Preclinical evaluation of tyrosine kinase 2 inhibitors for human beta-cell protection in type 1 diabetes

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Abstract

Aim: Type 1 diabetes (T1D) is a chronic autoimmune disease leading to progressive loss of pancreatic beta cells. Interferon (IFN)-α plays a critical role in the crosstalk between pancreatic beta cells and the immune system in early insulitis. In human beta cells IFNα signals through JAK1 and TYK2, leading to endoplasmic reticulum stress, inflammation and HLA class I overexpression. IFNα, acting synergistically with IL-1β, induces apoptosis. Polymorphisms in TYK2 that decrease its activity are associated with protection against T1D, and we hypothesized

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that pharmacological inhibitors that specifically target TYK2 could protect human beta cells against the deleterious effects of IFNα.

Materials and Methods: Two TYK2 inhibitors provided by Nimbus Lakshmi were tested in human insulin-producing EndoC-βH1 cells and human islets to evaluate their effect on IFNα signalling, beta-cell function and susceptibility to viral infection using RT-qPCR, western blot, immunofluorescence, ELISA and nuclear dyes.

Results: The two TYK2 inhibitors tested prevented IFNα-induced human beta-cell gene expression in a dose-dependent manner. They also protected human islets against IFNα + IL-1β-induced apoptosis. Importantly, these inhibitors did not modify beta-cell function or their survival following infection with the potential diabetogenic coxsackieviruses CVB1 and CVB5.

Conclusions: The two TYK2 inhibitors tested inhibit the IFNα signalling pathway in human beta cells, decreasing its pro-inflammatory and pro-apoptotic effects without sensitizing the cells to viral infection. The preclinical findings could pave the way for future clinical trials with TYK2 inhibitors for the prevention and treatment of type 1 diabetes.

1 | INTRODUCTION

Interferon-α (IFNα)-mediated signalling is an integral component of type 1 diabetes (T1D) pathophysiology. In line with this concept, type I IFN is expressed in human islets from donors with T1D,[1] and children genetically at risk for the disease present a type I IFN transcriptomic signature in the circulation before the development of autoantibodies.[2] In addition, laser-captured islets obtained from living donors with recent-onset T1D show a significant increase in the expression of IFN-stimulated genes[3] and human islets exposed in vitro to IFNα or to interleukin-1β (IL-1β) + IFNγ have gene signatures that are similar to those observed in islets obtained from patients affected by T1D, but not by type 2 diabetes. [4,5] We recently found that exposure of human beta cells to IFNα induces three hallmarks of human T1D, namely, release of chemokines, endoplasmic reticulum (ER) stress and a massive human leukocyte antigen (HLA) class I overexpression. IFNα also triggers human beta-cell apoptosis when combined with IL-1β.[6] These effects are mediated via the two kinases, janus kinase 1 (JAK1) and tyrosine kinase 2 (TYK2), and the transcription factors, signal transducer and activator of transcription 1 and 2 (STAT1 and STAT2). We identified the TYK2/STAT2/IFN regulatory factor 9 (IRF9) axis as a critical pathway responsible for the action of IFNα in human beta cells.[6,7] TYK2 is a candidate gene for T1D and genetic variants that decrease TYK2 activity and which are protective against the disease.[8,9] Notably, individuals with the protective variants of TYK2 do not have increased prevalence of infections and are not immunodeficient, as is the case for individuals with a complete TYK2 deficiency.[9,10] Consistent with this observation, a partial inhibition of TYK2 expression in human beta cells using siRNAs protects them against the viral by-product, double-stranded RNA (dsRNA) used in the synthetic version poly-IC-induced apoptosis,

lowering the production of IFNα and the chemokine, C-X-C motif chemokine ligand 10 (CXCL10), and expression of major histocompatibility complex (MHC) class I proteins.[7]

Specific small molecule inhibitors of TYK2 have the potential to reproduce the beneficial effects of the genetic variants that protect against T1D. Notably, TYK2 inhibitors are under development and being tested in other immune diseases. A specific TYK2 inhibitor (BMS-986165) has recently shown clinical efficacy in a phase 2 trial for psoriasis[11] as well as in preclinical studies for lupus nephritis and inflammatory bowel disease.[12] In the current study, we evaluated the ability of two TYK2 inhibitors developed by Nimbus Lakshmi (TYKi2A and TYK2iB) to protect human beta cells against the deleterious effects of IFNα. The results obtained indicate that these inhibitors prevent IFNα-induced HLA class I overexpression, chemokine production and beta-cell apoptosis without modifying human beta-cell function or susceptibility to potentially diabetogenic viruses.

2 | MATERIALS AND METHODS

2.1 | Culture of human EndoC-βH1 cells and human islets

The human beta cell line EndoC-βH1[13] (kindly provided by Dr. R. Scharfmann, University of Paris, France) was cultured in Matrigel fibronectin-coated plates as previously described. [14] The MycoAlert Mycoplasma Detection kit (Lonza, Basel, Switzerland) was used to regularly assure that the cells were free from mycoplasma infection.

Human islets from 15 non-diabetic organ donors (see Table S1; beta-cell purity was determined by immunohistochemistry for insulin, as described[5]) were isolated in Pisa, Italy, based on methods previously developed[15] with the approval of the local ethics committee and sent to Brussels for dispersion and experiments.[7]

All experiments with EndoC-βH1 cells or human islets are shown as independent biological data (i.e. considering EndoC-βH1 cells from different passages or human islets from different donors as n = 1). Cells were treated with TYKiA and TYKiB provided by Nimbus Lakshmi (Cambridge, MA, USA), the JAK1/2 inhibitor Baricitinib (Selleckchem, Munich, Germany), used as a positive control, and human IFNα (PeproTech, Rocky Hill, NJ, USA) 2000 U/mL (concentration selected based on our previous studies[6]).

2.2 | TYK2i development and characterization

TYK2iA and TYK2iB were developed by Nimbus Lakshmi as part of a drug development campaign using structure-based drug design. Both inhibitors bind to the JH2 pseudokinase domain of TYK2 and act as allosteric inhibitors of the kinase activity of the enzyme. The JH2-directed allosteric inhibition also provides the high specificity for TYK2 over other JAK family members.[16] Summarized information on the two TYK2 inhibitors presently used is provided in Table S2.

