Research Article

Distinct regulation of CXCL10 production by cytokines in human salivary gland ductal and acinar cells

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ABSTRACT (224 words)

CXCL10, a CXC chemokine induced by interferon-gamma [IFN-γ], has been observed in a wide variety of chronic inflammatory disorders and autoimmune conditions. Although CXCL10 is known to be overexpressed in the salivary glands of individuals with primary Sjögren's syndrome (pSS), it is unclear which cells produce CXCL10 under what types of stimulations. Here we investigated the precise molecular mechanisms by which CXCL10 was produced in human salivary gland ductal (NS-SV-DC) and acinar (NS-SV-AC) cell lines. Our results demonstrated that NS-SV-DC cells produced higher levels of CXCL10 compared to NS-SV-AC cells. In addition, our findings demonstrated that the regulator of the enhancement of CXCL10 was different between NS-SV-DC and NS-SV-AC cells; i.e., interferon-gamma (IFN-γ) had more potential than interferon-alpha (IFN-α), tumor necrosis factor (TNF)-α, and interleukin (IL)1-β in the induction of CXCL10 production in NS-SV-DC cells, whereas TNF-α had potential to induce CXCL10 production in NS-SV-AC cells. A Western blot analysis demonstrated that IFN-γ enhanced the production of CXCL10 via both the JAK/STAT1 pathway and the NF-κB pathway in NS-SV-DC cells, whereas TNF-α enhanced the production of CXCL10 via the NF-κB pathway in NS-SV-AC cells. The results of study suggest that the CXCL10 overexpression in the salivary glands is caused mainly by IFN-γ-stimulated salivary gland ductal cells. The enhanced production of CXCL10 by IFN-γ from ductal cells may result in the inflammation of pSS lesions.

KEY WORDS:

CXCL10, IFN-γ, TNF-α, primary Sjögren's syndrome, salivary gland ductal cells, salivary gland acinar cells

INTRODUCTION

Chemokines are a specialized family of cytokines that function as potent mediators of inflammation based on their ability to recruit and activate specific leucocyte subpopulations by modifying the expression and affinity status of adhesion molecules on the leucocyte surface [1]. CXCL10 is a non-ELR (lacking the Glu-Leu-Arg tripeptide motif) CXC chemokine [2]. CXCL9, CXCL10, and CXCL11 utilize the same chemokine receptor, CXCR3 [2], a G protein-coupled receptor. CXCR3 is induced on naïve T cells following activation, and preferentially remains highly expressed on type-1 helper (Th1)-type $CD4^+$ T cells, effector $CD8^+$ T cells, and innate-type lymphocytes, such as natural killer (NK) and NKT cells [2].

Sjögren's syndrome (SS), one of the most common autoimmune diseases [3], is characterized by the eventual total replacement of the acinar structure by marked lymphocytic infiltrates in the salivary and lacrimal glands [4]. Histological studies show that $CD4^+$ T cells predominantly infiltrate the salivary glands at an early stage of primary SS (pSS) [5]. It has been reported that CXCL9, CXCL10, and CXCL11 were involved in the accumulation of T-cell infiltrates in the salivary glands of pSS patients [6, 7]. In addition, the inoculation of a CXCL10 antagonist into MRL/*lpr* mice during the early stage of sialadenitis significantly reduced the mononuclear cell infiltration and parenchymal destruction [8].

Interferons (IFNs) play significant roles in the pathogenesis of autoimmune diseases, including SS. IFNs are subdivided into three distinct types: type I IFN (IFN-I; mainly

IFN-α/β), type II IFN (IFN-II; mainly IFN-γ), and type III IFN (IFN-III; mainly IFN-λ/interleukin [IL]-28/IL-29) [9]. Although the genes involved in SS were originally considered to be mediated via only type I interferons, recent findings demonstrated that genes upregulated by both type I and type II interferons are involved in pSS [10]. In addition, microarray studies in the labial salivary glands (LSGs) of patients with pSS revealed that several interferon-regulated genes were up-regulated [11, 12]. Several other cytokines, including IL-6, IL-7, IL-10, IL-12, IL-17, IL-21, tumor necrosis factor-alpha (TNF-α) [13], and IL-1β [14, 15], have been suggested to play roles in the pathogenesis of pSS.

It has thus been hypothesized that several cytokines cause autoimmune salivary diseases, and that CXCL10 plays an important role in the pathogenesis of SS. However, it is not yet known which cells produce CXCL10 by what types of cytokines. To the best of our knowledge, no study has evaluated the effects of IFN-α, IFN-γ, or several cytokines on the secretion of CXCL9, CXCL10, and CXCL11 by human salivary ductal cells or acinar cells. We therefore examined the effects of IFN-α, IFN-γ, TNF-α, and IL-1β stimulation on the expressions of CXCL9, CXCL10, and CXCL11 by using immortalized human salivary gland cell clones in an *in vitro* experiment. We also investigated the signal transduction pathways that induced the production of CXCL10.

MATERIALS AND METHODS

Cell culture

The characteristics of the cell lines NS-SV-DC (immortalized human salivary gland ductal cells) and NS-SV-AC (immortalized human salivary gland acinar cells) are described in detail elsewhere [16]. These cell clones were cultured at 37°C in serum-free keratinocyte medium (Gibco Laboratories, Gaithersburg, MD) in an incubator with an atmosphere containing 5% CO_2.

Reagents

Recombinant human IFN-α, IFN-γ, TNF-α and IL-1β were purchased from R&D Systems (Minneapolis, MN). Bay11-7082 was purchased from Wako (Osaka, Japan). 50-deoxy-50-(methylthio)-adenosine (MTA) was obtained from Sigma-Aldrich Co. (St. Louis, MO). Bay11-7082 [17] and MTA [18] were used for the inhibition of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) and signal transduction and activator of transcription1 (STAT1), respectively.

