

**Inhibition of JAK-STAT signaling by baricitinib reduces
interferon- γ -induced CXCL10 production in human salivary gland ductal
cells**

Keiko Aota*, Tomoko Yamanoi, Koichi Kani, Shinji Ono, Yukihiro Momota, and Masayuki
Azuma

Department of Oral Medicine, Tokushima University Graduate School of Biomedical
Sciences, 3-18-15 Kuramoto-cho, Tokushima 770-8504, Japan.

*Corresponding author: Keiko Aota

Tel: +81-88-633-7352; Fax: +81-88-633-7388

E-mail: aota.keiko@tokushima-u.ac.jp

ORCID ID: 0000-0001-5413-5750

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Abstract

Sjögren's syndrome (SS) is a chronic autoimmune disease targeting salivary and lacrimal glands. C-X-C motif chemokine ligand 10 (*CXCL10*) expression is upregulated in lip salivary glands (LSGs) of primary SS (pSS) patients, and *CXCL10* involved in SS pathogenesis via immune-cell accumulation. Moreover, interferon (IFN)- γ enhances *CXCL10* production via the Janus kinase (JAK)/signal transducer and activator of transcription (STAT) pathway. We investigated the effects of baricitinib, a selective JAK1/2 inhibitor, on both IFN- γ -induced *CXCL10* production and immune-cell chemotaxis. We used immunohistochemical staining to determine the expression levels and localization of JAK1 and JAK2 in LSGs of SS patients (n=12) and healthy controls (n=3). We then evaluated effect of baricitinib in an immortalized normal human salivary gland ductal (NS-SV-DC) cell line. Immunohistochemical analysis of LSGs from pSS patients revealed strong JAK1 and JAK2 expression in ductal and acinar cells, respectively. Baricitinib significantly inhibited IFN- γ -induced *CXCL10* expression as well as the protein levels in an immortalized human salivary gland ductal-cell clone in a dose-dependent manner. Additionally, western blot analysis showed that baricitinib suppressed the IFN- γ -induced phosphorylation of STAT1 and STAT3, with a stronger effect observed in case of STAT1. It also inhibited IFN- γ -mediated chemotaxis of Jurkat T cells. These results suggested that baricitinib suppressed IFN- γ -induced *CXCL10* expression and attenuated immune-cell chemotaxis by inhibiting JAK/STAT signaling, suggesting its potential as a therapeutic strategy for pSS.

KEY WORDS: baricitinib, Janus kinase, CXCL10, IFN- γ , Sjögren's syndrome, salivary gland ductal cells

Declarations

Funding

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Conflicts of interest/Competing interests

The authors declare that they have no conflict of interest.

Ethics approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the Institutional Review Board of Tokushima University Hospital (#2802) and with the 1964 Helsinki declaration and its later amendments.

Consent to participate

Written informed consent was obtained from all individual participants included in the study, and this process was documented by the Institutional Review Board of Tokushima University Hospital. The informed consent procedure was approved by the Ethics Committee of Tokushima University Hospital.

Consent for publication

Authorization has been given from all authors to use unpublished data.

Availability of data and material

Not applicable.

Code availability

Not applicable.

Authors' contributions

Keiko Aota and Masayuki Azuma contributed to the study conception and design. Material preparation, data collection, and analysis were performed by Keiko Aota, Tomoko Yamanoi, Koichi Kani, Shinji Ono, and Yukihiro Momota. The first draft of the manuscript was written by Keiko Aota and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

INTRODUCTION

Sjögren's syndrome (SS) is among the most common autoimmune diseases [1, 2] and is characterized by the destruction of acinar structure by marked lymphocytic infiltrates in the salivary and lacrimal glands, which results in sicca symptoms in affected patients [3–5]. SS pathogenesis is complicated and involves genetic contributions, frequency of the particular infection, and individual susceptibility to activating immune responses [6].

Recent data suggest central roles for interferon (IFN) types I (IFN- α and - β) and II (IFN- γ) in SS pathogenesis based on the upregulation of their target genes in peripheral blood [7] and lip salivary glands (LSGs) [8, 9] from SS patients. In LSGs from SS patients, plasmacytoid dendritic cells are the main source of IFN- α , whereas CD4⁺ T cells and natural killer cells are the main IFN- γ producers [10]. Furthermore, the type I IFN signature is prevalent in the peripheral blood of SS patients, whereas the type II IFN signature predominates in LSGs [11].

C-X-C motif chemokine ligand 10 (CXCL10) is a chemokine induced by IFN- γ and produced by diverse cell types, including peripheral blood mononuclear cells, fibroblasts, and endothelial cells, during T helper 1 cell-mediated immune responses [12]. CXCL10 and its receptor, CXC receptor 3 (CXCR3) apparently contribute to the pathogenesis of many autoimmune diseases, including SS [13]. Gene-expression profiling of LSGs from healthy subjects and primary SS (pSS) patients demonstrated that *CXCL10* is clearly upregulated in the latter [8]. Furthermore, CXCL10 is involved in the accumulation of infiltrating immune

cells in the salivary glands of pSS patients [14, 15], and recent *in vitro* experiments showed that IFN- γ enhanced CXCL10 production via both the Janus kinase (JAK)/signal transducer and activator of transcription (STAT) and nuclear factor-kappaB (NF- κ B) pathways [16].

