Piaszyk-Borychowska A, Széles L, Csermely A, et al. Signal integration of IFN-I and IFN-II With TLR4 involves sequential recruitment of STAT1-complexes and NFκB to enhance pro-inflammatory transcription. *Front Immunol*. 2019;10(JUN):1253. doi:10.3389/fimmu.2019.01253
42. Majoros A, Platanitis E, Kernbauer-Hölzl E, Rosebrock F, Müller M, Decker T. Canonical and non-canonical aspects of JAK-STAT signaling: lessons from interferons for cytokine responses. *Front Immunol*. 2017;8(January):29. doi:10.3389/fimmu.2017.00029
43. Kang K, Bachu M, Park SH, et al. IFN-γ selectively suppresses a subset of TLR4-activated genes and enhancers to potentiate macrophage activation. *Nat Commun*. 2019;10(1):3320. doi:10.1038/s41467-019-11147-3

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

Supplementary Material

How to cite this article: Vincken NLA, Welsing PMJ, Silva-Cardoso SC, et al. Suppression of IL-12/IL-23 p40 subunit in the skin and blood of psoriasis patients by Tofacitinib is dependent on active interferon-γ signaling in dendritic cells: Implications for the treatment of psoriasis and interferon-driven diseases. *Exp Dermatol*. 2022;31:962–969. doi:10.1111/exd.14566