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Baricitinib treatment in a patient with a gain-of-function mutation in signal transducer and activator of transcription 1 (*STAT1*)

To the Editor:

Heterozygous gain-of-function (GOF) mutations in signal transducer and activator of transcription 1 *(STAT1)* have been reported increasingly worldwide.[1] Chronic mucocutaneous candidiasis (CMC) is the hallmark of *STAT1* GOF mutations, but bacterial infections, mainly caused by *Staphylococcus aureus*; viral infections, predominantly Herpesviridae; and autoimmune manifestations are also commonly present.[1] Enhanced STAT1 phosphorylation in patients with *STAT1* GOF mutations is associated with overexpression of programmed death ligand 1 (PD-L1) and abolishes T_H17 responses, which are considered to represent the immunologic cause of CMC.[2-4]

Treatment of CMC in patients with *STAT1* GOF mutations includes long-term systemic antifungal therapy.[1] Considering the underlying immunologic defect, immunomodulatory treatment options are also explored, although sparsely, and their effectiveness is still indecisive.[5] The Janus kinase (JAK) 1/2 inhibitor ruxolitinib was reported to be beneficial in 1 patient, but increased IL-17 production was not demonstrated in this case.[6] Baricitinib is a novel and orally available JAK1/2 inhibitor that hampers interferon-induced JAK-STAT1 signaling in patients with immune-mediated diseases and was recently approved for the treatment of rheumatoid arthritis.[7] Based on its mechanisms of action, it was hypothesized that baricitinib could be of benefit in the treatment of patients with *STAT1* GOF mutations. In this report we describe a patient treated with baricitinib and show its potential clinical implications for treatment of patients with *STAT1* GOF mutations.

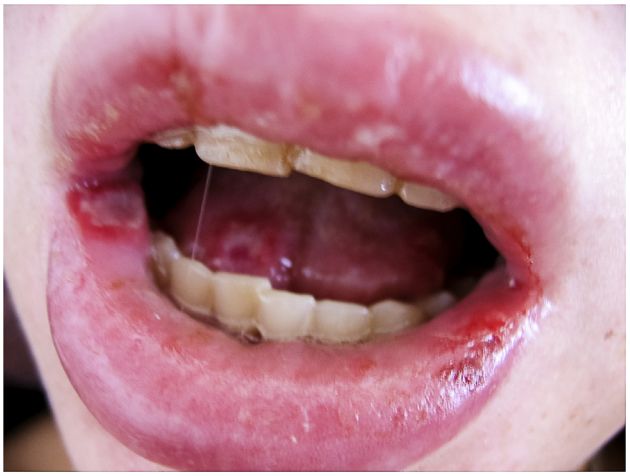

FIG 1. Recurrent oral ulcers in the patient. Multiple shallow ulcers with erythematous borders were found at the upper lip, lower lip, and tongue.

The patient is a 24-year-old Dutch woman given a diagnosis of CMC based on heterozygous *STAT1* GOF mutations at c.1957G>A (p.[V653I]) of the Src homology 2 domain. She also experienced other infectious complications and autoimmune phenomena, as described in detail previously.[4] Over the years, the main clinical problems included recurrent oral and esophageal *Candida albicans* infections. Candidiasis erupted repetitively within 2 weeks after termination of a course of antifungal therapy. Therefore prophylactic antifungal therapy was required.

Our patient also encountered oral and vaginal ulcers that appeared to be of a noninfectious nature and were assumed to represent autoimmune manifestations related to *STAT1* GOF mutations (Fig 1). Treatment with steroids led to rapid improvement of these ulcers, but steroid-sparing agents, including azathioprine, hydroxychloroquine, and mycophenolate mofetil, were only of minor benefit. On treatment with the anti–TNF-α adalimumab (in combination with antifungal therapy), the ulcers did not reoccur.

