Immunobiology 226 (2021) 152114

Contents lists available at ScienceDirect

Immunobiology

journal homepage: www.elsevier.com/locate/imbio

Induction of CIITA by IFN-γ in macrophages involves STAT1 activation by JAK and JNK

Juan Tur [1], Consol Farrera [1], Ester Sánchez-Tilló [1], Tania Vico, Paula Guerrero-Gonzalez, Ainhoa Fernandez-Elorduy, Jorge Lloberas [*], Antonio Celada [*]

Macrophage Biology Group, Department of Cellular Biology, Physiology and Immunology, Universitat de Barcelona, Barcelona, Spain

ARTICLE INFO

Keywords:
Monocytes/macrophages
Transcription factors
MHC
Gene regulation
Antigen presentation

ABSTRACT

The induction of major histocompatibility complex (MHC) class II proteins by interferon gamma (IFN-γ) in macrophages play an important role during immune responses. Here we explore the signaling pathways involved in the induction by IFN-γ of the MHC II transactivator (*CIIta*) required for MHC II transcriptional activation. Cyclophilin A (CypA) is required for IFN-γ-dependent induction of MHC II in macrophages, but not when it is mediated by GM-CSF. The effect of CypA appears to be specific because it does not affect the expression of other molecules or genes triggered by IFN-γ, such as FcγR, NOS2, *Lmp2*, and *Tap1*. We found that CypA inhibition blocked the IFN-γ-induced expression of *CIIta* at the transcriptional level in two phases. In an early phase, during the first 2 h of IFN-γ treatment, STAT1 is phosphorylated at Tyrosine 701 and Serine 727, residues required for the induction of the transcription factor IRF1. In a later phase, STAT1 phosphorylation and JNK activation are required to trigger *CIIta* expression. CypA is needed for STAT1 phosphorylation in this last phase and to bind the *CIIta* promoter. Our findings demonstrate that STAT1 is required in a two-step induction of *CIIta*, once again highlighting the significance of cross talk between signaling pathways in macrophages.

1. Introduction

Macrophages play key roles in many bacterial and parasitic infections. Microbial stimuli like lipopolysaccharides induce the production of reactive oxygen species that control bactericidal activity through phagocytosis, cytokine release, and toxic molecule release (Tur et al., 2020). In HIV as well as in other viral infections, macrophages are important for efficient anti-viral immune responses (Burdo et al., 2015). However, in some cases of pneumonia associated with severe COVID-19, patients may exhibit features of potentially fatal systemic hyper inflammation (e.g., macrophage activation syndrome or cytokine storm) (McGonagle et al., 2020).

Macrophages also play key roles in various other inflammatory processes. For example, tumor-associated macrophages are the major infiltrating leukocytes of the tumor microenvironment and are key to the link between inflammation and cancer, making them a promising target for cancer treatment (Pathria et al., 2019). Macrophages are also important in the resolution of inflammation and in tissue remodeling, repair, and fibrosis (Mantovani et al., 2013). In atherosclerosis, macrophages become foam cells that contribute to atherosclerotic plaque formation (Guerrini and Gennaro, 2019). The infiltration of adipose tissue macrophages correlates with the degree of obesity and is important in the development of obesity-associated pathology, such as type 2 diabetes (Castoldi et al., 2015). Finally, macrophages are important to the pathogenesis of many chronic inflammatory and autoimmune diseases, including rheumatoid arthritis, multiple sclerosis, autoimmune hepatitis, and inflammatory bowel disease (Wynn et al., 2013).

In macrophages, interferon gamma (IFN-γ) induces the expression of major histocompatibility complex (MHC) class II proteins that allows peptides coming from phagocytosed infectious agents to present to T cells. In this way, macrophages provide a switch between innate and acquired immunity (Roche and Furuta, 2015), with the intensity of the inflammatory immune response correlating with class II antigen (MHC II) expression (Otten et al., 1998). The lack of expression of these

Abbreviations: 5AC, 5-aza-2′-deoxycytosine; CsA, cyclosporin A; CypA, Cyclophilin A; DRB, dichlorobenzimidazole riboside; GAS, IFN-γ activation site; PIAS, protein inhibitor of activated STAT; PKA, Protein kinase A; RFX, regulatory factor X; SfA, Sanglifehrin A; SOCS, suppressant of cytokine signalling; TSA, Trichostatin.

* Corresponding authors at: Parc Cientific Barcelona, Baldiri Reixac 10, 08028 Barcelona, Spain.
E-mail addresses: jlloberas@ub.edu (J. Lloberas), acelada@ub.edu (A. Celada).

[1] These three authors contributed equally to this work.

https://doi.org/10.1016/j.imbio.2021.152114
Received 16 February 2021; Received in revised form 2 July 2021; Accepted 9 July 2021
Available online 14 July 2021

proteins in humans is associated with severe combined immunodeficiency (Reith and Mach, 2001), while increased expression has been associated with autoimmune disease (Trowsdale and Knight, 2013).

The induction of MHC II genes by IFN-γ in macrophages is regulated at both the transcriptional level (Benoist and Mathis, 1990; Mach et al., 1996; Reith and Mach, 2001; Ting and Trowsdale, 2002) and the post-transcriptional level (Cullell-Young et al., 2001; Gonalons et al., 1998). The tissue specificity of MHC II expression is due to the recruitment of the MHC class II transactivator (CIITA), which forms an enhanceosome with the transcription factors that bind to the MHC II promoter (Masternak et al., 2000; Reith et al., 2005; Serrat et al., 2010; Ting and Trowsdale, 2002). Due to the critical role of MHC II molecules in immune responses, it is important to have a detailed knowledge of the molecular mechanisms required for CIITA expression and MHC II molecule induction. This will provide opportunities to identify new therapeutic targets in several diseases (Turesson, 2004).