The JAK family of cytoplasmic protein tyrosine kinases comprises JAK1, JAK2, JAK3, and tyrosine kinase 2. JAKs bind to type 1 and type 2 cytokine receptors and transmit extracellular cytokine signals to activate STAT proteins (STAT1/2/3/4/5A/5B/6), which translocate to the nucleus and modulate transcription of effector genes [17]. JAK-STAT signaling is initiated by binding of cytokines to their respective receptors on the cell surface and utilized by >50 cytokines [18]. Therefore, JAKs represent excellent targets for therapeutic interventions for various cytokine-mediated disorders. Baricitinib, a selective inhibitor of JAK1 and JAK2 [19], has been approved for the treatment of moderate-to-severe active rheumatoid arthritis. Although this drug is administered orally due to its small molecular weight, it has comparable efficacy with biological disease-modifying anti-rheumatic drugs, which require intravenous or subcutaneous injection [20].

In this study, we tested the hypothesis that baricitinib can suppress *CXCL10* overexpression in LSGs of pSS patients. Additionally, we determined the *in vitro* effect of baricitinib on IFN- γ -induced *CXCL10* expression and chemotaxis using a human salivary gland cell clone.

MATERIALS AND METHODS

Patients

Twelve female patients with pSS and three healthy female subjects were enrolled in this study. All individual participants enrolled in the study were treated at Tokushima University Hospital between 2011 and 2017. All pSS patients satisfied the revised Japanese Ministry of Health criteria for the diagnosis of SS [21] and the American College of Rheumatology classification criteria for SS [22]. Based on the biopsy scoring system described by Tarpley et al. [23], we categorized LSG biopsy samples from pSS patients into four groups according to the grade of infiltration: Grade 1, Tarpley score 1 (1–2 lymphocytic aggregates/lobule), Grade 2, Tarpley score 2 (2–3 lymphocytic aggregates/lobule); Grade 3, Tarpley score 3 (diffuse infiltration through acini associated with partial destruction of acinar tissue); and Grade 4 Tarpley score 4 (diffuse infiltration associated with complete loss of tissue architecture). Healthy controls were subjects who had experienced subjective symptoms of oral dryness but met none of the objective criteria for SS diagnosis.

All procedures performed in studies involving human participants were in accordance with the ethical standards of the Institutional Review Board of Tokushima University Hospital (#2802) and with the 1964 Helsinki declaration and its later amendments. Written informed consent was obtained from all individual participants included in the study, and this process was documented by the Institutional Review Board of Tokushima University Hospital. The informed consent procedure was approved by the Ethics Committee of Tokushima University Hospital.

Immunohistochemical staining

Formalin-fixed paraffin-embedded LSG sections from pSS patients and healthy subjects were deparaffinized in xylene and rehydrated with graded ethanol (100%, 95%, 70%, and 50%). Antigen retrieval was performed by microwave treatment using a citrate-based antigen unmasking solution (Vector Laboratories, Burlingame, CA, USA) according to manufacturer instructions. Endogenous biotin was blocked using Blocking One reagent (Nacalai Tesque, Kyoto, Japan) according to manufacturer instructions. Sections were then incubated overnight at 4 °C with rabbit polyclonal anti-human JAK1 (1:100; Cell Signaling Technology, Beverly, MA, USA) and rabbit polyclonal anti-human JAK2 (1:50; Cell Signaling Technology) primary antibodies. After three washes with phosphate-buffered saline, the sections were incubated for 30 min at 20 °C with horseradish peroxidase (HRP)-conjugated second antibody (Nichirei Biosciences Inc., Tokyo, Japan). HRP was reacted with the 3,3'-diaminobenzidine substrate using the Histofine Simple Stain MAX PO kit (Nichirei Biosciences Inc.), and the sections were observed using a light microscope equipped with a digital camera (BZ-X700; Keyence, Tokyo, Japan).

Cell culture

The detailed characteristics of the immortalized normal human salivary gland ductal (NS-SV-DC) cell line have been described previously [24]. The cell line was cultured in

keratinocyte serum-free medium (Gibco, Gaithersburg, MD, USA) in an incubator at 37 °C with a humidified atmosphere containing 5% CO₂. Jurkat human leukemic T cells (Riken Cell Bank, Ibaraki, Japan) were maintained in RPMI-1640 medium (Gibco) supplemented with 10% fetal bovine serum (Gibco) in a 5% CO₂-humidified incubator at 37 °C.

Reagents

Baricitinib was purchased from MedChemExpress (Monmouth Junction, NJ, USA), and recombinant human IFN- γ was purchased from R&D Systems (Minneapolis, MN, USA).

Cell viability assay

NS-SV-DC cells (1×10^4 cells/well) were seeded in 96-well plates (Falcon, Oxnard, CA, USA) in serum-free keratinocyte medium. After 24 h, the cells were treated with baricitinib (10, 100, 1000, 2500, or 5000 nM), and following an appropriate incubation period, an 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reagent (Roche, Basel, Switzerland) was added to each well for an additional 4-h incubation. The cells were then dissolved in solubilization solution (Roche) and analyzed at 570 nm using a Multiskan JX microplate reader (Thermo Fisher Scientific, Waltham, MA, USA).

Real-time quantitative reverse transcription polymerase chain reaction (RT-qPCR)

NS-SV-DC cells were treated for 6, 12, or 24 h with 10 ng/mL IFN- γ in the presence or absence of baricitinib (10, 100, or 1000 nM), followed by isolation of total RNA using TRIzol reagent (Life Technologies, Carlsbad, CA, USA) according to manufacturer instructions. Total RNA was then converted into cDNA using the PrimeScript RT reagent kit (TaKaRa, Kusatsu, Japan) according to manufacturer instructions. mRNA levels of *CXCL10* and glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) were analyzed using corresponding Assays-on-Demand Gene Expression products (Applied Biosystems, Tokyo, Japan) and TaqMan Universal PCR master mix (Applied Biosystems) with an ABI Prism 7000 sequence detection system (Applied Biosystems) according to manufacturer instructions. The thermal cycler conditions were 95 °C for 10 min, followed by 40 cycles of 95 °C for 5 s and 60 °C for 30 s. Gene-expression data were analyzed using the $2^{-\Delta\Delta CT}$ method in Sequence Detection System software (v.1.1.0; Applied Biosystems). The relative quantification (RQ) of the fold change in gene expression was calculated using the formula $RQ = 2^{-\Delta\Delta CT}$ and normalized using *GAPDH* as an internal reference. The relative mRNA levels were expressed as fold increases relative to *GAPDH* level.