To demonstrate an evidence-based rationale to initiate baricitinib treatment, we first examined the effectiveness of baricitinib *in vitro* using fresh blood from the patient and 1 age/sex/race-matched healthy control subject. The concentrations of baricitinib used in this study were based on serum levels achieved in patients receiving 2 to 20 mg/d oral baricitinib.[8] T lymphocytes from the patient displayed higher STAT1 phosphorylation levels when stimulated with IFN-α, IFN-β, or IFN-γ than T lymphocytes from the healthy control subject. Interferon-induced phosphorylated STAT1 (pSTAT1) levels in the patient were decreased on addition of baricitinib (see Fig E1, *A*, in this article's Online Repository at www.jacionline.org). STAT3 phosphorylation, which is crucial for T_H17 development,[9] was also measured. No clear effects of baricitinib on both IFN-α– and IL-6–induced phosphorylated STAT3 (pSTAT3) levels in T lymphocytes from the patient were found (see Fig E1, *B*). Baricitinib at a

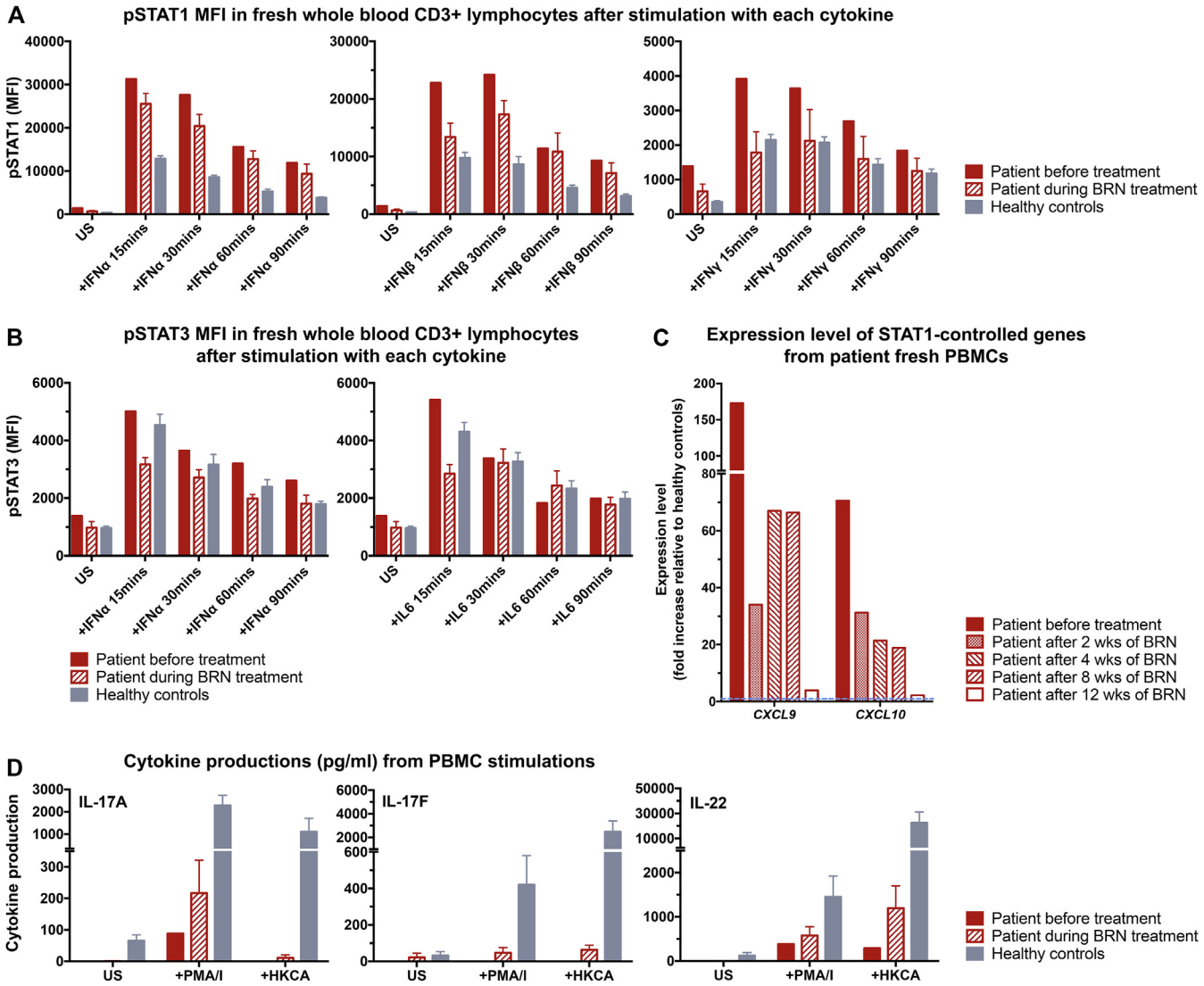

FIG 2. Immunologic responses of peripheral blood cells obtained from the patient before and during baricitinib treatment. Blood samples were collected just before baricitinib was started and after 2, 4, 8, and 12 weeks of treatment. **A** and **B,** Kinetics of STAT1 (Fig 1, *A*) and STAT3 (Fig 1, *B*) phosphorylation in fresh whole blood $CD3^+$ T lymphocytes on stimulation with IFN-α, IFN-β, IFN-γ, or IL-6 for the indicated time periods. Enhanced pSTAT1 levels in the patient observed before baricitinib treatment *(solid red bars)* were decreased after treatment (*red striped bars* depict the average MFI from the 4 time points of blood sampling after baricitinib initiation because samples from all time points showed comparable results). Inducible pSTAT3 in the patient also decreased under baricitinib treatment (*gray bars* in Fig 1, *A* and *B*, indicate average MFI from healthy control subjects; n = 5). **C,** Bar graph depicting mRNA expression levels of *CXCL9* and *CXCL10* in the patient's PBMCs relative to expression in healthy control subjects (n = 5) determined by using real-time quantitative