Binding affinity for the JH2 pseudokinase domain of human and mouse TYK2 was evaluated by measuring the ability of the TYK2 inhibitors to compete with the binding of an immobilized kinase ligand to a recombinant partial protein consisting of the JH2 pseudokinase domain region of TYK2 (Eurofins DiscoverX, Freemont, CA, USA). The

amount of TYK2 JH2 pseudokinase protein blocked from binding to the kinase ligand was measured following incubation with TYK2i at various concentrations. TYK2iA and TYK2iB showed concentration-dependent competitive inhibition of binding of the immobilized kinase ligand to the TYK2 JH2 pseudokinase domains with Kd values shown in Table S2.

A series of in vitro studies investigated the functional consequences of the binding of the TYK2 inhibitors to the TYK2 JH2 pseudokinase domain in isolated human peripheral blood mononuclear cells (PBMCs; Confluence Discovery Technologies, St. Louis, MO, USA). In this system, TYK2iA and TYK2iB inhibited TYK2-dependent IL-12-induced STAT4 phosphorylation with an IC50 of 36.3 and 13.3 nM, respectively. The potential 'off-target' effects of the TYK2 inhibitors were also examined in the same human PBMC system using JAK1/3-dependent IL-2-induced STAT5 phosphorylation and JAK2-dependent GM-CSF-induced STAT5 phosphorylation. In these studies, both TYK2 inhibitors showed no inhibition at concentrations of up to 50 μM.

The effects of the TYK2 inhibitors on TYK2-dependent production and release of CXCL10 in response to IFNα receptor activation was tested in freshly isolated heparinized human and mouse whole blood samples (HD Biosciences, San Diego, CA, USA). TYK2iB inhibited TYK2-dependent IFNα-induced CXCL10 protein production in human whole blood with an IC50 of 325 nM. TYK2iA was not tested in the human whole blood assay. TYK2iA and TYK2iB inhibited TYK2-dependent IFNα-induced CXCL10 protein production in mouse whole blood with IC50s of 1.3 uM and 460 nM, respectively.

2.3 | mRNA extraction and real-time PCR

Polyadenylated mRNA was isolated from cultured cells using the Dynabeads mRNA DIRECT purification kit (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions, and reverse transcription was performed using the Reverse Transcriptase Core kit (Eurogentec, Liège, Belgium). The real-time PCR amplification reactions were performed using SYBR Green and the data were compared with a standard curve. Gene expression values were corrected by the housekeeping gene β-actin, as its expression is not affected by the conditions used in this study.[7] The primers used in this study are listed in Table S3.

2.4 | Protein extraction and western blot analysis

Cells were washed with PBS and lysed using 1X Laemmli Sample Buffer (60 mM tris[hydroxymethyl]aminomethane pH 6.8, 10% glycerol, 1.5% dithiothreitol, 1.5% 2-mercaptoethanol, 2% sodium dodecyl sulphate and 0.005% bromophenol blue). Immunoblot analyses were performed using antibodies against pSTAT1, pSTAT2, VP1, α-tubulin and β-actin as housekeeping. We used a secondary antibody coupled with the horseradish peroxidase. Immunoreactive bands were visualized using the SuperSignal West Femto chemiluminescent substrate (Thermo Scientific, Waltham, MA, USA), detected using ChemiDoc XRS+ (Bio-Rad, Hercules, CA, USA), and quantified with Image Studio Lite version 5.2 software (LI-COR Biosciences). A list of all the antibodies used in this study is provided in Table S4.

2.5 | Immunofluorescence

Immunofluorescence was performed as previously described,[6] using anti-MHC class I antibody, anti-insulin antibody, Hoechst 33342 (14 533, Sigma-Aldrich, Bornem, Belgium) and Alexa Fluor-conjugated secondary antibodies (Table S4).

2.6 | ELISA

An enzyme-linked immunosorbent assay (Quantikine ELISA kit, R&D Systems, Minneapolis, MN, USA) was used to determine the release of CXCL10 to the culture medium (by 30 000 human islet cells/200 μL; culture time as indicated in the legends for the figures).

2.7 | Assessment of cell viability

Cell viability was evaluated by nuclear dye staining (Hoechst 33342 and propidium iodide [P4170, Sigma-Aldrich, Bornem, Belgium]), as previously described.[7] These measurements were executed by two different observers, one of them blinded to sample identity.

2.8 | Viral infection and titration

The prototype strains of enterovirus (CVB5/Faulkner; CVB1/Conn-5) were obtained from the American Type Culture Collection (Old Town Manassas, VA, USA). These viruses were passaged in green monkey kidney (GMK) cells and their identity was confirmed by a plaque neutralization assay with type-specific anti-sera. EndoC-βH1 cells and dispersed human islets were infected with CVB1 (multiplicity of infection [MOI] 0.1) or CVB5 (MOI 5) in culture medium; these MOIs were selected based on our previous studies.[17] After adsorption for 1 hour at 37°C, the inoculum virus was removed and the culture medium containing fœtal bovine serum was added to the plates. The virus was then allowed to replicate for 24 hours.[17]

Infectivity was evaluated in the medium of infected EndoC-βH1 cells and human islets using endpoint dilutions in microwell cultures of GMK cells. After 6 days, cytopathic effects were determined by microscopy, and 50% tissue infection dose titers were calculated using the Kärber formula.[18] We used relative viral titers obtained from the ratio between the viral titers measured in the treated condition and those measured under the control condition.

2.9 | Insulin secretion

EndoC-βH1 cells were preincubated with culture medium containing 2.8 mM glucose for 24 hours. Cells were then incubated in Krebs-Ringer buffer (β-KREBS serum-free medium, Univercell-Biosolutions, Toulouse, France) for 1 hour and sequentially stimulated with 0 mM glucose, 20 mM glucose, or 20 mM glucose + 20 μM forskolin for 40 minutes (modified from[19,20]). Insulin release and insulin content were measured using the human insulin ELISA kit (Mercodia, Uppsala, Sweden) in cell-free supernatants and acid-ethanol extracted cell lysates, respectively. Results were normalized by total protein content.[20]

2.10 | Statistical analysis

Data are expressed as means ± SEM. A normality test was performed to assess the Gaussian distribution. In case of normality, parametric ANOVA tests were used, followed by paired or unpaired *t* test with Bonferroni correction. When the distribution was considered not normal, a non-parametric ANOVA test was used. Results with *P* < .05 were considered statistically significant.