We previously described that incubating macrophages with IFN-γ activated not only STAT-1 but also IFN-γ, with the possibility of activating members of the MAPK family (p38, ERK-1/2, and JNK-1) involved in different gene expressions (Valledor et al., 2008). This provide an opportunity to explore new avenues to regulate the expression of MHC II genes in macrophages. In this research, we show that the IFN-γ-dependent induction of MHC II in macrophages occurs via an early phase of signal transducer and activator of transcription 1-alpha/beta (STAT1) activation/phosphorylation that induces interferon regulatory factor 1 (IRF1) and a late stage in which both IRF1 and STAT1 to induce CIITA. The late phosphorylation of STAT1 requires the immunophilin cyclophilin A (CypA).

2. Materials and methods

2.1. Reagents

Recombinant granulocyte macrophage colony-stimulating factor (GM-CSF) and IFN-γ were obtained from R&D Systems Inc. (Minneapolis, MN). SP600125, a JNK inhibitor, and KT 5720 (400 nM), a protein kinase A (PKA) inhibitor, were supplied by Calbiochem (San Diego, CA). Trichostatin (TSA) (50 nM) and 5-aza-2′-deoxycytosine (5AC) (1 μM) were obtained from Tocris (Bristol, UK). Actinomycin D and 5,6-dichlorobenzimidazole1-β-D-ribofuranoside (DBR) were provided by Sigma (St. Louis, MO) and used to block transcription. Novartis (Basel, Switzerland) kindly provided the sanglifehrin (SfA), cyclosporin A (CsA), and rapamycin, and Debiopharm Group (Lausanne, Switzerland) kindly provided the alisporivir. Recombinant CypA was obtained from Creative Biomart (Shirley, NY). All reagents were prepared following the manufacturer's recommendations, and preliminary studies were performed with the vehicle alone.

2.2. Cell culture

A murine lymphoma cell line, A20-2 J (Kim et al., 1979), was cultured in a DMEM medium (BioWhittaker, Radnor, PA) with 2 mM glutamine, 50 μM 2-β-ME, 10% heat-inactivated fetal calf serum (FCS) (PAA Laboratories, Pasching, Austria), 100 U/mL penicillin, and 100 μg/mL streptomycin. The Animal Research Committee of the University of Barcelona (number 2523) ratified the use of Balb/C mice, from which bone marrow-derived macrophages were isolated at age 8–10 weeks old (Charles River Laboratories, Wilmington, MA) (Celada et al., 1984). Femurs, tibias, and humeri were flushed to obtain the bone marrow cells, which were then grown in 150 mm plastic tissue culture dishes in DMEM containing 20% FCS plus 30% L-cell conditioned media as a source of M-CSF. The medium was complemented with 100 U/mL penicillin and 100 μg/mL streptomycin. Cells were incubated at 37 °C in a humidified 5% CO_2 atmosphere. A homogeneous population of adherent macrophages was obtained after 7 days of culture (>99% F4/80$^+$ CD11b$^+$). Before use, and after this 7-day period, macrophages were starved of M-CSF growth factor for 18 h.

2.3. Quantification of cell surface expression molecules

Using specific antibodies, cell surfaces were stained and cytofluorimetric analysis was performed (Casals et al., 2007). Cells were washed in phosphate-buffered saline (PBS) and suspended in 100 μL PBS with 5% FCS. To block Fc receptors for IgG (FcγRs), cells were incubated for 15 min at 4 °C with 1 μg/10^6 cells of anti-CD16/CD32 monoclonal antibody (BD Pharmingen, San Jose, CA). Then, the primary antibodies for macrophages, antibodies 11–5.2.1.9 (anti-I-Ak; PharMingen, San Diego, CA) and for A20 B cells 34–5-3 (anti-I-Ad; PharMingen) were added and incubated at 4 °C for 45 min. Cells were washed by centrifugation through an FCS cushion and incubated for 45 min at 4 °C with a fluorescein-conjugated secondary antibodies (FITC-labeled sheep anti-mouse IgG; Cappel, ICN Biomedicals, Irvine, CA). Finally, cells were fixed with 2% paraformaldehyde in PBS. Cytometric analysis was performed using Epics XL (Coulter, Brea, CA). To analyze the FcγR II and III expression on the macrophage surface, we used the anti-CD16/CD32 monoclonal antibody with FITC goat anti-rat Ig (554016 BD Pharmigen) as a secondary antibody.

2.4. RNA extraction and RT-PCR

We extracted RNA using TriReagent (Sigma), as described by the manufacturer. RNA (1 μg) was retro-transcribed using Moloney murine leukemia virus reverse transcriptase RNase H Minus (Promega, Madison, WI), and RT-PCR was performed (Serrat et al., 2010). Gene expression was normalized to three reference housekeeping genes: *Hprt1*, *L14*, and *Sdha*. The stability of these reference genes was determined by checking that their geNorm M value was less than 0.5 (Hellemans et al., 2007). The primers used are shown in Supplementary Table I.

2.5. Chromatin immune-precipitation assay

This assay was done as previously described (Casals et al., 2007; Serra et al., 2011). Cells were cross-linked with paraformaldehyde and lysed. Lysates were sonicated on ice, and the size of fragments obtained (200–1200 base pairs) was confirmed by electrophoresis. Soluble chromatin was collected and pre-cleared with salmon sperm. Nonspecific IgGs, pre-immune serum and protein-A-Sepharose were then added. After overnight incubation, the samples were centrifuged, supernatants were collected, and specific antibodies were added (anti-CIITA from Sigma; anti-IRF1 and anti-STAT1 p84/p91 from Santa Cruz Biotech (Santa Cruz, CA); anti-AcH3 from Merck Millipore (Billerica, MA). A control was made with nonspecific IgGs (Sigma). Protein-A-Sepharose was added to all the samples and mixtures were incubated in rotation. Beads were collected, and after being washed, immune precipitates were eluted three times. By heating samples at 65 °C overnight, reversion of cross-linking and input controls was achieved and the DNA was purified. RT-PCR was performed using the following primers for promoter IV of *CIIta:* 5′-GGCTCAAATCTGTCGTCCTC-3′ and 5′-AGTATCTGTGGCGCTTTTCC-3′. In some controls, we used a 2035 base-pair fragment of *I-aβ* containing an adaptor protein complex 1 box at 21,722 base pairs from the transcription start site (Casals et al., 2007).