TaqMan PCR *(qRT-PCR)*. Data were normalized to the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase *(GAPDH)*. **D,** PBMCs from the patient and healthy control subjects (n = 5) were stimulated with phorbol 12-myristate 13-acetate and ionomycin *(PMA+I)* or HKCA. IL-17A, IL-17F, and IL-22 production was measured (by means of ELISA) in culture supernatants after 5 days of culture. *BRN*, Baricitinib; *MFI*, mean fluorescence intensity; *US*, unstimulated. All the results with error bars indicate average values with SEMs.

concentration of 20 nmol/L significantly enhanced heat-killed *Candida albicans* (HKCA)–induced IL-17A production by PBMCs from the patient. However, this was not observed for higher concentrations of baricitinib (100 and 500 nmol/L), suggesting an immunomodulatory effect within a specific dose range (see Fig E1, *C*). Baricitinib did not enhance IL-17F or IL-22 production (data not shown). In long-term T-lymphocyte cultures, baricitinib significantly reduced expression of the STAT1-regulated genes *CXCL9*, *CXCL10*, and *CD274* (PD-L1) after IL-27 stimulation (see Fig E1, *D*, and see this article's Methods section in the Online Repository at www.jacionline.org).

Based on *in vitro* data, baricitinib treatment (2 mg once daily) was initiated in the patient after adalimumab treatment had been terminated for 2 weeks. At the start of baricitinib treatment, no active candidiasis or ulcers were reported. Prophylactic antifungal therapy (fluconazole, 200 mg once daily) was initially

continued. As the patient's clinical condition remained stable, the fluconazole dose was reduced and fully tapered after 3 months of baricitinib. During 8 months of follow-up, no oral or vaginal ulcers reoccurred. Mucocutaneous candidiasis did not reappear, even without prophylactic antifungal therapy. Although (re) occurrence of viral infections, especially herpes zoster, and other opportunistic infections has been reported on treatment with JAK inhibitors,[E1] our patient did not encounter any of these up to 8 months after initiation of treatment. Also, no other clinical or biochemical complications were reported.