Enzyme-linked immunosorbent assay (ELISA)

NS-SV-DC cells were incubated for 6, 12, or 24 h with 10 ng/mL IFN- γ in the presence or absence of baricitinib (10, 100, or 1000 nM). The supernatants were collected, and secreted CXCL10 levels were analyzed using a human CXCL10 ELISA kit (R&D Systems) according

to manufacturer instructions. Absorbance at 450 nm was measured using a Multiskan JX microplate reader (Thermo Fisher Scientific), and CXCL10 levels were determined using a standard curve.

Protein isolation and western blot analysis

NS-SV-DC cells were treated for 5, 10, 30, or 60 min with 10 ng/mL IFN- γ in the presence or absence of 100 nM baricitinib, followed by preparation of cytoplasmic lysates using NE-PER nuclear and cytoplasmic extraction reagents (Thermo Fisher Scientific) according to manufacturer instructions. Cytoplasmic lysate proteins (100 μ g) were separated by electrophoresis using 10% sodium dodecyl sulfate polyacrylamide gels (Bio-Rad, Hercules, CA, USA), followed by transfer onto nitrocellulose membranes (Bio-Rad). The membranes were blocked with 3% bovine serum albumin in Tris-buffered saline containing 0.1% Tween 20 (TBS-T) and incubated for 1 h at 20 °C with anti-STAT1, anti-phospho-STAT1, anti-STAT3, anti-phospho-STAT3, and anti- β -actin primary antibodies (all from Cell Signaling Technology) diluted 1:1000 in Can Get Signal Solution 1 (TOYOBO, Osaka, Japan). After several washes with TBS-T, the membranes were incubated for 1 h at 20 °C with appropriate secondary antibodies (Cell Signaling Technology) diluted 1:1000 in Can Get Signal Solution 2 (TOYOBO). The immune complexes were visualized using an enhanced chemiluminescence western blotting detection reagent (GE Healthcare, Little Chalfont, UK). Densitometric analysis was performed using an Amersham Imager 600 (GE Healthcare) to

determine the relative intensity of the immune complexes and using β -actin as an internal reference.

Cell migration assay

We analyzed Jurkat T cell directional migration, which was induced by conditioned medium (CM) derived from IFN- γ -treated NS-SV-DC cells, using a CytoSelect 96-well cell migration assay (5 μ m; Fluorometric Format; Cell Biolabs, San Diego, CA, USA) according to manufacturer instructions. Briefly, NS-SV-DC cells were treated with 10 ng/mL IFN- γ in the presence or absence of 100 nM baricitinib for 24 h, followed by transfer of 150 μ L of CM into the bottom wells (feeder tray). Serum-free medium containing 5×10^5 Jurkat T cells (100 μ L) was then placed in the migration chamber, and the chemotaxis plate was cultured at 37 °C for 24 h. After incubation, the cells that had migrated to the lower chambers were incubated for 20 min with 50 μ L of lysis buffer/dye Solution (Cell Biolabs). Fluorescence was read at 480/520 nm, and values were expressed as relative fluorescence units (RFUs). The experiments were performed in triplicate.

Statistical analyses

All statistical analyses were performed using SPSS (v.15.0; SPSS Inc., Chicago, IL USA). Data were analyzed using the non-parametric two-tailed Mann–Whitney U test, and statistical significance was defined as a $p < 0.05$.

RESULTS

Expression of JAK1 and JAK2 in LSG sections from pSS patients and healthy controls

To assess the efficacy of a highly selective JAK1 and JAK2 inhibitor (baricitinib) as a therapeutic agent for pSS patients, we used immunohistochemical staining to examine expression levels and localization of JAK1 and JAK2 in LSGs from SS patients and healthy controls. Representative data from both groups are shown in Fig. 1. In both healthy controls and pSS patients, JAK1 showed particularly intense staining in ductal cells and to a lesser extent in acinar cells. By contrast, although JAK2 showed intense staining in acinar cells from healthy controls, it was also observed in ductal cells. In Grade 1 pSS patients showing slight lymphocytic infiltration, JAK2 expression appeared similar to that in healthy controls, whereas Grades 2 through 4 pSS patients with moderate-to-severe lymphocytic infiltration showed diminished JAK2 expression in acinar cells. Additionally, we observed enhanced JAK2 levels at a lesion displaying immune-cell infiltration around the ductal structure. These results indicated that JAK1 and JAK2 were present in both healthy controls and pSS patients.

Effects of baricitinib on NS-SV-DC cell viability

We then examined the effects of baricitinib on NS-SV-DC cell viability by MTT assay. Following treatment of NS-SV-DC cells with various concentrations of baricitinib for up to 3 days, we observed no effect on NS-SV-DC viability and no significant difference in viability at doses ranging from 10 nM to 5000 nM (Fig. 2).