2.6. Western blot analysis

Total cytoplasmic extracts were obtained by lysing cells (Serra et al., 2011). SDS-PAGE was performed and gels were transferred to nitrocellulose membranes (Hybond-C; Amersham Biosciences). After blocking the extracts, they were incubated with the following: primary phospho-STAT1 (Ser727) or STAT1 from Cell Signalling (Danvers, MA); phospho-STAT1 (Tyr701) from Thermo Fisher Scientific (Waltham, MA); IRF1 from Santa Cruz Biotech; NOS2 from Merck Millipore (Billerica, MA) and I-Aα antiserum (FF282-4, kindly provided by Dr. R. N. Germain,

National Institutes of Health, Bethesda, MD) with secondary antibodies IgG rabbit horseradish peroxidase conjugated from Jackson or IgG mouse peroxidase conjugated from MP (Biomedicals, Irvine, CA). The antibodies were detected using the EZ-ECL kit (Biological Industries, Cromwell, CT) and extracts were exposed to x-ray films (Fujifilm, Tokio, Japan). We used β-actin as loading control and was detected with antibodies from Sigma. The Molecular Analyst System (Bio-Rad, Hercules, CA) was used to analyze expression.

2.7. Enzyme-linked immunosorbent assay

CypA levels in supernatants of bone marrow-derived macrophage cultures were measured by enzyme-linked immunosorbent assay (ELISA) according to the manufacturers' protocols (MyBioSource, San Diego, CA). Bone marrow-derived macrophages (10^6) were dispensed in triplicate into 12-well plates and cultured for 24 h in medium containing IFN-γ with or without SfA. Triplicates of supernatants (90 μL per sample) were used for ELISA analysis.

2.8. Statistical calculations

Data were analyzed using unpaired Student t-tests in GraphPad Prism 9.1.

3. Results

3.1. Sanglifehrin A inhibits the induction of MHC II by IFN-γ

Primary cultures of macrophages were used because these are a homogeneous cellular population that responds to both proliferative and pro-inflammatory stimuli. After interacting with the corresponding receptor, IFN-γ induces genes that produce morphological and functional modifications in a process known as macrophage activation.

After starving macrophages of M-CSF growth factor for 18 h, the cells were treated for 1 h with SfA and then stimulated with IFN-γ in the presence or absence of SfA for set times. An SfA concentration of 15 μM was used based on research with Annexin V showing that this was not toxic for macrophages (Sànchez-Tilló et al., 2006). Flow cytometry revealed that, while IFN-γ induced the expression of the MHC II molecule I-Aβ on the macrophage surface, adding SfA dramatically reduced this expression (Fig. 1A and 1B). To assess whether this effect occurred with all immunophilin-binding drugs, we tested the effects of cyclosporine A (CsA) and rapamycin on MHC II by IFN-γ. However, neither drug affected I-Aβ expression (Fig. 1B), indicating that the observed effect was specific to SfA. Using Western blot, we then measured I-Aα protein expressed in macrophages treated with IFN-γ in the presence and absence of SfA. In relation to unstimulated macrophages, IFN-γ induced a large amount of protein expression that fell in response to treatment