Blood samples collected before the start of baricitinib and every 2 to 4 weeks afterward were examined for STAT1/STAT3 phosphorylation, STAT1-regulated gene expression, and cytokine production. Before treatment, pSTAT1 levels in T lymphocytes from the patient were greater than pSTAT1 levels in 5 age/sex/race-matched healthy control subjects, as reported previously.[4] Baricitinib treatment reduced pSTAT1 levels at every time point examined (Fig 2, *A*). Although baricitinib did not clearly affect STAT3 phosphorylation in our initial *in vitro* studies (see Fig E1, *B*), IFN-α– and IL-6–induced STAT3 phosphorylation was reduced in T lymphocytes from the patient during baricitinib treatment (Fig 2, *B*). The remarkably high expression levels of STAT1-regulated genes (*CXCL9* and *CXCL10*) from patients' PBMCs before baricitinib treatment were strongly decreased on treatment (Fig 2, *C*). Before initiation of baricitinib treatment, PD-L1 *(CD274)* expression in PBMCs from the patient was approximately 3-fold greater compared with PBMCs from 5 healthy control subjects, but its expression level did not decrease during the treatment period (data not shown). PBMCs from the patient obtained during baricitinib treatment showed greater production of IL-17A, IL-17F, and IL-22 on stimulation with phorbol 12-myristate 13-acetate and ionomycin or HKCA than PBMCs obtained before treatment. However, these levels were much lower than those observed in healthy control subjects (n = 5; Fig 2, *D*, and see this article's Methods section in the Online Repository at www.jacionline.org).

This study provides the first evidence that baricitinib could be of value in the treatment of patients with *STAT1* GOF mutations. We demonstrate this at several levels. First, our patient showed remarkable clinical improvement with baricitinib treatment. Systemic prophylactic antifungal therapy and immunosuppressive treatment could be terminated without reoccurrence of candidiasis or ulcers. Second, baricitinib reduced STAT1 hyperphosphorylation and STAT3 phosphorylation and improved the capacity of PBMCs to produce IL-17A, IL-17F, and IL-22, cytokines crucial for antifungal immune responses. Third, baricitinib reduced expression of the IL-27/STAT-1–regulated genes *CXCL9*, *CXCL10*, and PD-L1 *(CD274)* in long-term T-lymphocyte cultures from the patient. PBMCs obtained from the patient during 3 months of baricitinib therapy also expressed *CXCL9* and *CXCL10* at levels approaching those of the healthy control subjects. Overexpression of PD-L1 in naive T lymphocytes from patients with a *STAT1* GOF mutation is associated with enhanced IL-27/STAT1 signaling and contributes to inhibition of T_H17 differentiation.[2,3] An anti-human PD-L1 inhibitory antibody was found to partially rescue IL-17A production in T lymphocytes from patients with a *STAT1* GOF mutation.[2] We found no reduction in PD-L1 *(CD274)* mRNA expression in unstimulated PBMCs from the patient during baricitinib treatment. However, PD-L1 is known to be upregulated on activated T lymphocytes,[E2] and our data show that specific activation of T lymphocytes from the patient with IL-27 results in strong upregulation of PD-L1, which can be abrogated by baricitinib (see Fig E1, *D*). Therefore the observed partial restoration of IL-17A production by PBMCs obtained from the patient during baricitinib treatment could result from less pronounced upregulation of PD-L1 on activated T lymphocytes under the phorbol 12-myristate 13-acetate and ionomycin and HKCA stimulation conditions.

In conclusion, for the first time, we show therapeutic benefit of the clinically available drug baricitinib in a patient with GOF *STAT1* mutation. Further studies are required to evaluate its clinical implications in other patients with *STAT1* GOF mutations.

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Hypoallergenic casein hydrolysate for peptide-based oral immunotherapy in cow's milk allergy

To the Editor:

Cow's milk allergy (CMA) is one of the most common food allergies and a critical public health problem in the case of children.[1] Oral immunotherapy and oral food challenges have been developed for treating and diagnosing food allergies, but