3 | RESULTS

3.1 | TYK2 inhibitors block IFNα-induced upregulation of inflammatory markers and chemokines in EndoC-βH1 cells in a dose-dependent manner

Because IFNα signals via both JAK1 and TYK2, baricitinib, a JAK1/2 inhibitor[21] was used as a positive control. Pretreatment of EndoC-βH1 cells with different doses of the two TYK2 inhibitors prevented IFNα-induced STAT1 and STAT2 phosphorylation (Figure 1A–C and Figure S1A–C) and induction of *HLA-ABC* (Figure 1D and Figure S1D), the chemokine *CXCL10* (Figure 1E and Figure S1E) and the antiviral MX dynamin-like GTPase 1 (*MX1*) (Figure 1F and Figure S1F) expression in a dose-dependent manner. However, TYK2 inhibitors did not prevent IFNα-induced expression of the ER stress marker CCAAT/ enhancer-binding protein homologous protein (*CHOP*, also known as *DDIT3*) (Figure 1G and Figure S1G). Based on these results, we selected 1 μM as the dose of TYK2 inhibitors to be tested in subsequent experiments.

Next, we evaluated the potential of these inhibitors to reverse IFNα effects. EndoC-βH1 cells were pretreated with IFNα for 24 hours, and then TYK2 inhibitors were added (still in the presence of IFNα) for an additional 24 hours. Interestingly, TYK2iA and TYK2iB completely reversed the expression of the chemokine *CXCL10* and the antiviral protein *MX1* (Figure 2A,B). By contrast, *HLA-ABC* expression remained high in the presence of the inhibitors (Figure 2C), which is in agreement with our previous data showing the long-lasting expression of *HLA-ABC* after IFNα washout from the cell culture.[22] In agreement with the finding that TYK2 inhibitors did not prevent *CHOP* induction by IFNα (Figure 1G and Figure S1G), they also failed to reverse IFNα-induced *CHOP* expression (Figure 2D).

3.2 | TYK2 inhibition reduces IFNα-mediated upregulation of inflammatory and ER stress markers in dispersed human islets

In dispersed human islets, the two TYK2 inhibitors used at the selected dose of 1 μM prevented IFNα-mediated STAT1 and STAT2 activation (Figure 3A–C) and upregulation of *HLA-ABC* (Figure 3D), *CXCL10* (Figure 3E) and *MX1* (Figure 3F) mRNA expression, with the same efficacy as observed in EndoC-βH1 cells. In addition, and differently from the observations in EndoC-βH1 cells, TYK2iA and iB prevented IFNα-induced *CHOP* expression in human islets (Figure 3G). Inhibition of TYK2 also prevented IFNα-mediated CXCL10 secretion to the cell culture medium (Figure 3H) and MHC class I expression at the cell surface (Figure 3I). A higher dose of the TYK2 inhibitors (4 μM) induced similar effects as observed with 1 μM (Figure 3A; data not shown). We also evaluated the effect of lower concentrations of the TYK2 inhibitors—namely, 0.1 and 0.5 μM—in dispersed human islets and observed a clear protection with 0.1 and particularly 0.5 μM against IFNα-induced

STAT1 and STAT2 phosphorylation (Figure S2A–C) and *HLA-ABC* (Figure S2D), *CXCL10* (Figure S2E), *MX1* (Figure S2G), and *CHOP* (Figure S2H) mRNA expression. These low concentrations of TYK2 inhibitors also prevented IFNα-mediated CXCL10 secretion into the cell culture medium (Figure S2F).

3.3 | TYK2 inhibitors prevent apoptosis and the expression of inflammatory and ER stress markers induced by IFNα + IL-1β in dispersed human islets

We next evaluated the effect of TYK2 inhibitors on a combination of IFNα + IL-1β, two cytokines that are probably present in the islet milieu during insulitis,[23] and observed a clear protection by the TYK2 inhibitors against IFNα + IL-β–induced human islet apoptosis (Figure 4A). The TYK2 inhibitors by themselves did not affect islet cell viability. Moreover, similar to the effects on IFNα signalling (see above), TYK2 inhibitors prevented IFNα + IL-1β-induced STAT1 and STAT2 phosphorylation (Figure 4B–D) and *HLA-ABC* (Figure 4E), *CXCL10* (Figure 4F), *MX1* (Figure 4H), *CHOP* (Figure 4I) and the activating transcription factor 3 *ATF3* (Figure 4J) mRNA expression. IFNα + IL-1β-induced CXCL10 secretion to the medium (Figure 4G) and ATF3 protein expression (Figure 4K,L) were also reduced by the TYK2 inhibitors. These observations indicate that TYK2 inhibitors also have beneficial effects in the context of human islet exposure to both IFNα and IL-1β.

3.4 | TYK2 inhibitors do not sensitize human beta cells to viral infection

TYK2 plays an essential role in the cellular antiviral responses, as supported by the present observation that TYK2 blockade prevents IFNα-induced expression of the antiviral protein MX1 (see above), and individuals with a complete TYK2 deficiency are immunodeficient.[10] By contrast, individuals with a protective single nucleotide polymorphism (SNP) in the TYK2 gene that decreases its activity by ~ 50% are healthy.[9] We thus evaluated if TYK2 inhibitors sensitize human islets to CVB1 and CVB5, two viruses that may contribute to trigger autoimmunity and T1D.[24] Inhibition of TYK2 did not augment CVB1- and CVB5-induced apoptosis in both EndoC-βH1 cells (Figure 5A) or dispersed human islets (Figure 5B). In agreement with these data, TYK2 inhibition did not increase viral titers (Figure 5C,D) or the expression of the viral capsid protein VP1 (Figure 5E–H) in CVB1- and CVB5-infected EndoC-βH1 cells and dispersed human islets, showing that TYK2 inhibitors do not worsen the outcome of a viral infection in human beta cells.