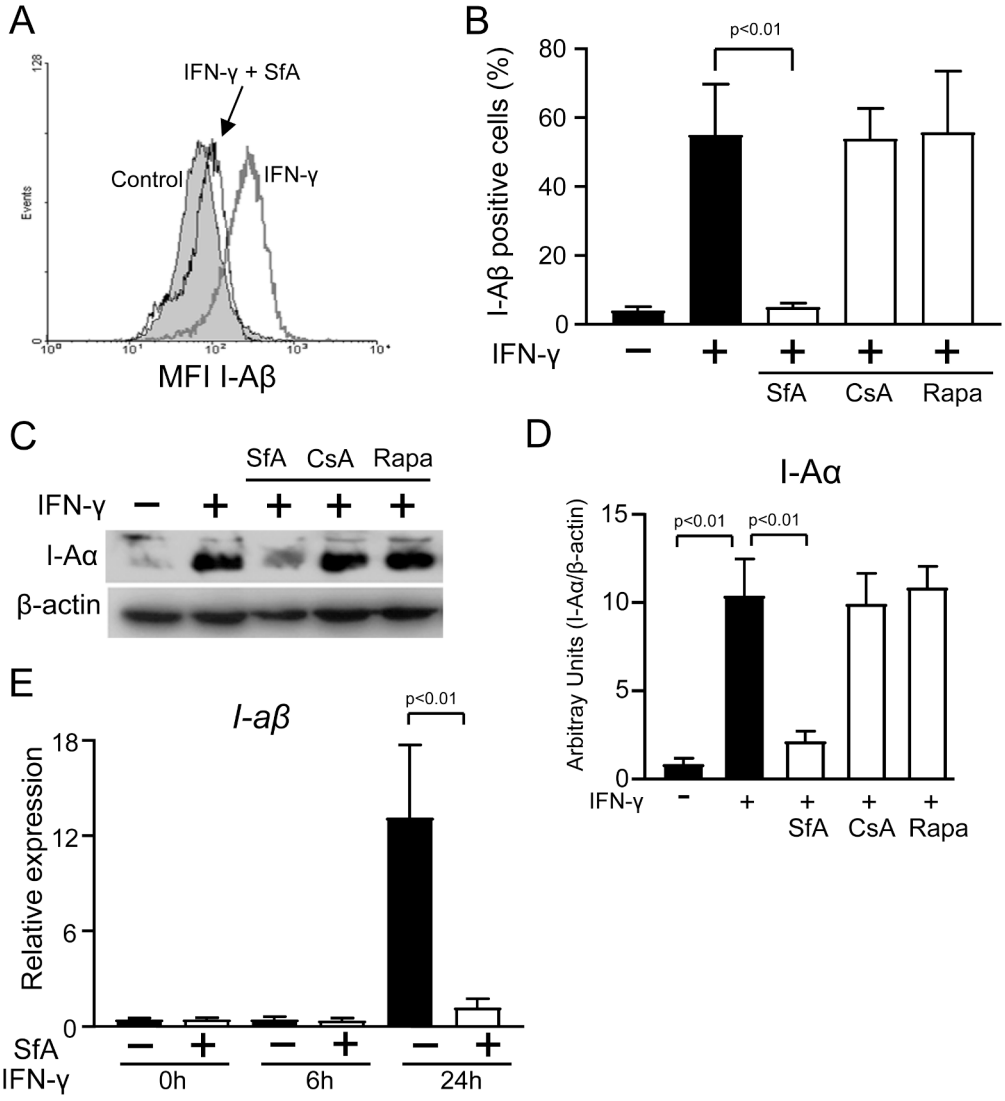


Fig. 1. SfA inhibits IFN-γ-dependent expression of MHC II molecules in macrophages. SfA inhibits IFN-γ-induced I-Aß surface expression. (A and B) Macrophages were incubated for 1 h in medium alone or with SfA (15 μM), CsA (10 μg/mL), or rapamycin (500 nM/mL), before adding IFN-γ (20 ng/mL) for 24 h. The expression of I-$A\beta^d$ on the macrophage surface was then measured by flow cytometry. (A) Histograms depicting the indicated conditions. (B) Quantification of I-$A\beta^d$-positive cells. (C) Macrophages were incubated for 1 h in medium alone or with SfA, CsA, or rapamycin before adding IFN-γ for 24 h and lysing cells. I-Aα was detected by Western blotting and β-actin was used as a loading and transfer control. (D) Quantification of the experiment detailed in C. (E) Macrophages were incubated for 1 h in medium alone or with SfA, and IFN-γ was added for the indicated times. The levels of *Ia-β* were determined by RT-PCR. Each graph shows the mean ± SD for three independent experiments.

with SfA, but not with CsA or rapamycin (Fig. 1C and 1D). Given that MHC II molecules are regulated at the transcriptional and post-transcriptional level (Gonalons et al., 1998), we also measured *I-aβ* expression by RT-PCR. We observed that treating macrophages with SfA caused a significant decrease in their RNA induction (Fig. 1E). The addition of different amounts of recombinant CypA (25, 50, and 100 ng/mL) (Zhu et al., 2015) to macrophages for 24 h did not induce *I-aβ* expression (data not shown), suggesting that CypA was necessary, but not sufficient, to induce MHC II expression.

Although previous studies by our group showed that *I-aβ* mRNA (Casals et al., 2007) and protein (Cullell-Young et al., 2001) were both highly stable, we hypothesized that a reduction in the half-life of its RNA caused the low level of *I-aβ* after SfA treatment. We therefore induced *I-aβ* mRNA with IFN-γ for 24 h in the presence or absence of SfA, blocking mRNA synthesis with 5 μg/mL Actinomycin D and 20 μg/mL DBR, as determined by $^{[3H]}$UTP incorporation (Celada et al., 1989). The mRNA was extracted at set times, *I-aβ* expression was determined, and the mRNA half-life was calculated. As a control, we measured the half-live of *c-Myc,* which was short. In controls and SfA-treated macrophages, *I-aβ* was stable (Fig. 2A). These data indicate that the reduced levels of *I-aβ* in cells treated with SfA was caused by a decreased transcription rate that was independent of the mRNA half-life.

Inhibiting MHC II genes by glucocorticoids has been shown to affect both IFN-γ-dependent and constitutive expression (Celada et al., 1993). Therefore, we evaluated the effect of SfA on the constitutive expression of *I-aβ,* using the B cell line A20-2J (Casals et al., 2007), to clarify whether the mechanism by which SfA represses MHC II is similar to that for glucocorticoids. The incubation of these cells with SfA for 24 h did not alter the expression of *I-aβ* (Fig. 2B and S1A), suggesting that the effect of SfA was specific to the inducible expression of this gene. When we then measured the effect of SfA on GM-CSF-induced MHC II genes in macrophages, no *I-aβ* inhibition was observed (Fig. 2C). Thus, the effect was deemed specific to the IFN-γ-dependent expression of *I-aβ*.

3.2. Sanglifehrin A and alisporivir inhibit the induction of CIIta by IFN-γ

All MHC II gene promoters contain three cis-acting elements 5′ upstream of the transcription start site that are denoted W, X, and Y boxes. These are the site of transcription factor binding, and their expression is constitutive and not regulated by IFN-γ (Kobayashi and van den Elsen, 2012). We previously showed that the inhibition of IFN-γ-induced expression of MHC II genes in the macrophages of aged mice was related to a decrease in a transcription factor (Herrero et al., 2001). To exclude an effect of SfA on the transcription factors that bind to the X box, we performed gel retardation assays with oligonucleotides covering the sequences of this box. The nuclear extracts from macrophages treated or