Quantitative real-time reverse transcription-polymerase chain reaction (qRT-PCR)

We treated NS-SV-DC and NS-SC-AC cells with 1000 IU/mL IFN-α, 10 ng/mL IFN-γ, 10 ng/mL TNF-α, and 1 ng/mL IL-1β for 6, 12, or 24 h. Total cellular RNA was isolated with TRIzol reagent (Life Technologies, Carlsbad, CA). The cDNA was synthesized from total RNA with the use of a PrimeScript RT reagent Kit (Takara, Otsu, Japan). We quantitatively analyzed the expression levels of mRNAs for CXCL9, CXCL10, CXCL11 and GAPDH using

an ABI Prism 7000 Sequence Detection System (Applied Biosystems Japan, Tokyo) and TaqMan® Universal PCR Master Mix (Applied Biosystems) and Assays-on-Demand™ Gene Expression Products (Applied Biosystems) according to the manufacturer's recommendations. The thermal cycler protocol was 95°C for 10 min, followed by 40 cycles of 95°C for 5 s and 60°C for 30 s.

We performed an analysis of the relative gene expression data using the $2^{-\Delta\Delta CT}$ method on Sequence Detection System Software (Applied Biosystems). We calculated the fold change in the studied gene expression, normalized to an endogenous control, using the formula $RQ = 2^{-\Delta\Delta CT}$. The relative expression levels of CXCR3 ligand mRNAs are expressed as the fold increase in the GAPDH mRNA expression.

Reverse transcription-polymerase chain reaction (RT-PCR)

The total cellular RNA was isolated from unstimulated NS-SV-DC and NS-SC-DC cells using TRIzol reagent. The cDNA was synthesized according to the above method. Specific primers were custom synthesized (Sigma, Deisenhofen, Germany). The sense and anti-sense primers for IFNGR1, IFNGR2, TNFR1, TNFR2 and GAPDH, respectively, were as follows: 5’-GCTGTATGCCGAGATGGAAAA-3’ and 5’-AGGAAAATGGCTGGTATGACG-3’, 5’-CGACAGTAAATGGTTCACGGC-3’ and 5’-TGGACATAATAACAAAAAAAGGC-3’, 5’-GCCTCTGCCTCAATGGGACCG-3’ and 5’-GGGGTGAAGCCTGGAGTGGGA-3’, 5’-GCCCACTCGGAACCAGCCACA-3’ and

5’-TCAGGCACTCCAAGGGGCAGG-3’, 5’-ACGCATTTGGCTGTATTGGG-3’ and 5’-TGATTTTGGAGGGATCTCGC-3’. The PCR reactions were conducted in a DNA Thermal Cycler (model LifeECO, Nippon Genetics, Tokyo). The reaction conditions were as follows: IFNGR1, after 1 min of denaturation at 94°C, 35 cycles of PCR were performed (94°C for 30 s and 68°C for 6 min), followed by a final 6 min extension at 68°C; IFNGR2, TNFR1, TNFR2, and GAPDH: after 1 min of denaturation at 94°C, 35 cycles of PCR were performed (94°C for 30 s and 68°C for 3 min), followed by a final 3 min extension at 68°C. The amplified products were electrophoresed in ethidium bromide-stained 1.5% agarose gel.

Enzyme-linked immunosorbent assay (ELISA)

We treated NS-SV-DC and NS-SC-AC cells with 1000 IU/mL IFN-α, 10 ng/mL IFN-γ, 10 ng/mL TNF-α, and 1 ng/mL IL-1β for 6, 12, or 24 h. The concentrations of CXCL9, CXCL10, and CXCL11were each determined with commercially available ELISA kits (R&D Systems) according to the manufacturer's instructions. Optimal absorbance was read at 450 nm in a microtiter plate reader. The CXCR3 ligand protein levels were determined using the standard curve prepared for each assay.

We performed blocking experiments using specific transcription factors. NS-SV-DC cells were incubated with IFN-γ (10 ng/mL) in the presence or absence of the STAT1 inhibitor, MTA (1 mM), or the NF-κB inhibitor, Bay11-7082 (10 μM), for 24 h. NS-SV-AC cells were incubated with TNF-α (10 ng/mL) in the presence or absence of Bay11-7082 (10

μM) or MTA (1 mM) for 24 h. CXCL10 secretion was analyzed by ELISA kits. Optimal absorbance was read at 450 nm in a microtiter plate reader.

Protein isolation and Western blot analysis

NS-SV-DC cells were treated with 10 ng/mL IFN-γ for 1, 5, 10, 30, 60, 120, or 360 min, and NS-SV-AC cells were treated with 10 ng/mL TNF-α for 5, 15, 30, 60, or 120 min. Whole-cell lysates were prepared using M-PER lysis solution (Thermo Fisher Scientific, Waltham, MA) supplemented with Halt™ Protease Inhibitor Cocktail (Thermo Fisher Scientific). Nuclear extracts were prepared using NE-PER Nuclear and Cytoplasmic Extraction Reagent (Thermo Fisher Scientific). Cytosolic extracts (20 μg) were subjected to electrophoresis on 10% sodium dodecyl sulfate (SDS)-polyacrylamide gels (Bio-Rad, Hercules, CA), and then transferred onto nitrocellulose membranes. The membranes were blocked with 3% bovine serum albumin and incubated with each of the following antibodies (all from Cell Signaling Technology, Beverly, MA; diluted at 1:1000): anti-janus kinase1 (JAK1), anti-Phospho-JAK1, anti-JAK2, anti-Phospho-JAK2, anti-STAT1, anti- Phospho-STAT1, anti-p65, anti-inhibitor of κB (IκB)-α, anti-Phospho-IκB-α, and anti-β-actin. After intervening rinses with TBS-T, the IgG secondary antibodies (Cell Signaling Technology; diluted at 1:1000) were used for the respective primary antibodies. The immune complexes were visualized by enhanced chemiluminescence (ECL) Western Blotting Detection Reagent (GE Healthcare,

Buckinghamshire, UK). The density of the visualized immune complexes was digitized using an Amersham Imager 600 (GE Healthcare).

Statistical analysis

The data were analyzed with the use of the non-parametric Mann-Whitney U-test. SPSS Statistics-15.0 J software was used for the analyses. Statistical tests were two-sided, and significance was defined as a *p*-value <0.05.