3.5 | TYK2 inhibitors do not affect human beta-cell function and insulin secretory capacity

In dispersed human islets, IFNα and the combination of IFNα + IL-1β had no effect on the expression of insulin (*INS*) and the pancreatic duodenal homeobox 1 (*PDX1*), a key regulator of *INS* expression; and TYK2 inhibition had no effect as well (Figure S3A–D), indicating that these inhibitory compounds have no impact on human beta-cell function. In addition, IFNα or IFNα + IL-1β reduced the expression of the pro-insulin processing enzyme PC1/3 (*PCSK1*) (Figure S4A,D) and PC2 (*PCSK2*) (Figure S4B,E), as previously described,[25] but not the carboxypeptidase E (*CPE*) (Figure S4C,F); again, TYK2 inhibition did not alter the expression of these genes.

In line with the observations with *INS* and *PDX1* mRNA expression (Figure S3A–D), neither IFNα nor the TYK2 inhibitors affected glucose or glucose + forskolin-induced insulin release in EndoC-βH1 cells (Figure S5). These observations indicate that IFNα has a mild/limited effect on human beta-cell function, and that, importantly, TYK2 inhibitors have no deleterious impact on the functional phenotype of these cells.

4 | DISCUSSION

We presently show that TYK2 inhibitors can prevent the key deleterious effects of IFNα in human beta cells, namely, HLA class I overexpression, inflammation and ER stress, and, together with IL-1β induction of apoptosis. Importantly, these compounds neither alter the beta-cell function nor the susceptibility to infections by CVB1 and CVB5. These data provide proof of principle that TYK2 inhibitors block the IFNα pathway in human pancreatic beta cells and may therefore prevent inflammatory signalling in the beta cells and amplification of the innate immune response during the initiation and evolution of T1D.[23]

T1D is a complex disease that remains clinically silent for many years before appearance of symptoms.[26] IFNα is present in the islets of individuals with T1D and is believed to play a crucial role at the very early stages of disease pathogenesis by mediating MHC class I overexpression, ER stress and inflammation.[1,6] In children at risk of developing T1D, a type I IFN transcriptional signature is detected ahead of seroconversion for autoantibodies, suggesting that activation of the innate type I IFN pathway occurs very early in the development of the disease.[2] Furthermore, islets obtained from patients affected by T1D show a gene signature similar to human islets treated in vitro with pro-inflammatory cytokines.[4,5] It remains to be proven by clinical trials, however, whether and/or to what extent INFα is a key cytokine for the induction of insulitis and the autoimmune killing of beta cells in T1D.

IFNα acts via the activation of JAK1 and TYK2 and the subsequent phosphorylation and activation of STAT1 and STAT2. Thus, targeting the key downstream effectors of IFNα, i.e. JAK1 and TYK2, for the early treatment of T1D is a logical strategy. Four different JAK inhibitors have been approved for the treatment of different autoimmune diseases, including rheumatoid arthritis, systemic lupus erythematosus and dermatitis.[27] Repurposing drugs that are already in use for other diseases is of great interest as the safety profiles of these molecules have already been evaluated, and this reduces the delay from the laboratory to the clinic.[28] This possibility was evaluated in non-obese diabetic (NOD) mice treated with the JAK1/JAK2 inhibitor AZD1480 that targets the same active site as ruxolitinib and baricitinib, two clinically approved JAK inhibitors.[21,29] AZD1480 blocked IFNγ signalling in mouse beta cells in vitro and in vivo, reducing beta-cell MHC class I expression and immune infiltration. Mice were protected from autoimmune diabetes and the inhibitor also reverted disease in newly diagnosed mice.[29] AZD1480 is not in clinical use, and we evaluated the efficiency of ruxolitinib, baricitinib and cerdulatinib, another JAK inhibitor, and showed that they block IFNα signalling in a dose-dependent manner in EndoC-βH1 cells[22] (present data and unpublished data). These results support the use of JAK inhibitors for the treatment of diabetes. It is of concern, however, that these first-generation JAK inhibitors are non-selective for JAK1 as they target the highly homologous catalytic domain

of all JAKs. Therefore, there is a risk of important side effects such as infections, anaemia, neutropenia and thrombocytopenia. Despite these side effects, the clinically approved JAK inhibitors seem to be comparatively well tolerated,[30,31] but the discovery of more selective JAK inhibitors should narrow the spectrum of action and improve safety.[21]

In the context of T1D, it may be more interesting to target TYK2 instead of JAK1. Indeed, SNPs in the TYK2 gene have been associated with protection against autoimmune diseases, including T1D.[8,9] These SNPs reduce TYK2 activity, but, importantly, individuals homozygous for the minor allele (protective allele) are healthy and not prone to develop more infectious diseases,[9,10] suggesting that a drug-induced partial inhibition of TYK2 could have beneficial effects in autoimmune diseases without increasing the risk of secondary infections. Recently, pharmaceutical compounds were found to bind to the autoinhibitory pseudokinase (JH2) domain of TYK2, stabilizing it and maintaining the autoinhibition on the catalytic domain of TYK2, even under the stimulation condition.[32] This effect might be unique for TYK2 and thus provides the opportunity to develop new and very specific TYK2 inhibitors, such as the presently tested ones (see Table S2).

Recently, the TYK2 inhibitor BMS-986165—which also acts via stabilization of JH2—showed positive results in the treatment of psoriasis in a phase 2 clinical trial. Importantly, during the 12 weeks of the study there were no important side effects on blood counts, immunoglobulins, liver enzymes, electrocardiograms and vital signs, as is the case for JAK inhibitors.[11] The same compound also showed positive effects in preclinical models of lupus nephritis and inflammatory bowel disease.[12] In addition, preclinical evaluation of the TYK2 inhibitor NDI-031301 showed significant activity against T-cell acute lymphoblastic leukaemia both in vitro and in an in vivo mouse model, reducing tumour burden and improving survival.[33] These promising first results of TYK2 inhibitors in different diseases, together with the beneficial effects that we presently observed in human pancreatic beta cells, suggest that TYK2 inhibition might also be efficient for the treatment of T1D. Target populations would be high-risk individuals in stage 1 or 2 diabetes (i.e. autoantibody positivity) or in individuals with recent-onset stage 3 T1D, as it is crucial to intervene when there are still enough beta cells remaining to enable clinical improvement.