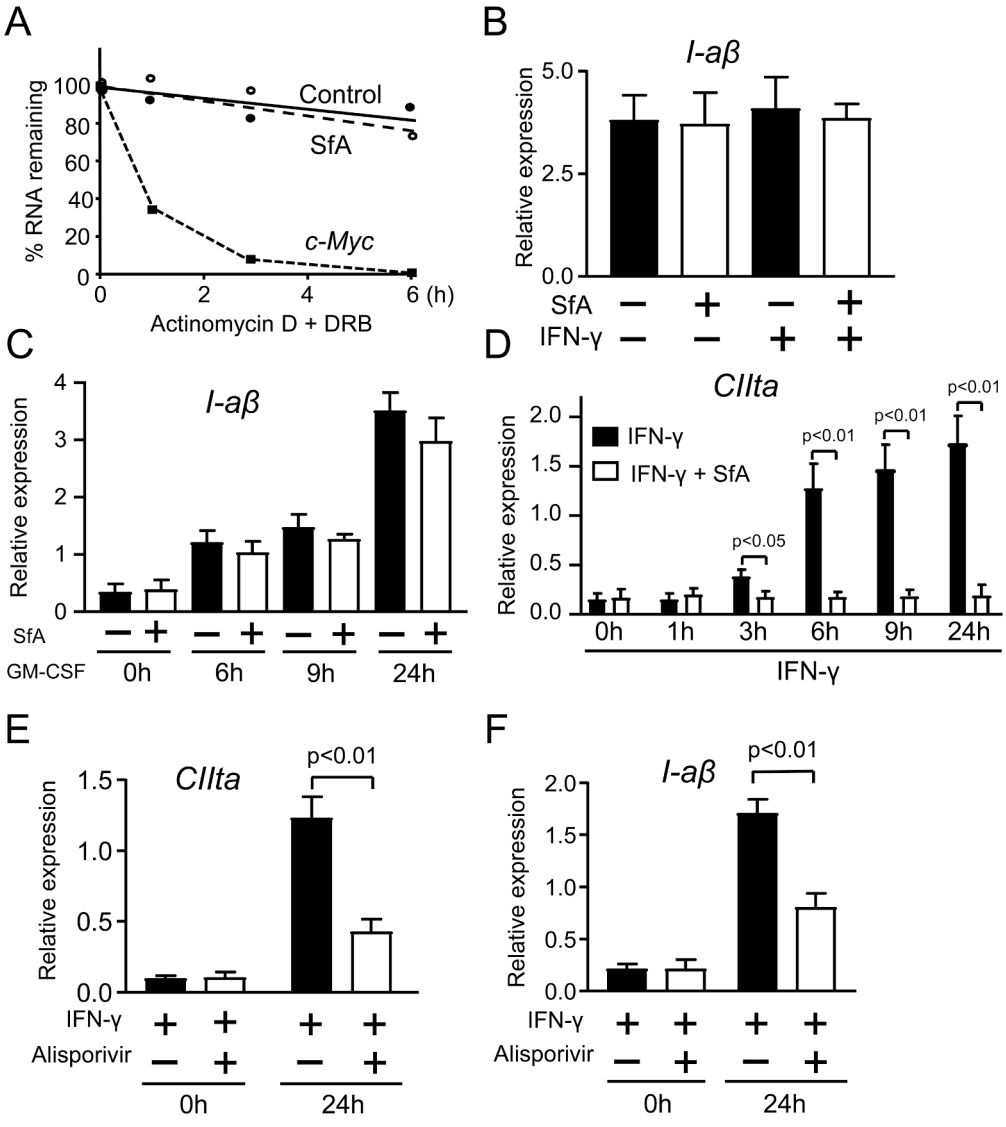


Fig. 2. SfA inhibits *CIIta* induction by IFN-γ at the transcriptional level. (A) Effect of SfA on mRNA stability of IFN-γ-induced *Ia-β*. Macrophages were incubated for 1 h in medium or SfA before adding IFN-γ for 24 h. Cells were then treated for the indicated periods with a combination of RNA synthesis inhibitors, actinomycin D (5 μg/mL), and DBR (20 μg/mL). In all these experiments, the gene expression levels were evaluated by RT-PCR. To evaluate the rate of mRNA degradation, the remaining mRNA was calculated as a percentage of the expression levels of that gene before RNA synthesis was blocked. Therefore, the graphics do not show differences in gene expression between treatments before the addition of RNA synthesis inhibitors. We compared the degradation of c-Myc as a positive control. (B) The effect of SfA on the constitutive expression of *Ia-β* was determined using the B cell line A20-2 J. Cells were incubated for 1 h in medium or SfA before adding IFN-γ for 24 h and then measuring *Ia-β* by RT-PCR. (C) To assess the effect of SfA on GM-CSF-dependent induction of *Ia-β*, macrophages were incubated for 1 h with medium or SfA and then GM-CSF (5 ng/mL) was added as indicated. The levels of *Ia-β* were determined by RT-PCR. (D) SfA also blocked the IFN-γ-dependent induction of *CIIta* (macrophages were incubated for 1 h with medium or SfA, and then IFN-γ was added for the indicated times, before *CIIta* levels were detected by RT-PCR. (E and F) Macrophages were incubated for 1 h with medium or Alisporivir (10 μM) and then IFN-γ was added for 24 h. The levels of *CIIta* E) and *Ia-β* F) were determined by RT-PCR. Alisporivir blocked the IFN-γ-dependent induction of *CIIta* and *Ia-β*. Each graph shows the mean ± SD of three independent experiments.

not with IFN-γ in the presence or absence of SfA produced similar protein-DNA complexes in all cases (Fig. S1B). The specificity of the binding was controlled by competition experiments with cold oligonucleotides.

The expression of MHC II genes is under the control of CIITA, which requires IFN-γ treatment to be induced and organize gene expression without direct binding to the promoter (Ting and Trowsdale, 2002). The interaction of CIITA with the transcription factors leads to an enhanceosome, permitting transcription by opening chromatin (Serrat et al., 2010). Next, we addressed whether IFN-γ-dependent *CIIta* induction was affected by SfA. As early as 3 h of macrophage incubation with IFN-γ, the levels of *CIIta* showed an increase in a time-dependent manner (Fig. 2D). SfA dramatically reduced the expression of *CIIta* mediated by IFN-γ (Fig. 2D). To assess the specificity of SfA on CypA, we used alisporivir at a non-toxic concentration of 10 μM, and showed that this also inhibited both *CIIta* and *Ia-β* gene expression (Fig. 2E and 2F). These results indicate that CypA is required for the induction of *CIIta*, and consequently *Ia-β*, by IFN-γ.

3.3. Sanglifehrin A does not inhibit the early activation of STAT1.

Macrophage activation by IFN-γ starts within a few minutes of exposure through the Jak-Stat cascade, with rapid STAT1 phosphorylation at tyrosine 701 that mediates its dimerization and translocation to the nucleus (Kramer et al., 2009). STAT1 phosphorylation started 15 min after incubating macrophages with IFN-γ and decreased after 120 min (Fig. 3A and 3B). Strikingly, adding SfA to IFN-γ did not alter STAT1 phosphorylation at tyrosine 701. STAT1 was also phosphorylated by IFN-γ at serine 727, a process compulsory for the formation of STAT-promoter complexes for complete transcriptional activity (Zhang et al., 1995). Thus, we analyzed the effects of SfA treatment on serine 727 phosphorylation in STAT1 (Fig. 3A and 3C). With or without SfA treatment, STAT1 phosphorylation started at 15 min of incubation with IFN-γ and continued up to 120 min (Fig. 3A).