Effects of baricitinib on *CXCL10* expression and protein secretion in IFN- γ -stimulated NS-SV-DC cells

We previously determined whether IFN- α , IFN- γ , tumor necrosis factor- α , and interleukin-1 β could regulate *CXCL10* expression in human salivary gland cell lines, revealing that IFN- γ -stimulated ductal cells were mainly responsible for *CXCL10* overexpression in the salivary glands of pSS patients [16]. In the present study, we performed RT-qPCR analyses to explore the potential involvement of baricitinib in regulation of IFN- γ -induced *CXCL10* expression. Following treatment of NS-SV-DC cells with 10 ng/mL IFN- γ in the presence or absence of baricitinib (10, 100, or 1000 nM), we observed significant suppression of *CXCL10* mRNA levels ($p < 0.05$) in IFN- γ -stimulated NS-SV-DC cells in a dose-dependent manner (Fig. 3a).

To further evaluate the effects of baricitinib, we performed an ELISA to measure secreted *CXCL10* levels in supernatants of NS-SV-DC cells treated with IFN- γ in the presence or absence of baricitinib. As expected, baricitinib induced in a significant decrease in secreted

CXCL10 levels ($p < 0.05$) in a dose-dependent manner (Fig. 3b), suggesting that JAKs were involved in IFN- γ -induced CXCL10 production in salivary gland ductal cells.

Effects of baricitinib on IFN- γ -induced STAT1 and STAT3 phosphorylation in NS-SV-DC cells

IFN- γ binds to its receptor (IFN γ R) and activates JAK1 and JAK2, leading to primary phosphorylation of STAT1 and secondarily of STAT3, resulting in their translocation to the nucleus in order to bind conserved DNA elements. We previously reported that IFN- γ stimulated the production of CXCL10 via the JAK/STAT pathway in salivary gland ductal cells [16]. To evaluate the effects of baricitinib on IFN- γ -induced STAT1 and STAT3 phosphorylation in NS-SV-DC cells, we performed western blot analyses of extracts from NS-SV-DC cells treated with 10 ng/mL of IFN- γ in the presence or absence of baricitinib (100 nM). The results demonstrated that baricitinib suppressed IFN- γ -induced phosphorylation of STAT1 and STAT3 in NS-SV-DC cells (Fig. 4a), with quantitative data revealing stronger suppression of STAT1 phosphorylation by baricitinib relative to STAT3 phosphorylation (Fig. 4b).

Effects of baricitinib on the chemotaxis of Jurkat T cells

Because a previous report indicated that CXCL10 promotes accumulation of CXCR3⁺ T cells in LSGs of SS patients [14], we assessed the ability of baricitinib to promote recruitment of

Jurkat T cells by IFN- γ -treated NS-SV-DC cells. Migration assays demonstrated that the chemotaxis of Jurkat T cells increased in the presence of IFN- γ -treated NS-SV-DC cells as compared with untreated NS-SV-DC cells. Additionally, baricitinib treatment significantly inhibited the observed IFN- γ -mediated chemotaxis of Jurkat T cells ($p < 0.05$) (Fig. 5). These results indicated that baricitinib inhibited the chemotaxis of CXCR3⁺ T cells by attenuating CXCL10 levels.

DISCUSSION

This is the first study to elucidate the effect of baricitinib, a selective inhibitor of JAK1 and JAK2, on IFN-induced *CXCL10* expression in salivary gland cell clones. Although SS is a chronic autoimmune disease that affects exocrine glands, such as salivary and lacrimal glands, it also causes systemic autoimmune lesions [1–3]. The clinical symptoms of SS include both extraglandular (systemic) and glandular symptoms, such as dryness and swelling [2]. Histologically, SS patients exhibit selective and progressive destruction of the acinar structures in LSGs, resulting in infiltration of various immune cells [4], with destruction of the acinar structures resulting in reduced salivary flow. Gene-expression profiling of LSGs shows that *CXCL10* expression is upregulated in pSS patients [8]. CXCL10 is involved in the accumulation of infiltrating CXCR3⁺ immune cells and SS pathogenesis in the salivary glands of pSS patients [14, 15]. We recently reported that IFN- γ is responsible for *CXCL10* overexpression in salivary glands, and that IFN- γ -stimulated ductal cells, but not acinar cells,

significantly secrete CXCL10 [16]. Furthermore, *in vitro* results showed that IFN- γ enhanced CXCL10 production via both the JAK/STAT and NF- κ B pathways [16]. These findings suggested that JAK inhibitors might inhibit CXCL10 production in salivary glands and suppress destruction of acinar cells.

JAK1 and JAK2 are ubiquitously expressed in various tissues [25]; however, to the best of our knowledge, their expression in LSGs has not been investigated. In the present study, immunohistochemical analyses using LSG sections from control subjects showed that JAK1 and JAK2 localization differed (i.e., JAK1 was strongly expressed in ductal cells, whereas JAK2 was strongly expressed in acinar cells). In the LSGs of pSS patients, the JAK1-expression pattern was similar to that of control subjects; however, JAK2 expression in acinar cells appeared to diminish along with increasing lymphocytic infiltration, possibly indicating that JAK2 phosphorylation increases along with infiltration grade. These findings suggested that JAK1 and JAK2 inhibition by baricitinib could represent a potential treatment for pSS.

Clinical trials for pSS have generally failed, because the majority of enrolled patients showed no extraglandular manifestations at the time of enrollment but rather suffered from fatigue, dryness, and pain that did not significantly respond to the study medication [26]. Improving xerostomia (dry mouth) and keratoconjunctivitis sicca (dry eye) are mostly required for patients with pSS. Therefore, we concluded that treatment of pSS is important to prevent the destruction of glandular tissues. Based on previous studies, we predicted that

blockade of JAK-STAT signaling might inhibit both IFN- γ -induced *CXCL10* expression and chemotaxis in the salivary glands of pSS patients. Therefore, we investigated the *in vitro* pharmacological profile of baricitinib, a novel selective inhibitor of JAK1 and JAK2, using a human salivary gland cell line in order to evaluate its therapeutic potential for treating pSS.