In conclusion, we have shown that TYK2 inhibitors protect human pancreatic beta cells against IFNα- and IFNα + IL-1β-induced MHC class I overexpression, inflammation, ER stress and apoptosis. The safety and efficacy of this class of inhibitors for other diseases has already been shown, and the present data thus provide a proof of principle for considering TYK2 inhibitors in future clinical trials for T1D.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGMENTS

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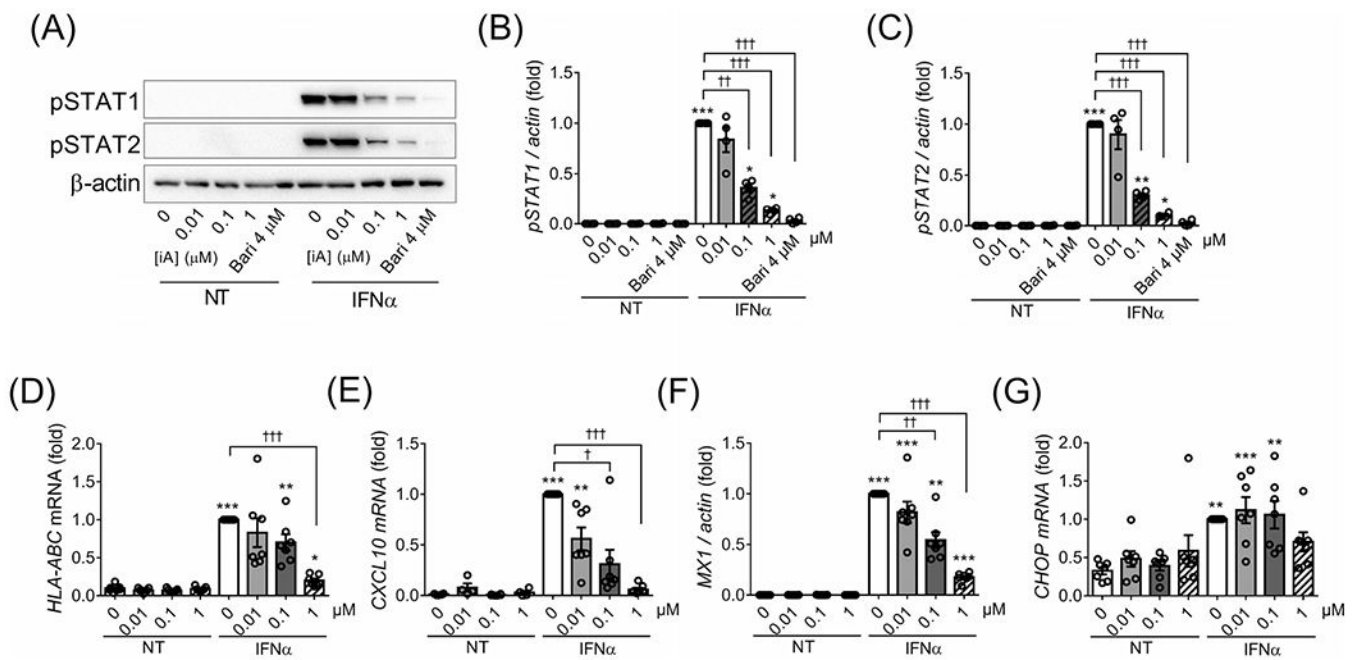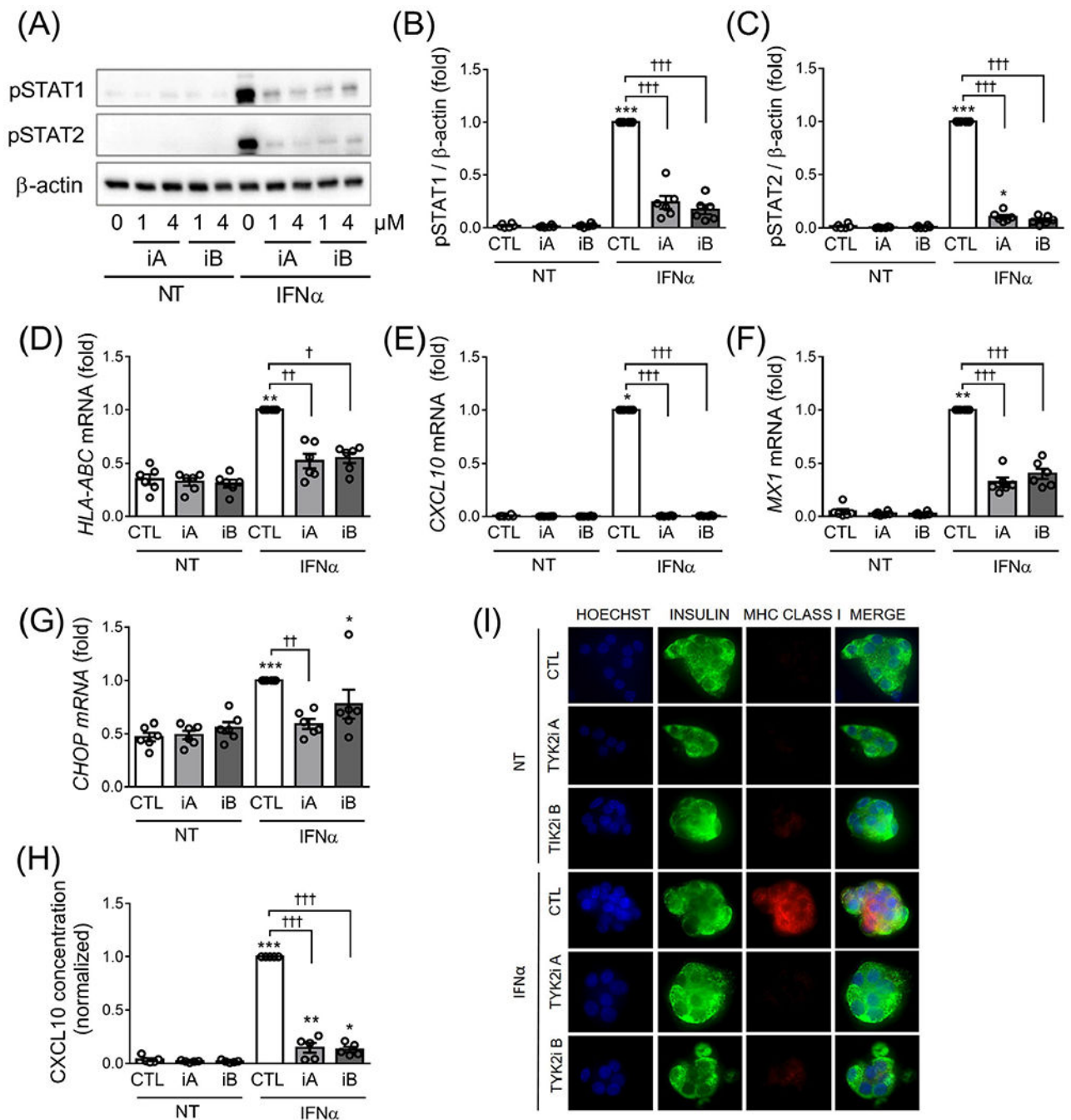
FIGURE 1.