RESULTS

The expressions of CXCL9, CXCL10, and CXCL11 mRNA and protein following IFN-α, IFN-γ, TNF-α, or IL-1β stimulation in NS-SV-DC and NS-SV-AC cells

We attempted to identify the factors that regulate the expressions of CXCL9, CXCL10, and CXCL11, using NS-SV-DC and NS-SV-AC cells. The CXCL10 gene has been reported to contain response elements for STAT1 and NF-κB [19]. We therefore selected IFN-α, IFN-γ, TNF-α, and IL-1β. Indeed, these cytokines were reported to be associated with pSS [10, 13–15]. We conducted a qRT-PCR to examine the expression levels of CXCL9, CXCL10, and CXCL10 mRNAs following IFN-α, IFN-γ, TNF-α, or IL-1β stimulation. As shown in Figure 1a, significant increases in the expressions of CXCL9, CXCL10, and CXCL11 mRNAs were observed in NS-SV-DC cells after treatment with IFN-γ compared to the unstimulated control cells. At 12 h of treatment, the mRNA levels of CXCL9, CXCL10, and CXCL11 were

24352.8-, 3026.4-, and 712.5-fold higher than the basal levels, respectively. In the case of TNF-α treatment, an increase in the expression of CXCL10 mRNA was detected only in NS-SV-AC cells (Fig. 1a). On the other hand, IFN-α had a marginal effect on the expression of only CXCL11 mRNAs in NS-SV-DC cells, and no effect in NS-SV-AC cells. IL-1β had no effect on the expression of these CXCR3 ligand mRNAs in either cell line (Fig. 1a).

To further evaluate the expressions of CXCL9, CXCL10, and CXCL11 proteins, we detected the protein levels by ELISA using the conditioned medium derived from NS-SV-DC and NS-SV-AC cells treated with IFN-α, IFN-γ, TNF-α, or IL-1β. The NS-SV-DC cells showed a significantly increased expression of the CXCL10 proteins after IFN-γ stimulation (Fig. 1b). The NS-SV-AC cells showed an increase in the expression of CXCL10 proteins after TNF-α stimulation (Fig. 1b). IFN-α and IL-1β had no significant effect on the expressions of these CXCR3 ligand proteins in either cell line (Fig. 1b). These results indicate that ductal cells play an important role in the secretion of CXCR3 ligand chemokines, especially CXCL10, in salivary gland cells. In addition, our findings demonstrated that IFN-γ and TNF-α are the key cytokines in CXCL10 production in salivary gland cells.

The detection of IFN-γ receptor and TNF-α receptor mRNA in NS-SV-DC and NS-SV-AC cells

NS-SV-DC and NS-SV-AC cells were examined for the presence of mRNA for IFN-γ receptor (IFNGR) and TNF-α receptor (TNFR). IFNGR consists of two subunits: IFNGR1

(also known as the IFN-γ receptor α chain) and IFNGR2 (also known as the IFN-γ receptor β chain) [20]. TNFR consists of two subunits: a 55 kDa type1 receptor (TNFR1) and a 75 kDa type2 receptor (TNFR2) [21]. As shown in Figure 2, NS-SV-DC and NS-SV-AC cells expressed the mRNAs for IFNGR1, IFNGR2, TNFR1 and TNFR2 almost same at the intense level in both cells.

The effect of IFN-γ on the CXCL10 production in NS-SV-DC cells and the effect of TNF-α on CXCL10 production in NS-SV-AC cells

We next conducted an ELISA assay to investigate the effect by the density of cytokines on the CXCL10 production in each cell line. As shown in Figure 3a, the CXCL10 production in NS-SV-DC cells was increased by the treatment with IFN-γ in a dose-dependent manner. Although the CXCL10 production in NS-SV-AC cells was increased by the treatment with TNF-α (>10 ng/mL), there was no dose-dependent increase (Fig. 3b). These results suggested that NS-SV-DC cells had a greater ability to produce CXCL10 compared to NS-SV-AC cells.

The involvement of STAT1 and NF-κB in CXCL10 production in NS-SV-DC and NS-SV-AC cells

The CXCL10 gene has been reported to contain response elements for STAT1 and NF-κB [19]. Accordingly, we next examined the involvement of the two transcription factors STAT1 and NF-κB in the production of CXCL10. We did this by using a STAT1 inhibitor, MTA [18],

and an NF-κB inhibitor, Bay11-7082 [17]. As shown in Figure 4a, the addition of MTA (1 mM) resulted in a significant decrease in the IFN-γ-induced CXCL10 production in NS-SV-DC cells ($p<0.05$). Bay11-7082 also significantly decreased the IFN-γ-induced CXCL10 secretion ($p<0.05$) (Fig. 4a). In contrast, the TNF-α-induced CXCL10 production in NS-SV-AC cells was significantly reduced by Bay11-7082 ($p<0.05$) but not by MTA (Fig. 4b). These data suggest that in NS-SV-DC cells, IFN-γ enhanced the CXCL10 production via both the JAK/STAT1 pathway and the NF-κB pathway, whereas in N S-SV-AC cells, TNF-α enhanced the CXCL10 production via the NF-κB pathway but not the JAK/STAT1 pathway.

IFN-γ promoted the phosphorylations of JAK1, STAT1, and IκB-α in NS-SV-DC cells, whereas TNF-α promoted the phosphorylation of IκB-α in NS-SV-AC cells.

To clarify the signal transduction pathway activated by IFN-γ in NS-SV-DC cells, we examined the expression levels of JAK1, JAK2, STAT1, phospho-JAK1, phospho-JAK2, phospho-STAT1, p65, IκB-α, and phospho-IκB-α. The Western blot analysis demonstrated that IFN-γ induced the phosphorylations of JAK2 and STAT1 (Fig. 5a); however, JAK1 was not observed in NS-SV-DC cells (data not shown). In addition, IFN-γ promoted the phosphorylation of IκB-α, and p65 was shifted to the nucleus in NS-SV-DC cells (Fig. 5b).

We then investigated the signal transduction pathway activated by TNF-α in NS-SV-AC cells. The expression levels of p65, IκB-α, and phospho-IκB-α were examined. The Western blot analysis showed that IκB-α was degraded by TNF-α, and p65 was shifted to

the nucleus in NS-SV-AC cells (Fig. 5c). These results indicate that IFN-γ promotes the phosphorylation of JAK2 and IκB-α, thereby resulting in the activation of both the JAK/STAT1 pathway and the NF-κB pathway in NS-SV-DC cells, whereas TNF-α promotes the phosphorylation of IκB-α, thereby resulting in the activation of the NF-κB pathway in NS-SV-AC cells.