In vitro experiments using immortalized human salivary gland ductal cells, RT-qPCR, and ELISA demonstrated that baricitinib treatment significantly suppressed IFN- γ -induced *CXCL10* expression in a dose-dependent manner. Additionally, IFN- γ induced rapid phosphorylation of both STAT1 and STAT3, whereas these activities were inhibited by baricitinib treatment. These results suggested that baricitinib inhibited IFN- γ -induced *CXCL10* expression in NS-SV-DC cells by suppressing the activation of JAK/STAT signaling.

Previous studies reported that *CXCL10* is expressed in the salivary ductal glands of SS patients and accumulates in CXCR3⁺ immune cells in the LSGs of SS patients [14, 15]. In the present study, we performed migration assays to investigate whether baricitinib can inhibit immune-cell accumulation. We found that the migration of Jurkat T cells was stimulated in response to CM obtained from IFN- γ -treated NS-SV-DC cells. This finding is consistent with the histopathology of LSGs from SS patients (i.e., showing periductal infiltration of T cells). Notably, in the presence of baricitinib, we observed significant suppression of Jurkat T cell migration. This result suggests that baricitinib contributed to inhibition of T cell chemotaxis through downregulation of IFN- γ -stimulated *CXCL10* secretion from salivary gland ductal cells.

In conclusion, this study provides new insight into the underlying molecular mechanism of baricitinib by demonstrating its ability to suppress IFN- γ -induced *CXCL10* expression via inhibition of JAK/STAT signaling in human salivary gland ductal cells. Furthermore, baricitinib inhibited the chemotaxis of Jurkat T cells by reducing CXCL10 production and secretion by salivary gland ductal cells. These findings suggest baricitinib as a potential therapeutic strategy for pSS patients.

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FIGURE LEGENDS

Fig. 1 Expression of JAK1 and JAK2 in LSG sections from pSS patients and healthy controls.

LSG biopsy samples from 12 SS patients categorized into four groups according to the grade of infiltration. Healthy controls included subjects who had experienced subjective symptoms of oral dryness but met none of the objective criteria for SS diagnosis. Representative images are shown. Scale bars: 100 μm .

Fig. 2 Effects of baricitinib on NS-SV-DC cell viability. NS-SV-DC cells (1×10^4 cells/well) were seeded into 96-well plates and after a 24-h incubation with baricitinib (10, 100, 1000, 2500, or 5000 nM). At the indicated time intervals, cell growth was evaluated by MTT assay.

Bar: mean \pm SD.

Fig. 3 Effects of baricitinib on *CXCL10* expression and protein secretion in IFN- γ -stimulated NS-SV-DC cells. (a) Histogram showing relative changes in *CXCL10* mRNA levels in NS-SV-DC cells treated for 6, 12, or 24 h with 10 ng/mL IFN- γ in the presence or absence of baricitinib (10, 100, or 1000 nM). Untreated cells were used as a control. Fold changes in mRNA levels were evaluated by RT-qPCR using *GAPDH* mRNA as an internal reference. Data represent the mean \pm SD of three independent experiments. $*p < 0.05$, two-tailed Mann–Whitney *U* test. (b) Histogram showing the concentration of CXCL10 measured by ELISA in the supernatants of NS-SV-DC cells treated for 6, 12, or 24 h with 10 ng/mL IFN- γ in the

presence or absence of baricitinib (10, 100, or 1000 nM). Untreated cells were used as a control. Data represent the mean \pm SD of three independent experiments. $*p < 0.05$, two-tailed Mann–Whitney *U* test.

Fig. 4 Effects of baricitinib on IFN- γ -induced STAT1 and STAT3 phosphorylation in NS-SV-DC cells. (a) Representative western blot analysis showing STAT1, phospho-STAT1 (P-STAT1), STAT3, and phospho-STAT3 (P-STAT3) levels in NS-SV-DC cells treated for various time periods (5, 10, 30, or 60 min) with 10 ng/mL IFN- γ in the presence or absence of 100 nM baricitinib. β -actin was used as an internal control. (b) Histogram showing relative protein levels normalized against that of β -actin.

Fig. 5 Effects of baricitinib on the chemotaxis of Jurkat T cells. NS-SV-DC cells were treated with 10 ng/mL IFN- γ in the presence or absence of baricitinib (100 nM) for 24 h. Serum-free Jurkat T cells were placed in the migration chamber, and the chemotaxis plate was incubated at 37 °C for 24 h. Fluorescence values (480/520 nm) are expressed as RFUs. Data represent the mean \pm SD of three independent experiments. $*p < 0.05$, two-tailed Mann–Whitney *U* test.