The TYK2iA prevents IFNα-induced STAT1/2 phosphorylation and gene expression in EndoC-βH1 cells in a dose-dependent manner. EndoC-βH1 cells were pretreated for 2 hours with the TYK2iA (0.01,0.1 and 1 μM) or with the JAK inhibitor baricitinib (4 μM; used as a positive control). IFNα (2000 U/mL) was then added for 8 (A-C) or 24 hours (D-G) in the continuous presence of the inhibitors. (A) Protein expression was measured by western blot and representative images of four independent experiments are shown. Densitometry results are shown for pSTAT1 (B) and pSTAT2 (C). The mRNA expression of *HLA-ABC* (D), *CXCL10* (E), *MX1* (F) and *CHOP* (G) was analysed by RT-qPCR. In all experiments values were normalized by β-actin and then by cells treated with IFNα without TYK2iA or baricitinib, considered as 1. Results are mean ± SEM of four (A-C) or seven (D-G) independent experiments. $*P < .05$, $**P < .01$ and $***P < .001$ vs. control (no inhibitor and no IFNα); $^{†}P < .05$, $^{††}P < .01$ and $^{†††}P < .001$ vs. IFNα; one-way ANOVA
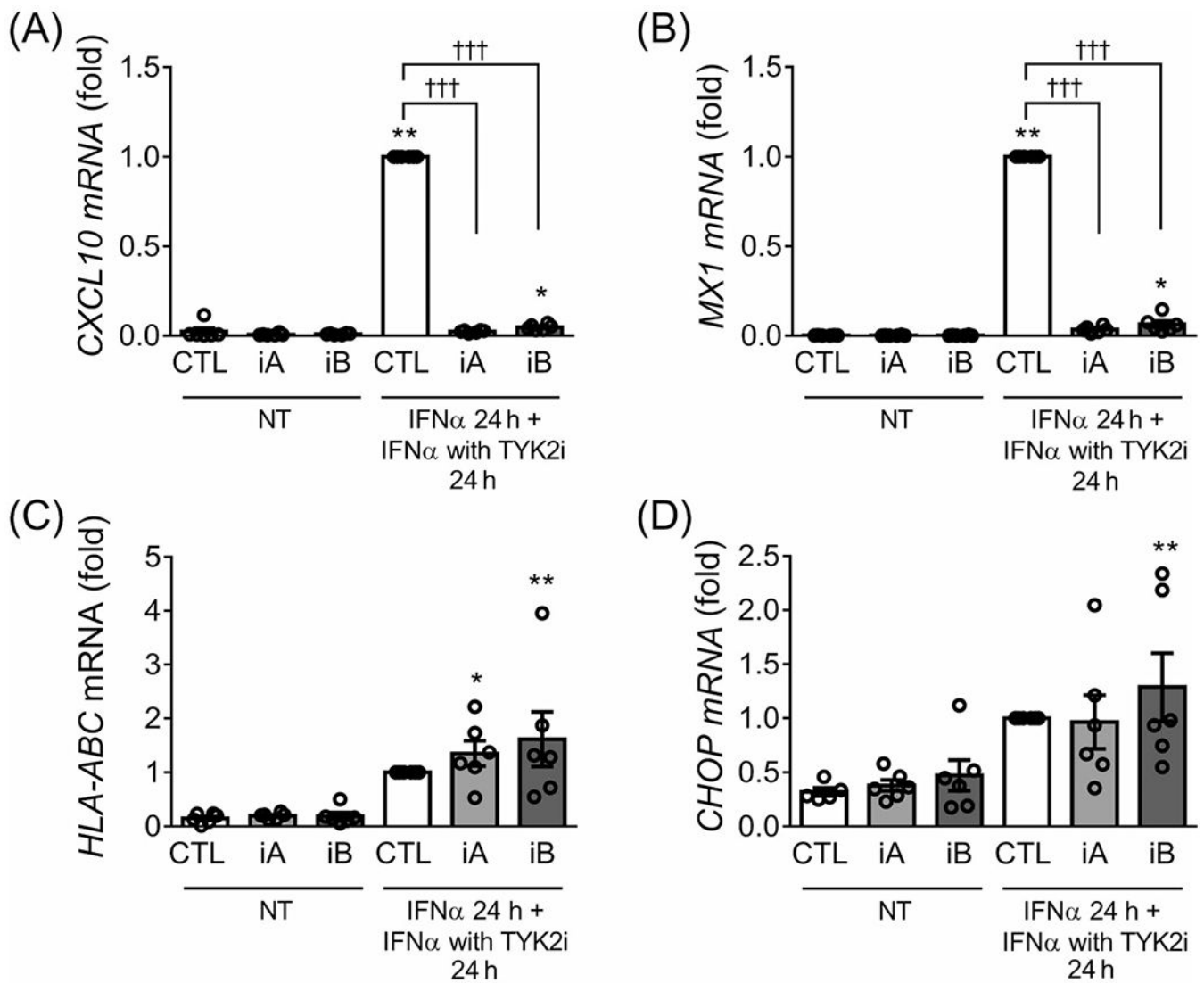
FIGURE 2.