DISCUSSION

SS is a chronic autoimmune disease characterized by lymphocytic infiltration and the destruction of salivary and lacrimal glands [3, 4]. Histologically, SS is characterized by an extensive target tissue infiltration of Th cells, predominantly $CD4^+$ Th cells but also $CD8^+$ Th cells [5]. Based on the expression patterns of cytokines, it has been shown that $CD4^+$ Th cells are distributed in Th1 and Th2 cells. Accumulated evidence indicates a close relationship between the cytokine expression in salivary gland tissues and the development and progression of pSS. There is supportive evidence that leukocyte activation and elicitation through a cytokine-driven network is one of the key processes in the autoimmune response [22]. A 2001 review indicated that chemokines are one of the families of effector molecules under the control of the cytokine-driven network [22].

Chemokines are a superfamily of cytokines that regulate immune cell migration under both inflammatory and normal physiological conditions [23]. The interactions between chemokines and their receptors play important roles in the induction of a selective local

infiltration of specific cells in various diseases [24]. The CXCR3 ligand chemokines (CXCL9, CXCL10, and CXCL11) are dual-function chemokines induced by IFN-γ, and they are produced during Th1-type immune responses [2] by diverse cell types, including neutrophils, monocytes, fibroblasts, keratinocytes, endothelial cells, and astrocytes [25].

The expression of CXCR3 and CXCR3 targeting chemokines has been demonstrated to be involved in several diseases and pathological states, including sarcoidosis, viral infections, allograft rejection, rheumatoid arthritis, diabetes, and glomerulonephritis [26–29]. Regarding SS, it was reported that CXCR3 ligand chemokines were expressed in the salivary glands of pSS [6, 7], and that the downregulation of CXCL10 reduced the mononuclear cell infiltration in MRL/*lpr* mice [8]. Although CXCR3 ligand chemokines and CXCR3 appear to contribute to the pathogenesis of pSS, the clinical significance of chemokines and their receptors and the underlying molecular mechanisms are not yet known.

In the present study, we performed an *in vitro* experiment using NS-SV-DC and NS-SV-AC cells to identify the precise molecular mechanism involved in the regulation of CXCL9, CXCL10, and CXCL11 in salivary gland cells. We selected IFN-α, IFN-γ, TNF-α, and IL-1β as reagents that may regulate the expression of CXCR3 ligands. The CXCL10 gene has been reported to contain NF-κB and STAT1-responsible elements in the promoter regions [19]. Interestingly, our study demonstrated that the regulator of the enhancement of CXCR3 ligand chemokines was different between NS-SV-DC and NS-SV-AC cells. Although both cells expressed IFN-γ receptor and TNF-α receptor, we detected a significant increase in the

expression of CXCL10 in NS-SV-DC cells after IFN-γ treatment, whereas the increase in the expression of CXCL10 in NS-SC-AC cells was detected after TNF-α treatment. These results indicated that IFN-γ and TNF-α are the key cytokines in CXCL10 production in salivary gland cells. Our findings also demonstrated that NS-SV-DC cells had a greater ability to produce CXCL10 compared to NS-SV-AC cells. These findings are in accord with the histopathological images of pSS that show the periductal infiltration of T cells. The CXCL10 secretion from ductal cells by IFN-γ stimulation may be caused by an infiltration of $CXCR3^+$ T cells in pSS lesion.

The blocking experiments using specific transcription factors revealed that a specific inhibitor of the STAT1 pathway (MTA) decreased the CXCL10 in IFN-γ-treated NS-SC-DC cells. The specific inhibitor of the NF-κB pathway (Bay11-7082) also decreased the CXCL10 in IFN-γ-treated NS-SV-DC cells. These results are consistent with those of a study reporting that IFN-γ potentiates the TNF-α-induced CXCL10 production in human monocytes by increasing the activation of STAT1 and NF-κB through the JAK1 and JAK2 pathways (i.e., by crosstalk between IFN-γ and TNF-α signal transduction) [30]. In contrast, the specific inhibitor of the NF-κB pathway (Bay11-7082) but not the specific inhibitor of the STAT1 pathway (MTA) decreased the CXCL10 in TNF-α-treated NS-SV-AC cells. Our findings demonstrated that the IFN-γ-induced CXCL10 expression was mediated by the JAK2/STAT1 and NF-κB pathways in NS-SV-DC cells, whereas the TNF-α-induced CXCL10 expression was transduced by the NF-κB pathway in NS-SV-AC cells.

In conclusion, the results of this study demonstrated that the regulator of the enhancement of CXCL10 was different between salivary ductal cells and acinar cells, and that salivary ductal cells had a higher ability to produce CXCL10 compared to acinar cells. The enhanced production of CXCL10 by IFN-γ from ductal cells may result in the migration of $CXCR3^+$ T cells. This may be one of the key processes in the inflammation of pSS lesions.

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COMPLIANCE WITH ETHICAL STANDARDS

Conflicts of interest:

None of the authors has any potential financial conflict of interest related to this manuscript.

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FIGURE LEGENDS

Fig. 1. The expressions of CXCL9, CXCL10, and CXCL11 mRNA and protein following IFN-α, IFN-γ, TNF-α or IL-1β stimulation in NS-SV-DC and NS-SV-AC cells. a: NS-SV-DC and NS-SC-AC cells were treated with IFN-α (1000 IU/mL), IFN-γ (10 ng/mL), TNF-α (10 ng/mL) or IL-1β (1 ng/mL) for 6, 12, or 24 h. Total RNAs were prepared, and the quantification of mRNA levels was evaluated by qRT-PCR. Unstimulated cells were used as a control. Bar: mean±SD. **b:** NS-SV-DC and NS-SC-AC cells were treated with IFN-α (1000 IU/mL), IFN-γ (10 ng/mL), TNF-α (10 ng/mL) or IL-1β (1 ng/mL) for 6, 12, or 24 h. An ELISA was performed to measure CXCL9, CXCL10, and CXCL11 protein in the conditioned medium. Unstimulated cells were used as a control. Bar: mean±SD.

Fig. 2. The detection of IFN-γ receptor and TNF-α receptor mRNA in NS-SV-DC and NS-SV-AC cells. NS-SV-DC and NS-SV-AC cells were examined for the presence of mRNAs for IFNGR1, IFNGR2, TNFR1, and TNFR2 by RT-PCR. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as housekeeping gene. The mRNAs of IFN-γ receptor and TNF-α receptor were detected in both cells.