Figure 1

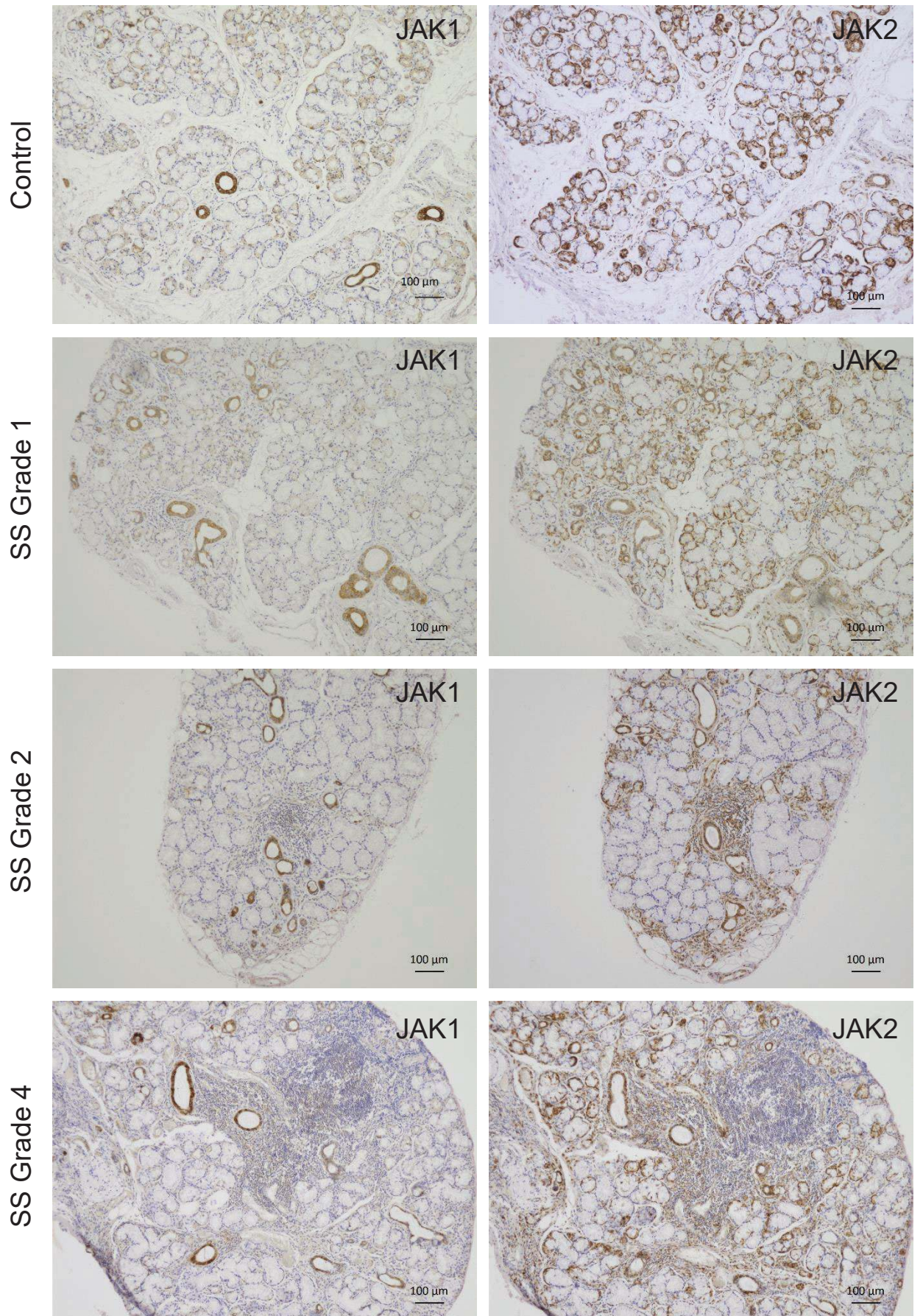


Figure 2