TYK2 inhibitors revert IFNα-induced *CXCL10* and *MX1* but not *HLA-ABC* and *CHOP* expression in EndoC-βH1 cells. EndoC-βH1 cells were pretreated for 24 hours with IFNα (2000 U/mL). The TYK2 inhibitors (iA and iB for TYK2iA and B, respectively, 1 μM) were then added for an additional 24 hours in the continuous presence of IFNα. The mRNA expression of *CXCL10* (A), *MX1* (B), *HLA-ABC* (C) and *CHOP* (D) was analysed by RT-qPCR and the values were normalized by β-actin and then by cells treated with IFNα without TYK2 inhibitors, considered as 1. Results are mean ± SEM of six independent experiments. *$P < .05$, **$P < .01$ and ***$P < .001$ vs. control (CTL NT); †††$P < .001$ vs. CTL IFNα; one-way ANOVA
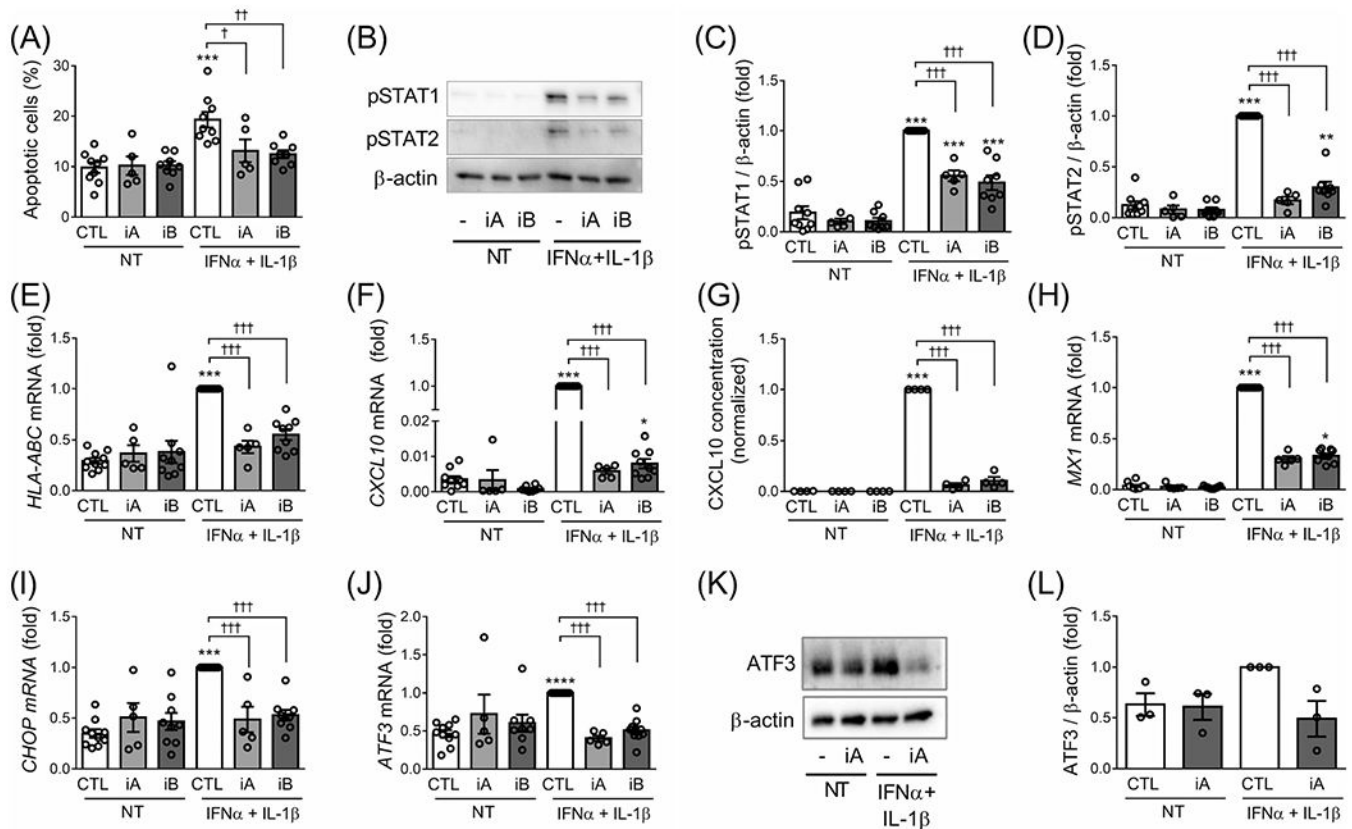
FIGURE 3.
TYK2 inhibition prevents IFNα-induced STAT1/2 phosphorylation and *HLA-ABC, CXCL10, MX1* and *CHOP* upregulation in human islet cells. Dispersed human islets were left untreated (CTL) or pretreated with 1 μM of TYK2 inhibitor (iA and iB for TYK2iA and B, respectively) for 2 hours. Then IFNα (2000 U/mL) was added for 24 hours in the continuous presence of the inhibitors. (A) Protein expression was measured by western blot and representative images of six independent experiments are shown. Densitometry results are shown for pSTAT1 (B) and pSTAT2 (C) and the values were normalized by β-actin. The

mRNA expression of *HLA-ABC* (D), *CXCL10* (E), *MX1* (F) and *CHOP* (G) was analysed by RT-qPCR and the values were normalized by β-actin. (H) CXCL10 protein secretion in the supernatant fraction was determined by ELISA. In all experiments, the values were normalized by cells treated with IFNα without TYK2 inhibitors, considered as 1. Results are mean ± SEM of six (A-G) or five (H) independent experiments. $*P < .05$, $**P < .01$ and $***P < .001$ vs. control (CTL NT); $^{†}P < .05$, $^{††}P < .01$ and $^{†††}P < .001$ vs. CTL IFNα; one-way ANOVA. (I) ICC of MHC class I (red), insulin (green) and Hoechst (blue) in human islets treated with IFNα with or without TYK2 inhibitors as described above (magnification ×40)

FIGURE 4.