Fig. 3. The effect of IFN-γ on the CXCL10 production in NS-SV-DC cells and the effect of TNF-α on the CXCL10 production in NS-SV-AC cells. a: NS-SV-DC cells were treated with 1, 10, 50, or 100 ng/ml of IFN-γ for 24 h. An ELISA was performed to measure

CXCL10 protein in the conditioned medium. Bar: mean±SD. *p<0.05. **b:** NS-SV-AC cells were treated with 1, 10, 50, or 100 ng/ml of TNF-α for 24 h. An ELISA was performed to measure CXCL10 protein in the conditioned medium. Bar: mean±SD. *p<0.05.

Fig. 4. The involvement of STAT1 and NF-κB in the CXCL10 production in NS-SV-DC and NS-SV-AC cells. a: NS-SV-DC cells were incubated with IFN-γ (10 ng/mL) in the presence or absence of the STAT1 inhibitor MTA (1 mM) or the NF-κB inhibitor Bay11-7082 (10 μM) for 24 h. CXCL10 secretion was analyzed with ELISA kits. Optimal absorbance was read at 450 nm in a microtiter plate reader. The IFN-γ-induced CXCL10 production in NS-SV-DC cells was significantly reduced by Bay11-7082 and MTA. Bar: mean±SD. *p<0.05. **b:** NS-SV-AC cells were incubated with TNF-α (10 ng/mL) in the presence or absence of Bay11-7082 (10 μM) or MTA (1 mM) for 24 h. The TNF-α-induced CXCL10 production in NS-SV-AC cells was significantly reduced by Bay11-7082, but not by MTA. CXCL10 secretion was analyzed with ELISA kits. Optimal absorbance was read at 450 nm in a microtiter plate reader. Bar: mean±SD. *p<0.05. ND: not determined.

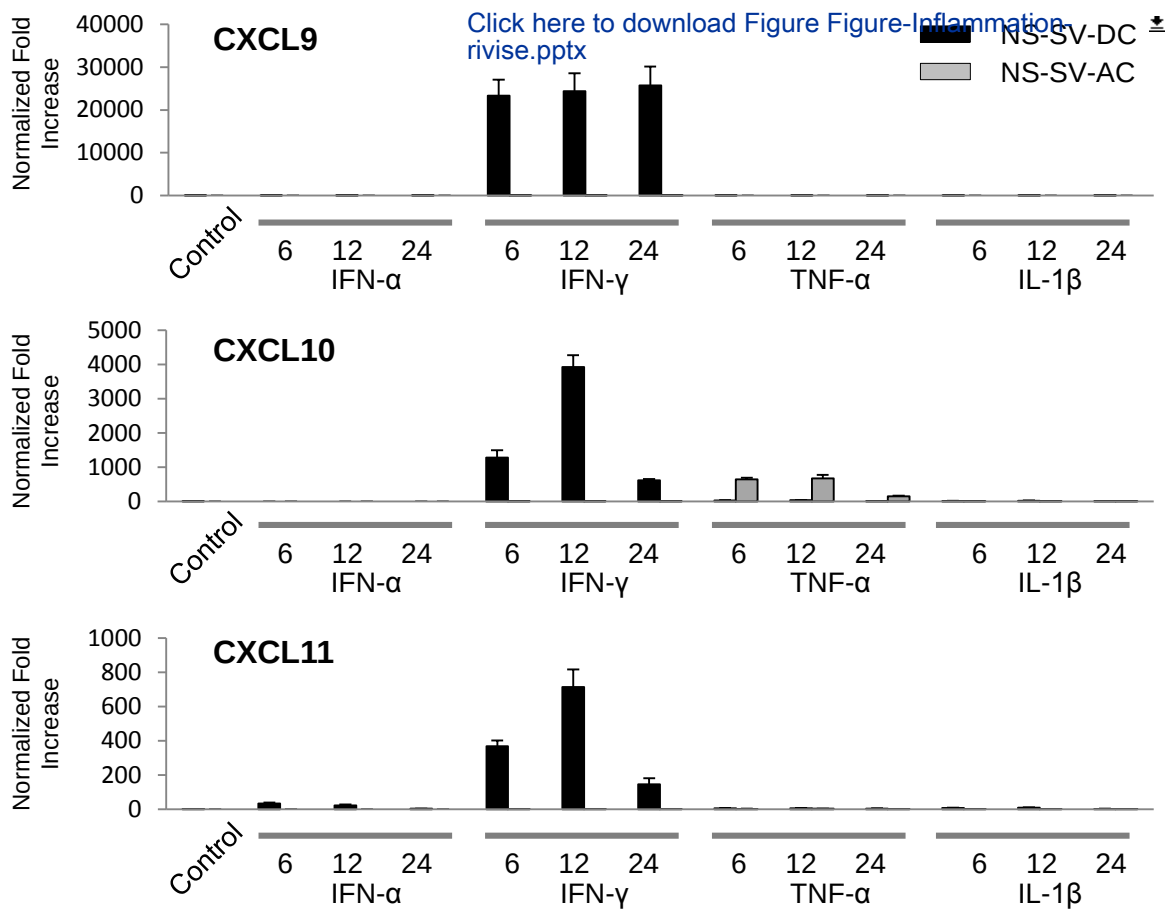
Fig. 5. IFN-γ promoted the phosphorylations of JAK1, STAT1, and IκB-α in NS-SV-DC cells, whereas TNF-α promoted the phosphorylation of IκB-α in NS-SV-AC cells. a: NS-SV-DC cells were treated with 10 ng/mL of IFN-γ. The Western blot analysis showed that phospho-JAK2 and phospho-STAT1 were significantly increased after IFN-γ stimulation. **b:**

NS-SV-DC cells were treated with 10 ng/mL of IFN-γ. IFN-γ promoted the phosphorylation of IκB-α, and p65 was shifted to the nucleus in NS-SV-DCcells. **c:** NS-SV-AC cells were treated with 10 ng/mL of TNF-α. The Western blot analysis showed that IκB-α was degraded by TNF-α, and p65 was shifted to the nucleus in NS-SV-AC cells.