METHODS

STAT phosphorylation analysis

Baricitinib (Selleck Chemicals, Houston, Tex) was added to cells 1 hour before stimulation. Stimulation with IFN-α (10^4 IU/mL; PeproTech, London, United Kingdom), IFN-β (10^3 IU/mL; Tebu-bio, Le-Perray-en-Yvelines, France), IFN-γ (10^5 IU/mL; R&D Systems, Abingdon, United Kingdom), or IL-6 (100 ng/ml; R&D Systems) was performed for various durations and as previously described.[E3] For flow cytometric analysis, cells were fixed and permeabilized with permeabilizing reagent (Phospho-Epitopes Exposure kit; Beckman Coulter, Fullerton, Calif). Cells were stained with allophycocyanin-conjugated antihuman CD3 (BD Biosciences, San Jose, Calif), Alexa Fluor 488–conjugated phospho-STAT1 Tyr701 (Cell Signaling Technology, Danvers, Mass), and phycoerythrin-conjugated pSTAT3 Tyr705 (Cell Signaling Technology) antibodies.

Cytokine production

PBMCs were cultured in RPMI-1640 medium (Lonza, Basel, Switzerland), containing 10% heat-inactivated FCS and penicillin and streptomycin (Cambrex BioWhittaker, Verviers, Belgium). Baricitinib was added to the cells for 1 hour before stimulation with phorbol 12-myristate 13-acetate (81 nmol/L) and ionomycin (1.3 μmol/L; eBioscience, San Diego, Calif) or HKCA (10^6 cells). After 5 days of culturing, supernatants were collected and analyzed for IL-17A, IL-17F, and IL-22 by using an ELISA (R&D Systems).

Long-term T-lymphocyte culture

Long-term T-lymphocyte cultures were established, as previously described.[E3] After 2 weeks of culturing, T-lymphocyte cultures with a purity of greater than 90% were obtained. Baricitinib was added to the cells for 1 hour before stimulation with IL-27 (200 ng/mL; R&D Systems) for 24 hours. Subsequently, cells were collected and analyzed for *CXCL9*, *CXCL10*, and PD-L1 *(CD274)* gene expression with real-time PCR.

Real-time PCR

Total RNA was extracted from cultured cells with the GenElute Mammalian Total RNA Miniprep Kit (Sigma-Aldrich, St Louis, Mo), according to the manufacturer's protocol. RNA was reverse transcribed into cDNA with random primers (Invitrogen, Thermo Fisher Scientific, Waltham, Mass). PCR for *CXCL9*, *CXCL10*, and PD-L1 *(CD274)* was performed with a primer probe mix (Thermo Fisher Scientific) and a 7900HT Fast Real-Time PCR System (Applied Biosystems, Foster City, Calif). Gene expression data were normalized to expression of the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase *(GAPDH)*.

Statistical analysis

Statistical analysis for cytokine production experiments was performed with a mixed model with statistical curve fit analysis and gene expression experiments with *t* tests. All assessments were conducted with GraphPad Prism (GraphPad Software, San Diego, Calif) and SPSS (v.25.0; IBM SPSS Statistics; IBM, Armonk, NY) software. Statistical significance was considered at a P value of less than .05 in all analyses.