TYK2 inhibition prevents apoptosis and the expression of inflammatory and ER stress markers induced by IFNα + IL-1β in dispersed human islets. Dispersed human islets were left untreated (CTL) or pretreated with 1 μM of TYK2 inhibitor (iA and iB for TYK2iA and B, respectively) for 2 hours. Afterwards, IFNα (2000 U/mL) + IL-1β (50 U/mL) were added for 24 hours in the continuous presence of the inhibitors. (A) Apoptosis was evaluated using HO/PI staining. (B, K) Protein expression was measured by western blot and representative images of three (K) or five to eight (B) independent experiments are shown. Densitometry results normalized by β-actin are shown for pSTAT1 (C), pSTAT2 (D) and ATF3 (L). The mRNA expression of *HLA-ABC* (E), *CXCL10* (F), *MX1* (H), *CHOP* (I) and *ATF3* (J) was analysed by RT-qPCR and the values were normalized by β-actin. (G) CXCL10 protein secretion in the supernatant fraction was determined by ELISA. In all experiments, values were normalized by cells treated with IFNα + IL-1β without TYK2 inhibitors, considered as 1. Results are mean ± SEM of three to nine independent experiments. $*P < .05$, $**P < .01$ and $***P < .001$ vs. control (CTL NT); $^{†}P < .05$, $^{††}P < .01$ and $^{†††}P < .001$ vs. CTL IFNα + IL-1β; one-way ANOVA
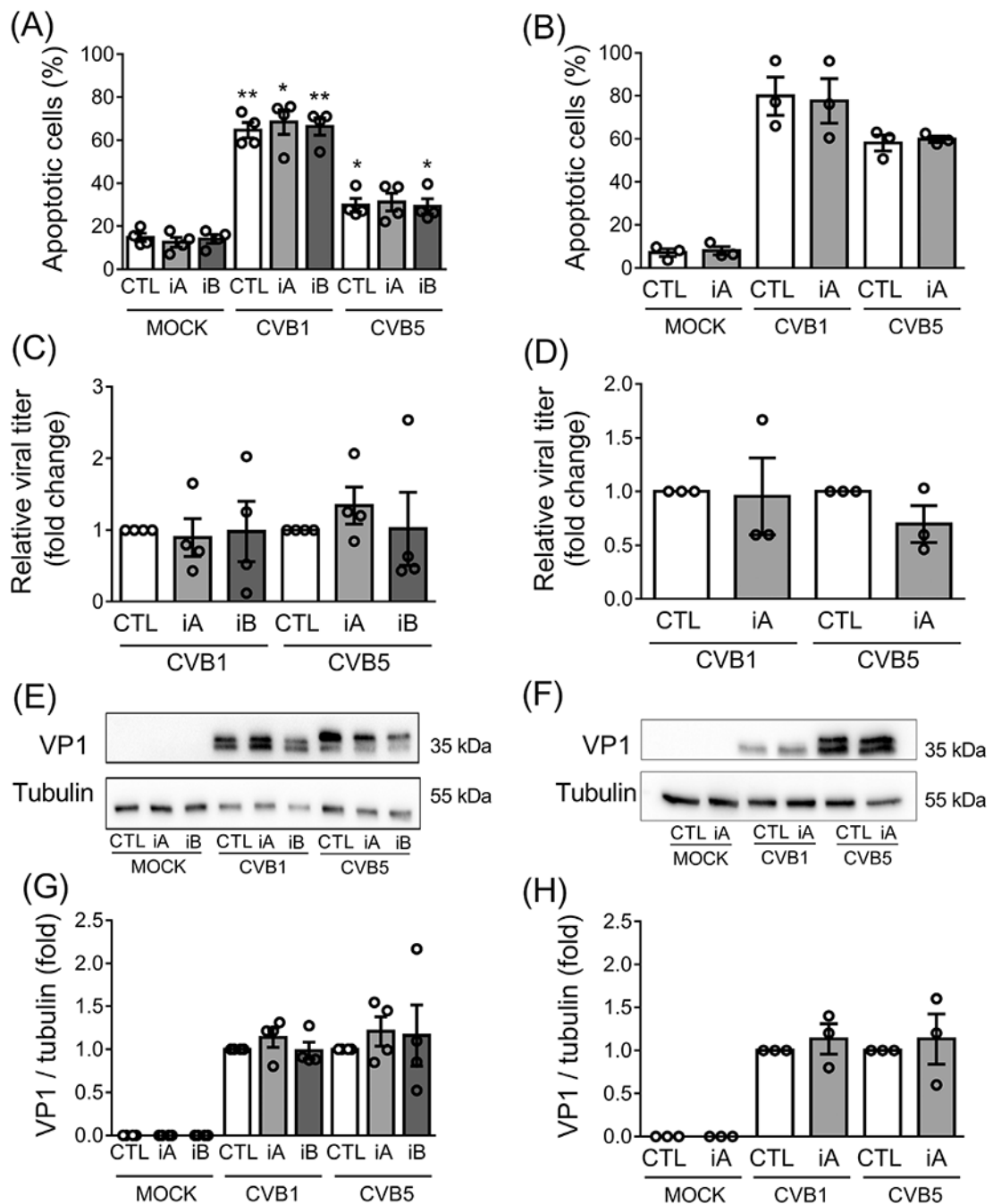
FIGURE 5.

TYK2 inhibitors do not sensitize human beta cells to viral infection. EndoC-βH1 cells (A, C, E, G) and dispersed human islets (B, D, F, H) were left untreated (CTL) or pretreated with 1 μM of TYK2 inhibitor (iA and iB for TYK2iA and B, respectively) for 2 hours. Afterwards, cells were infected or not (MOCK) with CVB1 (MOI 0.1) or CVB5 (MOI 5) for 24 hours in the continuous presence of the inhibitors. (A, B) Apoptosis was evaluated using HO/PI staining. (C, D) Viral replication was quantified by viral titration and presented as relative viral titer compared with the control condition (CTL) for each virus. (E, F) The expression

of the viral capsid protein (VP1) was evaluated by western blot and representative images of four (E) and three (F) independent experiments are shown. Densitometry results are shown (G, H) and the values were normalized by α-tubulin and then by each control condition (without TYK2 inhibitor), considered as 1. Results are mean ± SEM of four (EndoC-βH1 cells) and three (dispersed human islets) independent experiments. *$P < .05$ and **$P < .01$ vs. CTL MOCK; one-way ANOVA