Figure

Figure 1

a



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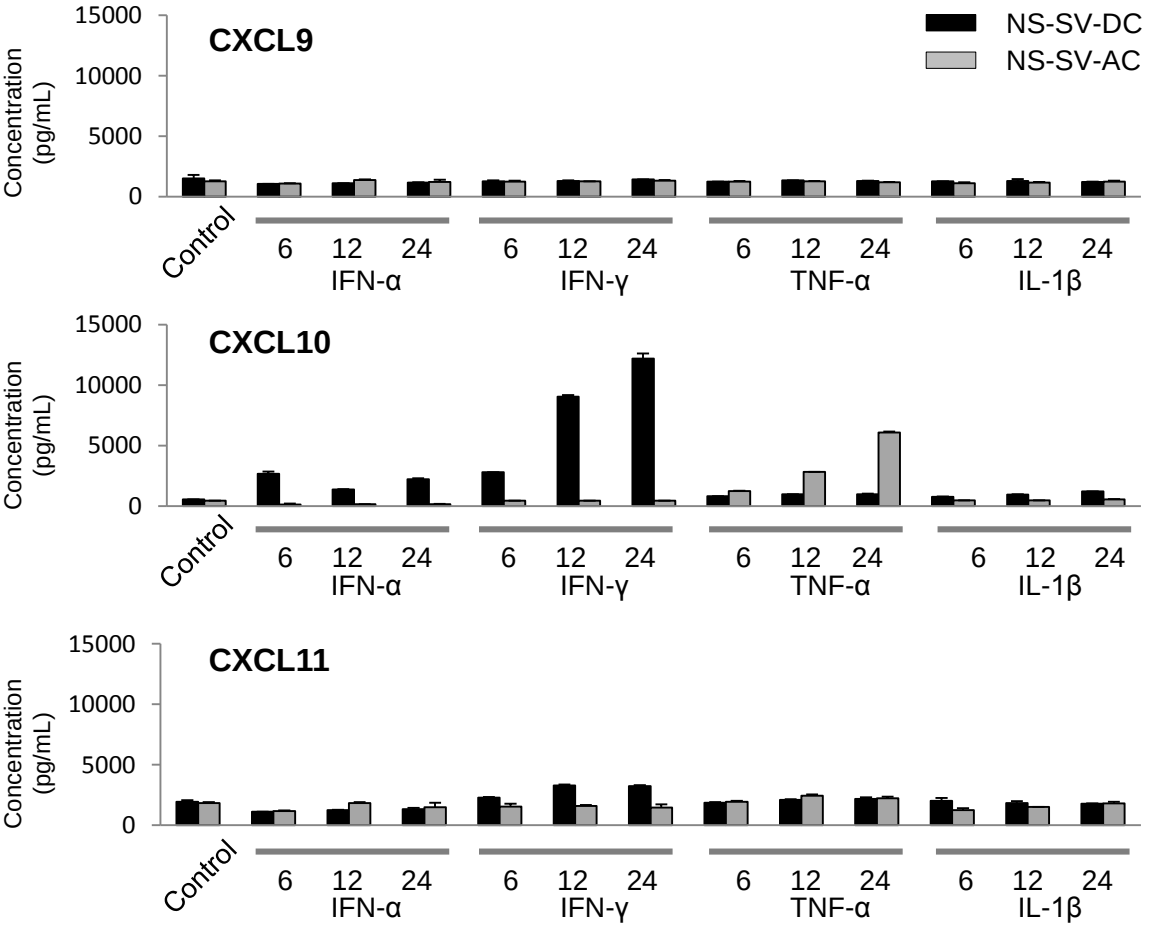


Figure 2

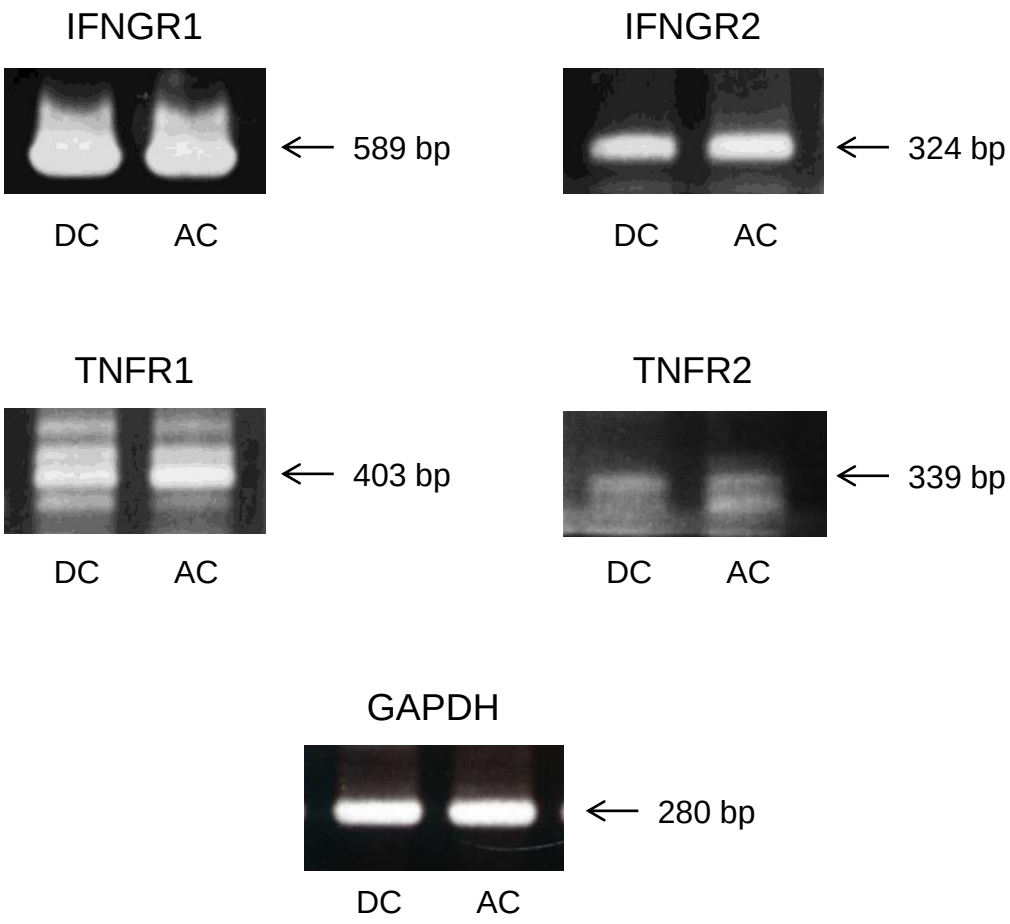
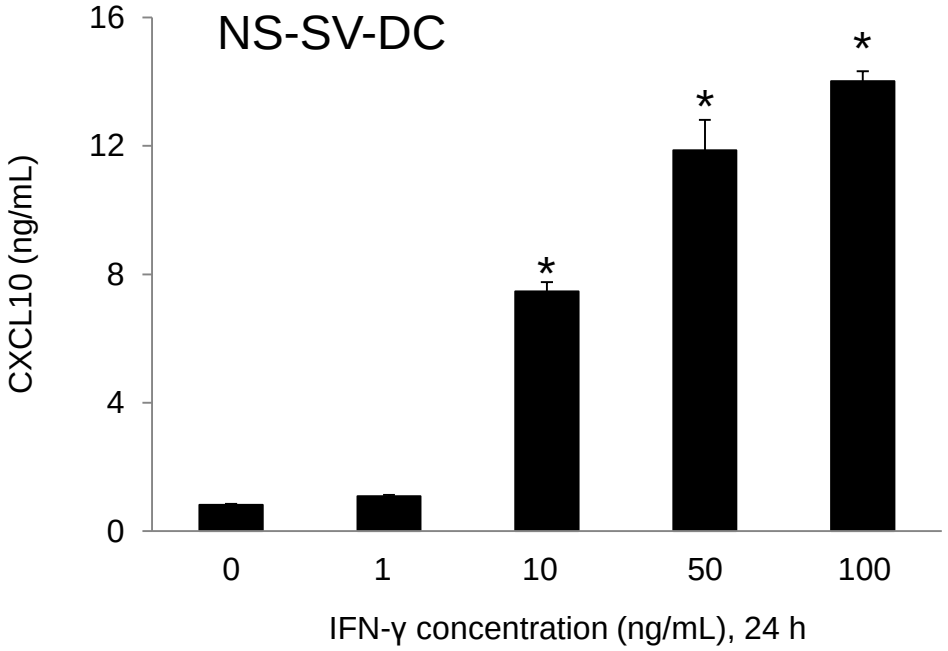