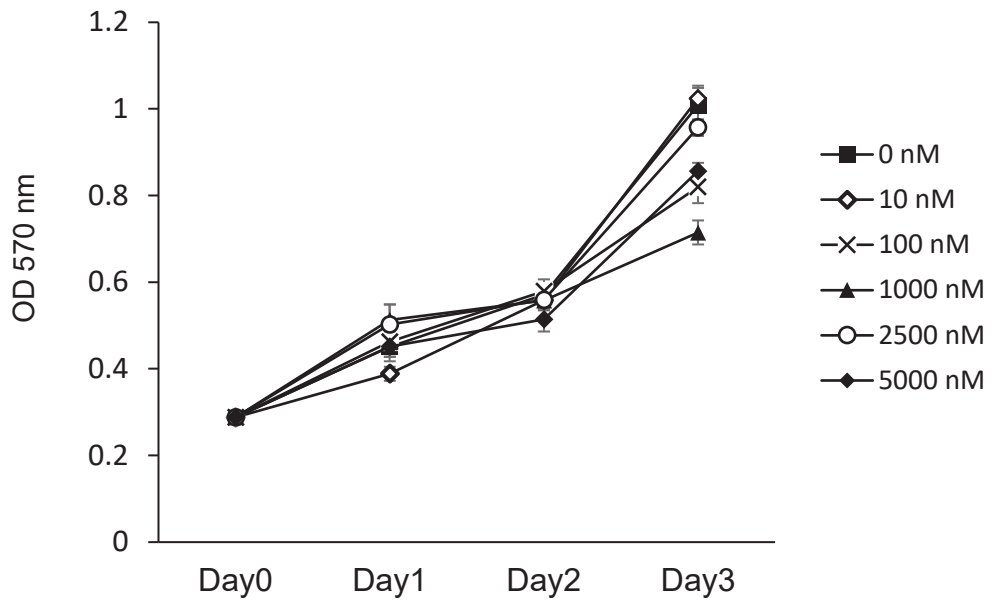
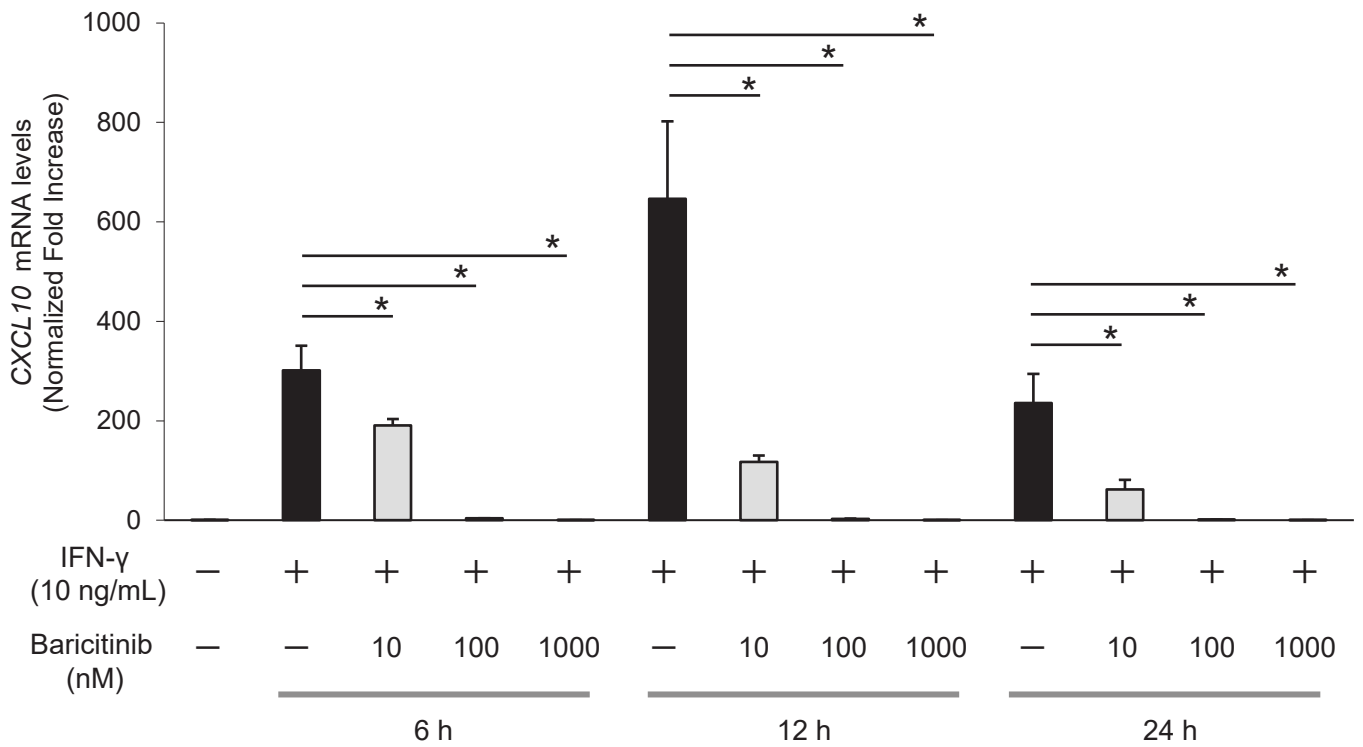