CIITA expression is finely regulated in various cells and against a range of stimuli by promoters that lack sequence homology (LeibundGut-Landmann et al., 2004). IFN-γ induction in macrophages and its constitutive expression in B lymphocytes occurs via distinct promoters. Promoter IV is inducible by IFN-γ and requires 300 base pairs of the proximal region that contain a gamma interferon activation site (GAS) element, where Stat1 binding is necessary for *CIIta* induction (Muhlethaler-Mottet et al., 1998).

To study the binding of STAT1 to the CIITA promoter *in vivo,* we used chromatin immunoprecipitation assays with antibodies against STAT1 and primers of promoter IV of *CIIta*. STAT1 binding was induced as early as 1 h after incubation with IFN-γ (Fig. 3D). The addition of SfA to the IFN-γ treatment did not alter STAT1 binding (Fig. 3D).

IFN-γ induction of *CIIta* requires an E box and an IRF1-binding site

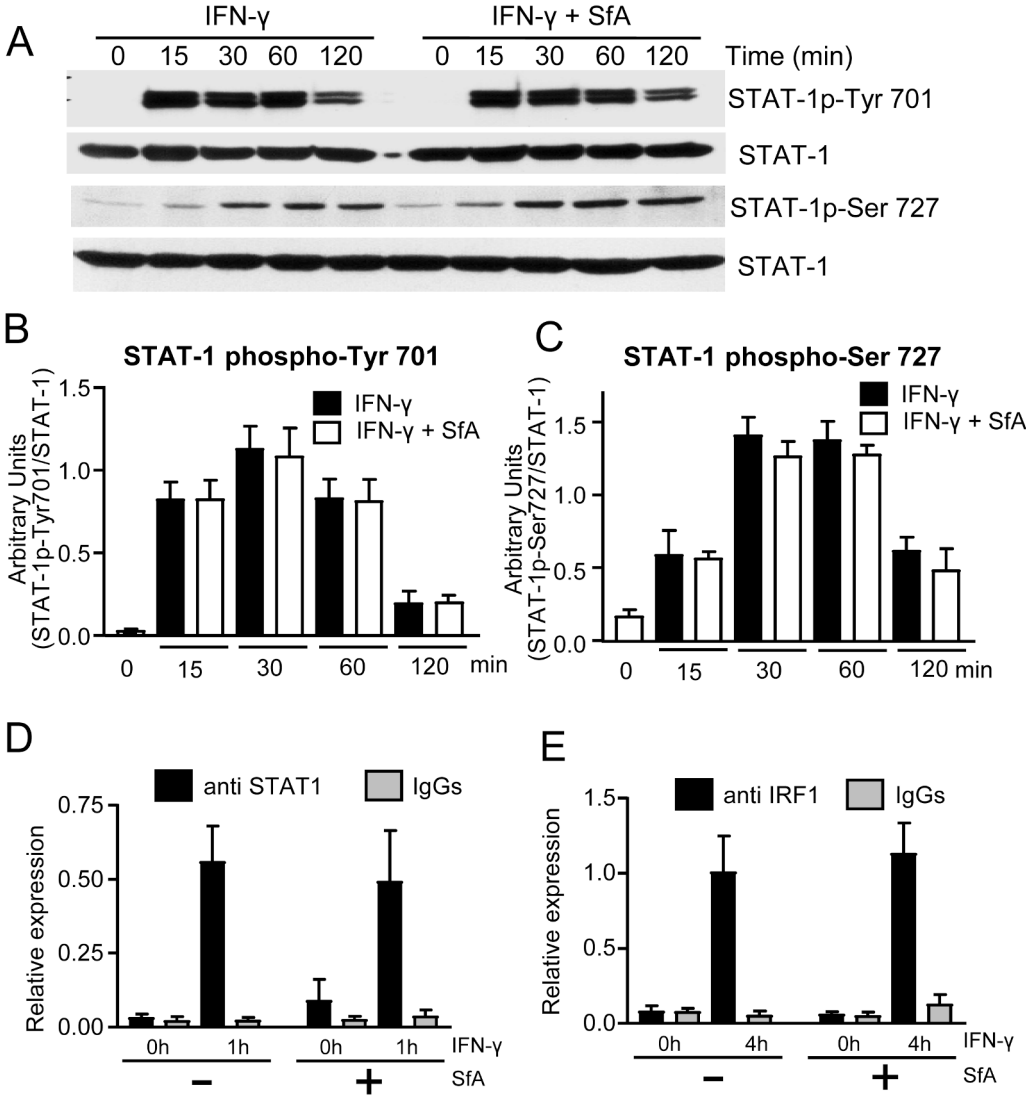


Fig. 3. The early IFN-γ-induced phosphorylation of STAT1 on tyrosine 701 and serine 727 was not affected by SfA treatment. (A) Macrophages were incubated for 1 h with medium or SfA, and then IFN-γ was added for the indicated times. The cell lysates were analyzed by Western blot for STAT1 phosphorylated at tyrosine 701 or at serine 727. (B) Quantification of the experiment in A for the phosphorylation of Tyrosine 701 of STAT-1. (C) Quantification of the experiment in A for the phosphorylation of Serine 707 of STAT-1. (D) Early binding of STAT1 to the *CIIta* promoter was not affected by SfA. Macrophages were incubated for 1 h with medium or SfA and IFN-γ was added for 1 h. The *in vivo* binding of STAT1 to *CIIta* promoter IV was determined by chromatin immunoprecipitation using specific antibodies against STAT1 or the IgG control. Immune-precipitated DNA was analyzed for the *CIIta* promoter by RT-PCR and the values obtained for each sample were normalized to their corresponding inputs. (E) IRF1 binding to the *CIIta* promoter at 4 h was not affected by SfA. Macrophages were incubated for 1 h with medium or SfA and then IFN-γ was added for 4 h, and the *in vivo* binding of IRF1 to *CIIta* promoter IV was determined by chromatin immunoprecipitation. Each graph represents mean ± SD of three independent experiments.