IFNGR1
589 bp
DC
AC
IFNGR2
324 bp
DC
AC
TNFR1
403 bp
DC
AC
TNFR2
339 bp
DC
AC
GAPDH
280 bp
DC
AC

Figure 3

a


NS-SV-DC
CXCL10 (ng/mL)
16
12
8
4
0
*
*
*
0
1
10
50
100
IFN-γ concentration (ng/mL), 24 h

b

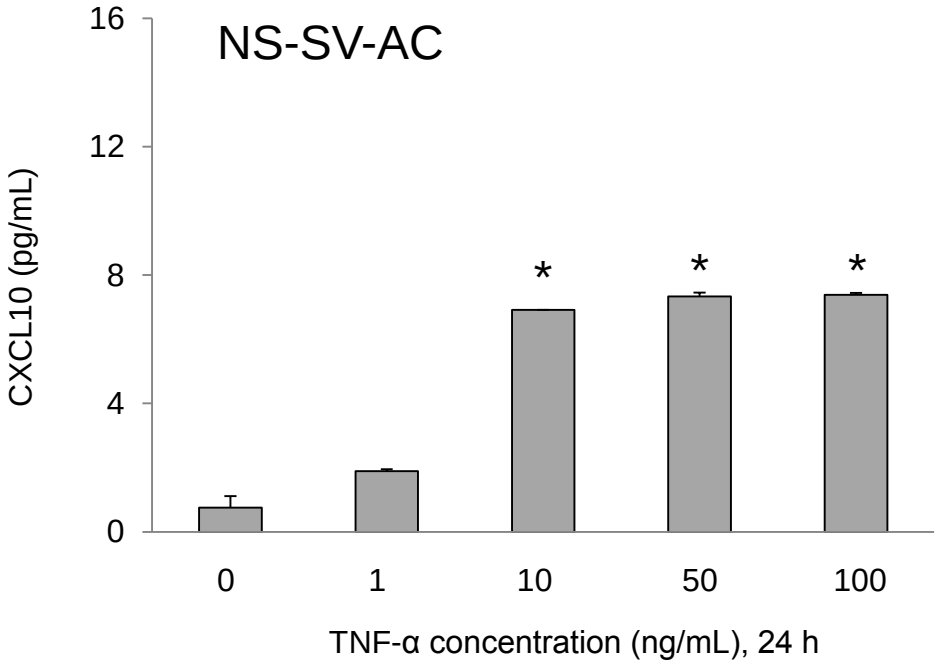

NS-SV-AC
CXCL10 (pg/mL)
16
12
8
4
0
*
*
*
0
1
10
50
100
TNF-α concentration (ng/mL), 24 h