Figure 3

a



b

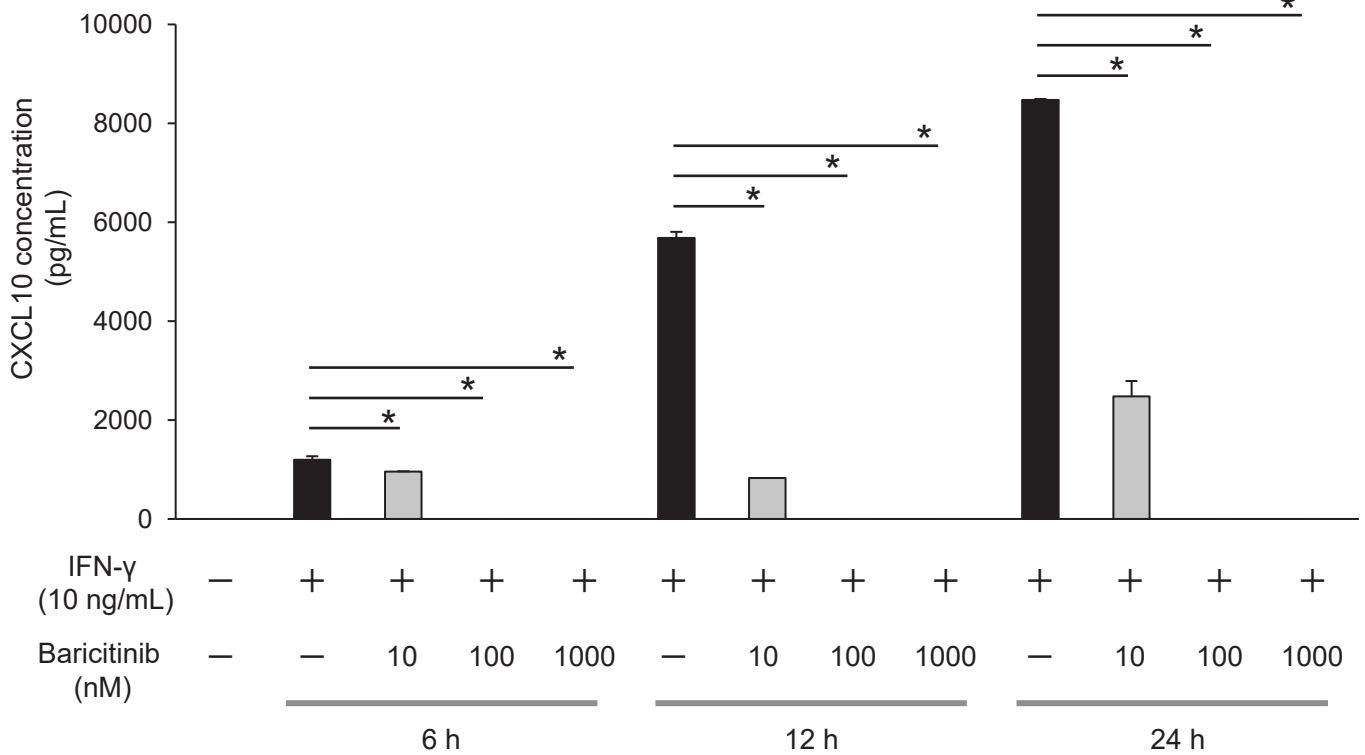
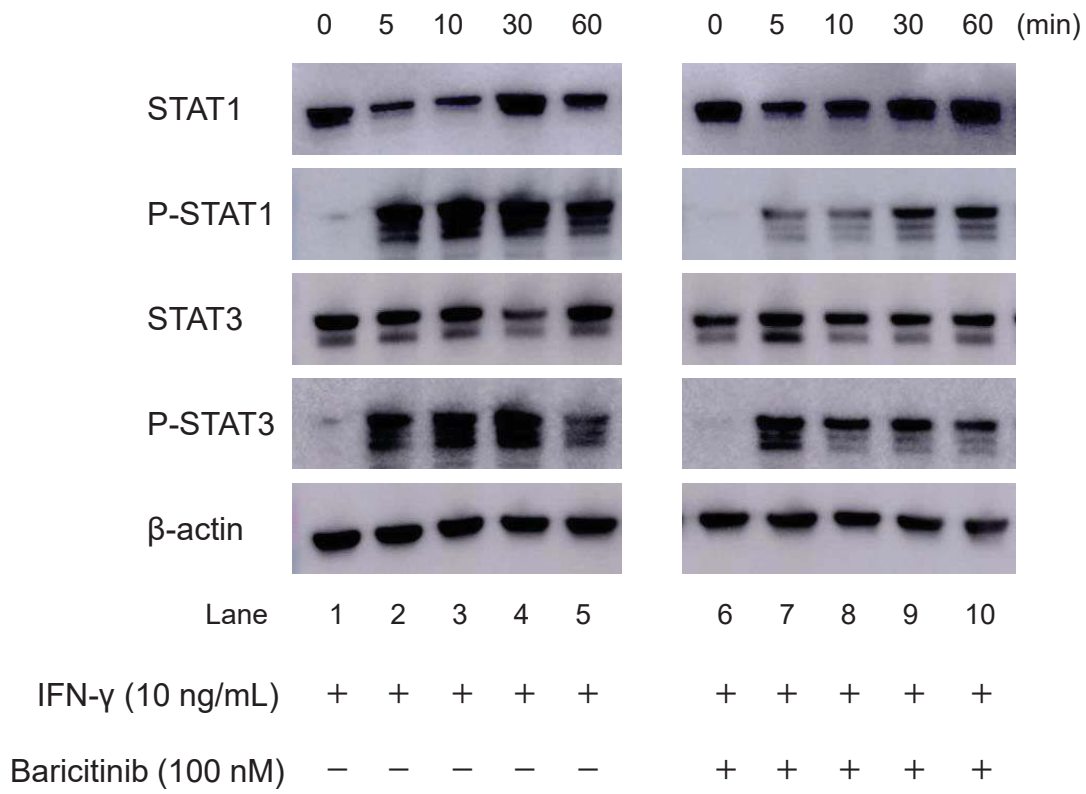


Figure 4

a



b

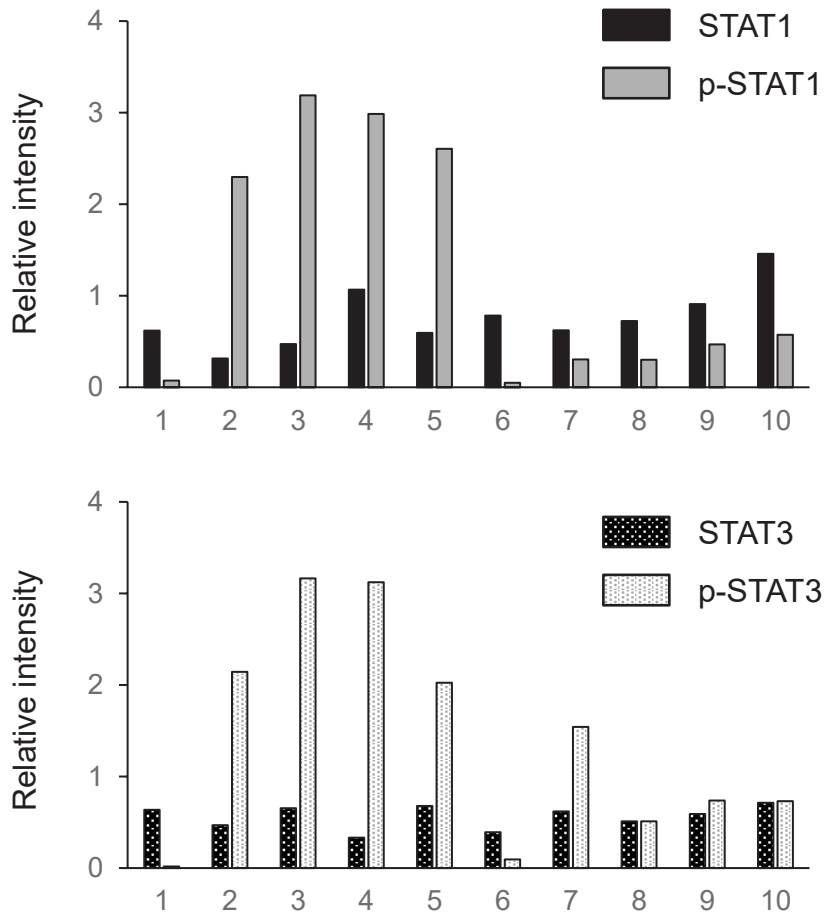


Figure 5