near the GAS element. CIITA activation by IFN-γ needs cooperative interaction between STAT1 and upstream regulatory factor-1 (USF-1), a constitutively expressed member of the basic helix-loop-helix/leucine zipper family (Muhlethaler-Mottet et al., 1998). IRF1 synthesis was induced by IFN-γ, explaining the delayed kinetics of *CIIta*. To determine if SfA affected these transcription factors, we measured their expression after IFN-γ treatment in the presence or absence of SfA. On the CIITA promoter, IRF1 and IRF2 co-occupied the interferon regulatory element, with IRF2 acting as a transcriptional repressor (Wang and Kubes, 2016). However, no immunosuppressant effect was observed for the expression of these transcription factors (Fig. S1C). *Irf2* expression did not increase in the presence of SfA, suggesting that this did not alter the balance of IRF1/IRF2 occupancy. Moreover, the kinetics of IRF1 induction were similar when macrophages were treated with IFN-γ alone and in the presence of SfA (Fig. S1D and S1E). Finally, the *in vivo* binding of IRF1 to promoter IV of *CIIta* was not affected by SfA, as measured by chromatin immunoprecipitation (Fig. 3 E).

Next, we studied the role of the suppressant of cytokine signaling (SOCS) that is induced by IFN-γ and the JAK/STAT pathway (Chen et al., 2000). The ectopic expression of SOCS-1 in macrophages inhibits IRF1 and STAT1 activation, thus blocking CIITA transcription (O'Keefe et al., 2001). The levels or induction of *Socs1*, *Socs2*, and *Socs3* by IFN-γ in the presence of SfA were also checked using RT-PCR. SfA did not alter the levels of these inhibitors, making it unlikely that the immunosuppressant obstructs *CIIta* expression through this mechanism (Fig. S2A).

3.4. Sanglifehrin A does not alter the epigenetic mechanisms of CIIta or PKA activation.

Epigenetic mechanisms, such as methylation of CpG sites or deacetylation on promoter IV of *CIIta*, can inhibit MHC II expression (Wright and Ting, 2006). STAT1 binding to promoter IV of *CIIta* is complemented by modest acetylation of histones H3 and H4 (Morris et al., 2002). In primary trophoblast cells, IFN-γ does not induce MHC II and the inhibition of histone deacetylases by TSA restores IFN-γ induction (Holtz et al., 2003). Tumor cells also avoid immune surveillance by inhibiting MHC II expression after triggering epigenetic modifications at the *CIIta* promoter (Wright and Ting, 2006). We hypothesized that a blockage in histone acetylation in the promoter region of CIITA could be responsible for SfA inhibiting its expression. The role of these mechanisms on SfA-mediated MHC II inhibition was studied using 5AC and TSA that inhibit methyl transferase activity and histone deacetylation, respectively. RT-PCR of *I-aβ* and *CIIta* showed that no treatment restored the inhibitory effect of SfA (Fig. 4A and B). Finally, using chromatin immunoprecipitation, we showed that histone H3 was acetylated at promoter IV of *CIIta*, even in the absence of IFN-γ, and that treatment with SfA did not affect binding (Fig. 4C).

The induction of CIITA by IFN-γ in macrophages can also be suppressed by other mechanisms, such as the increased production of cyclic adenosine monophosphate (cAMP) and concomitant activation of PKA (Cullell-Young et al., 2001). In fact, IFN-γ induces the expression of adenosine receptor A_{2B} in macrophages, thereby increasing the levels of cAMP and acting as a regulatory mechanism of macrophage deactivation (Xaus et al., 1999). The inhibition of PKA by KT 5720 did not restore the expression of I-Aα after stimulation by IFN-γ and SfA, indicating that the inhibition of MHC II by SfA was not mediated by the cAMP-PKA pathway (Fig. S2B and S2C).

These data suggest that the effect of SfA on CIITA expression was not dependent on PKA activation, CpG methylation, or deacetylation processes. Instead, our results demonstrated that the cyclophilin-binding immunosuppressant SfA participated in MHC II expression by inhibiting CIITA transcription.

3.5. Sanglifehrin A does not inhibit other genes induced by IFN-γ in macrophages

After observing that the effects of SfA are mediated by IFN-γ-induced *CIIta*, we performed a more thorough analysis of some relevant genes induced by this cytokine in macrophages. Surprisingly, no effect was observed when we measured the surface expression of Fcγ receptors II and III (Fig. 5A), the induction of *Lmp2* (Fig. 5B), and the stimulation of NOS2 (Fig. 5C and 5D). In other scenarios, SfA enhanced the response to IFN-γ, as occurred with *Tap1* (Fig. 5E). These data indicate that SfA was not a general suppressant of genes induced by IFN-γ, but that it underpinned a specific mechanism of MHC II gene activation.

3.6. Sanglifehrin A inhibits late STAT1 phosphorylation induced by IFN-γ, thereby inhibiting CIIta induction

We previously described that macrophages exhibit JNK activation after 2 to 5 h of incubation with IFN-γ (Valledor et al., 2008). Remarkably, a selective lack of functional JNK-1, but not p38, blocked the induction of *CIIta* in response to IFN-γ. This resulted in decreased levels of *I-aβ* and cell surface expression of I-Aβ (Valledor et al., 2008), with CypA reported to activate MAPK, including JNK (Jin et al., 2004). We therefore compared the effects of SfA and SP600125 on IFN-γ-induced *CIIta* and *I-aβ* to examine how SfA participates in late (4 h) JNK activation. This revealed that both drugs inhibited the expression of these genes (Fig. 6A), but that neither affected GM-CSF-dependent induction (Fig. 6B).

We next questioned whether the effects of SfA on CIITA induction are mediated by a late effect on STAT1 phosphorylation. Western blot analysis of tyrosine 701 phosphorylation of STAT1 showed that this phosphorylation was not affected until 2 h after IFN-γ treatment, after which it underwent decreased activation that persisted beyond 12 h (Fig. 6C). SfA treatment, which did not affect the initial phase of activation, completely suppressed STAT1 phosphorylation after 2 h of IFN-γ treatment (Fig. 6C and 6D). Furthermore, STAT1 phosphorylation at serine 727 did not modify these prolonged kinetics.