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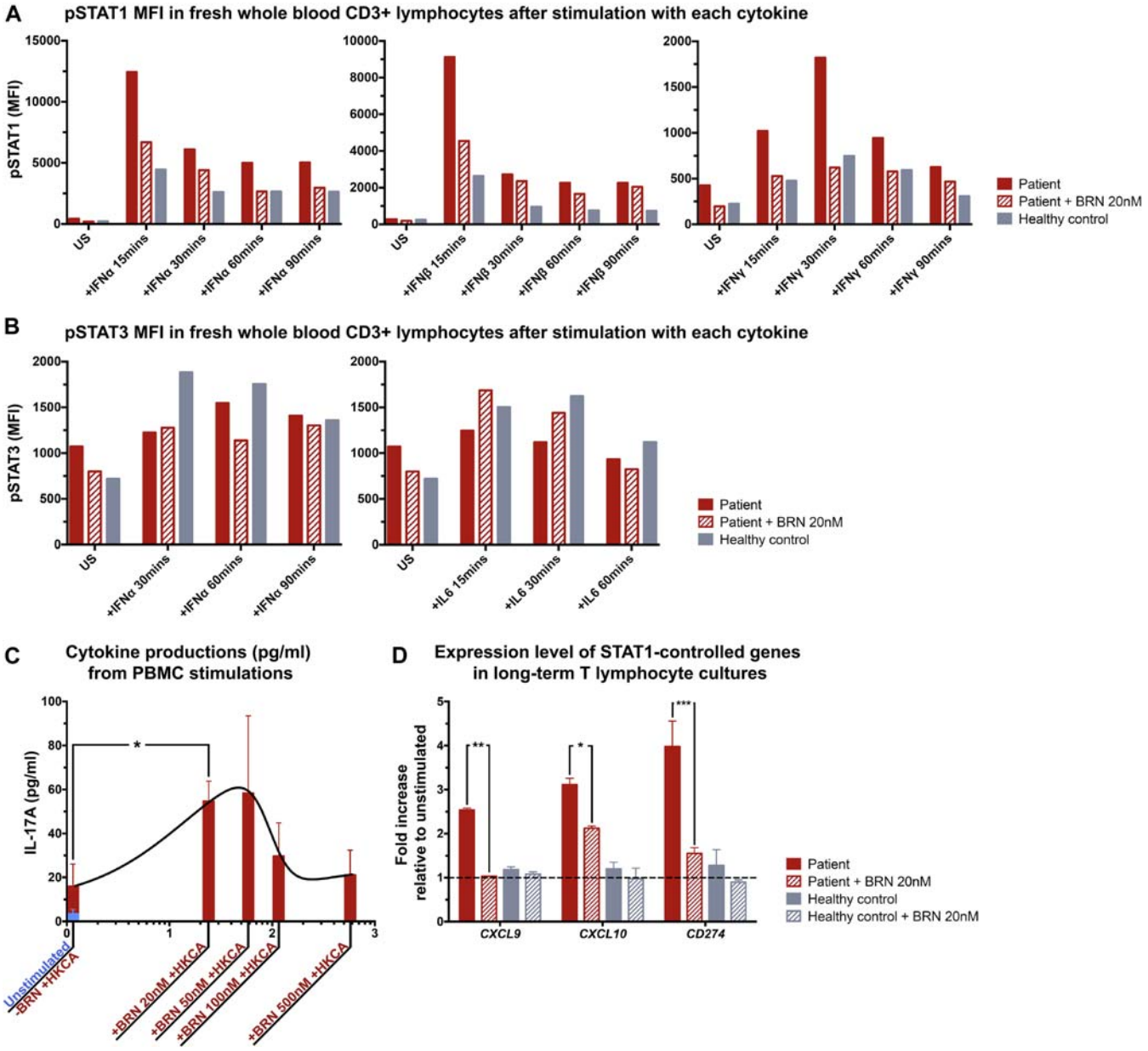

FIG E1. *In vitro* baricitinib efficacy studies. **A** and **B,** Kinetics of STAT1 phosphorylation (Fig E1, *A*) and STAT3 phosphorylation (Fig E1, *B*) in fresh whole blood $CD3^+$ T lymphocytes on stimulation with IFN-α, IFN-β, IFN-γ, or IL-6 for the indicated time periods. Samples were preincubated with baricitinib for 1 hour before stimulation. **C,** Bar graph with statistical curve fit analysis depicting IL-17A production in culture supernatants from the patient's PBMCs after 5 days of stimulation with HKCA. The *x-axis* shows the baricitinib concentration used in the experiment in log scale. Samples were preincubated with baricitinib for 1 hour before stimulation. **D,** Long-term T-lymphocyte cultures were established, preincubated with baricitinib for 1 hour, and stimulated for 24 hours with IL-27. mRNA expression levels of the STAT1-regulated genes *CXCL9*, *CXCL10*, and PD-L1 *(CD274)* were determined by using real-time quantitative TaqMan PCR *(qRT-PCR)* and normalized to the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase *(GAPDH).* In Fig E1, *C* and *D*, data depicted are means of 3 replicate experiments. *Error bars* indicate SEMs. *BRN*, Baricitinib; *MFI*, mean fluorescence intensity; *US*, unstimulated. $*P < .05$, $**P < .01$, and $***P < .001$.