Figure 4

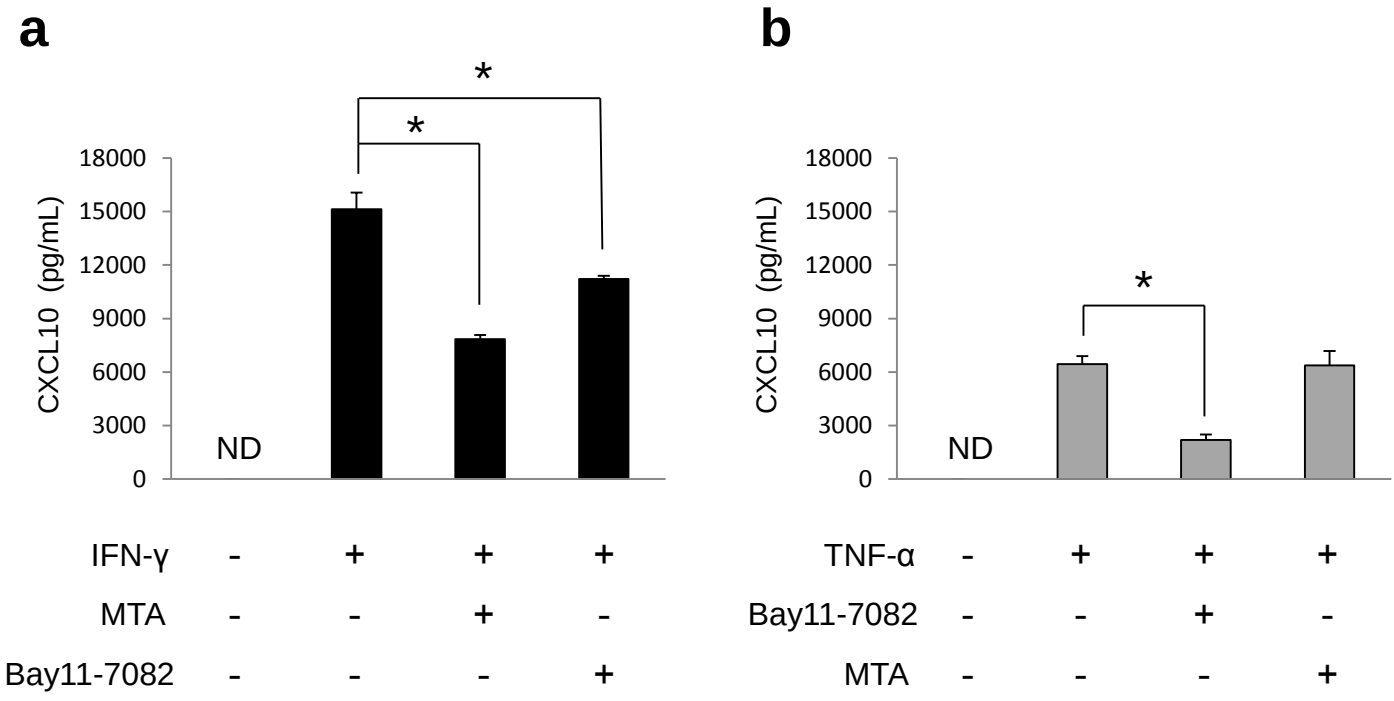
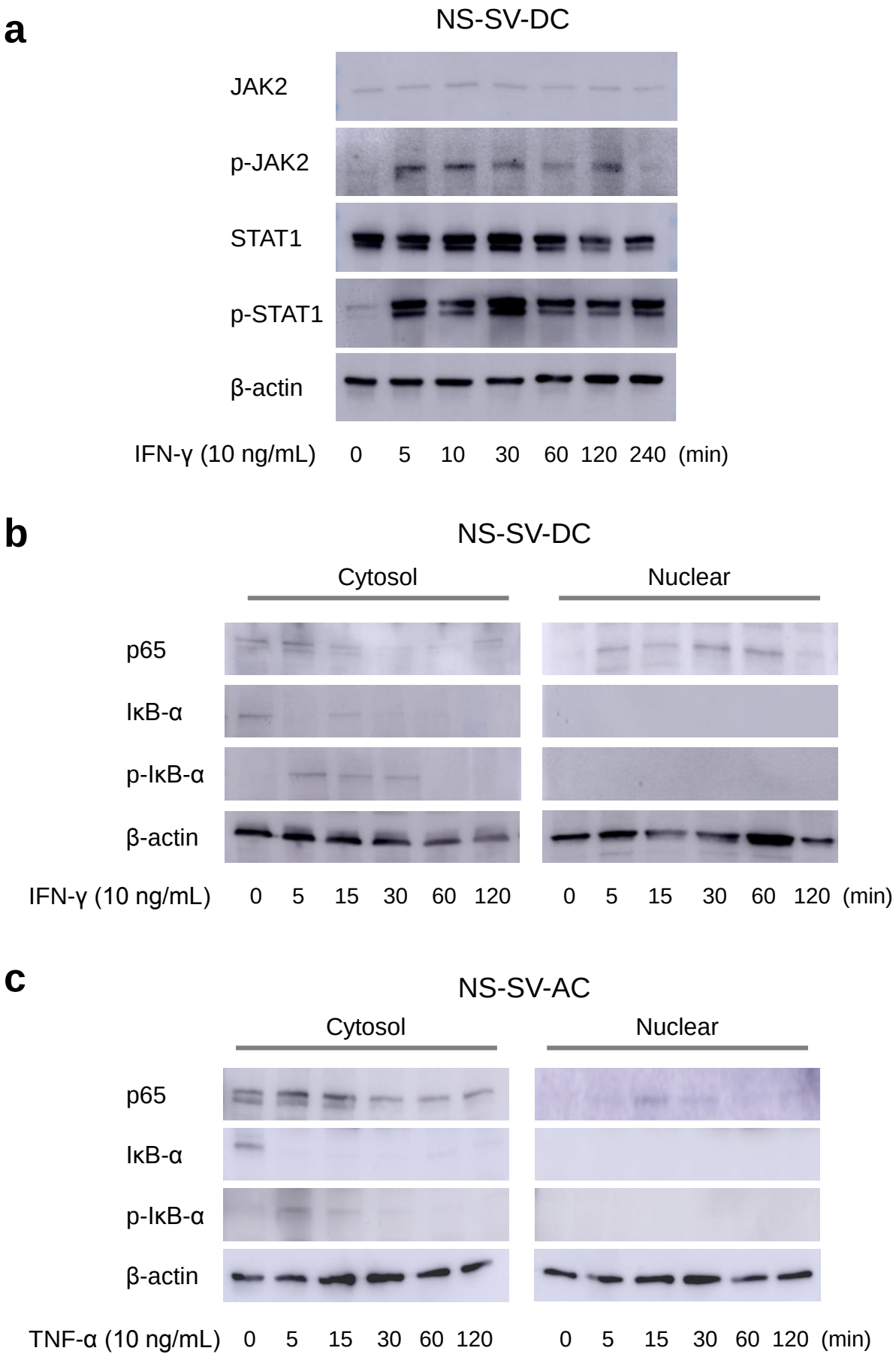

a
b
CXCL10 (pg/mL)
18000
15000
12000
9000
6000
3000
0
ND
*
*
IFN-γ - + + +
MTA - - + -
Bay11-7082 - - - +
TNF-α - + + +
Bay11-7082 - - + -
MTA - - - +

Figure 5


a
NS-SV-DC
JAK2
p-JAK2
STAT1
p-STAT1
β-actin
IFN-γ (10 ng/mL) 0 5 10 30 60 120 240 (min)
b
NS-SV-DC
Cytosol
Nuclear
p65
IκB-α
p-IκB-α
β-actin
IFN-γ (10 ng/mL) 0 5 15 30 60 120 0 5 15 30 60 120 (min)
c
NS-SV-AC
Cytosol
Nuclear
p65
IκB-α
p-IκB-α
β-actin
TNF-α (10 ng/mL) 0 5 15 30 60 120 0 5 15 30 60 120 (min)