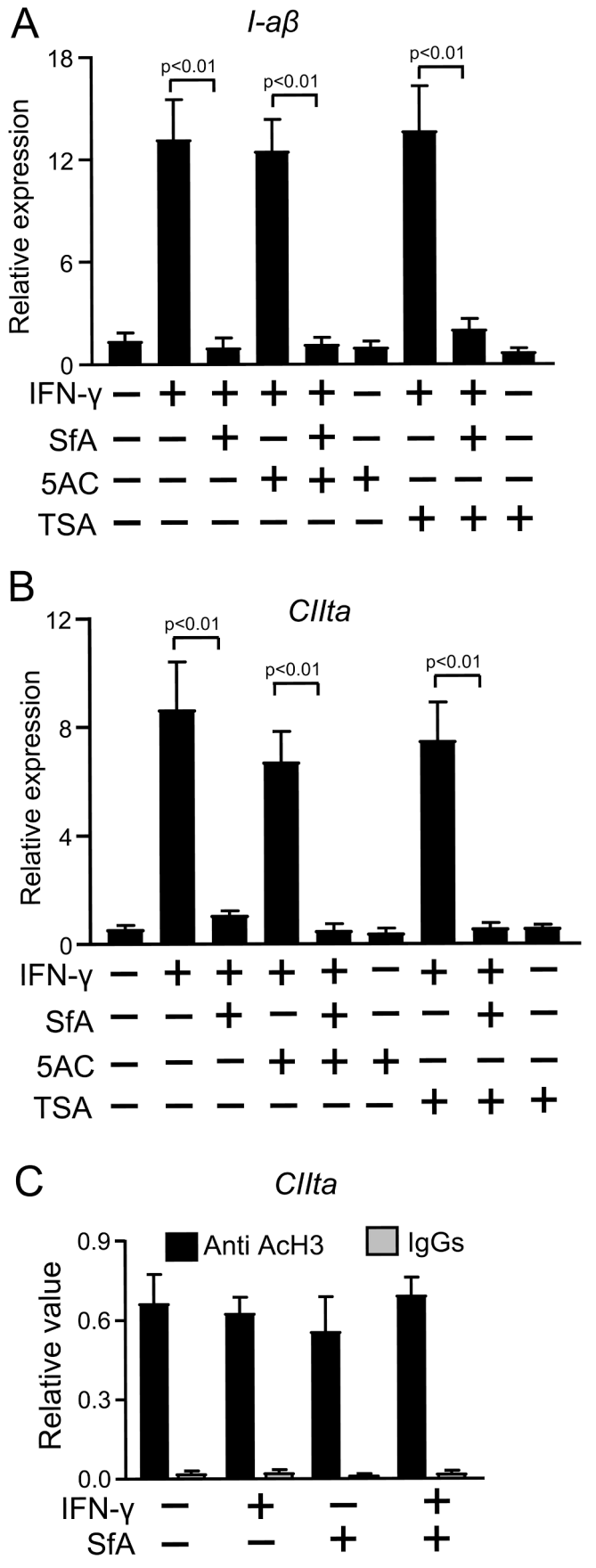
To confirm that the effect of SfA on STAT1 phosphorylation 4 h after IFN-γ treatment directly affected *CIIta* induction, we performed a ChIP assay of STAT1 and detected the *CIIta* promoter IV region. Although IFN-γ treatment induced STAT1 binding to this promoter after 4 h of treatment, a drastic decrease in STAT1 binding was detected when macrophages were later incubated with SfA (Fig. 6E). This observation was consistent with the lack of STAT1 phosphorylation after 2 h of stimulation and further confirmed the importance of CypA in the induction of CIITA by IFN-γ.

Finally, to determine the role of autocrine CypA production on I-Aβ expression induced by IFN-γ, macrophages were incubated with anti-CD147 to block the CypA binding to its receptor (Zhu et al., 2015). I-Aβ expression decreased significantly (Fig. 6F), demonstrating the role of CypA on MHC II induction. We also incubated IFN-γ-treated cells for 24 h in the absence or presence of SfA as a control to determine if SfA inhibited the release of CypA from macrophages. ELISA revealed no significant differences under these conditions (2.06 ± 0.12 versus 2.65 ± 0.38 ng/mL, based on three independent experiments).

4. Discussion

IFN-γ acts in both the innate and acquired immune responses mediated by STAT1 (Meraz et al., 1996), and recently, we showed that STAT1 responses are also regulated by MAPKs in a selective manner (Valledor et al., 2008). In the current study, we explored the role of CypA on IFN-γ-dependent responses in macrophages. Our results indicate that a yet unidentified system involving an isomerase plays a key role in the transcriptional induction of MHC II genes in these phagocytic cells.

We used SfA, a drug that binds and inhibits cyclophilin, to describe



(caption on next column)

Fig. 4. SfA did not induce epigenetic modifications. (A) Macrophages were incubated for 1 h with medium or SfA and IFN-γ was added for 18 h in the presence or absence of 5AC (1 μM), an inhibitor of methyl transferase activities, or TSA (50 nM), an inhibitor of histone deacetylation. The levels of *Ia-β* were then determined by RT-PCR. (B) A similar experiment to that in A, but measuring *CIIta*. (C) SfA did not modify the binding of the acetylated form of histone 3 to *CIIta* promoter IV. Macrophages were incubated for 1 h with medium or SfA and then IFN-γ was added for 12 h. The binding of the acetylated form of histone 3 was analyzed by chromatin immunoprecipitation using an antibody specific for the acetylated form of histone 3 or unspecific IgGs. Each graph represents the mean ± SD of three independent experiments.

the role of CypA (Sanglier et al., 1999). This effect seemed to be specific. First, exposure to the immunophilin-binding immunosuppressive drugs cyclosporin A and rapamycin did not inhibit the expression of MHC II molecules in macrophages. Second, the effect of SfA was exerted on induced mechanisms and not on constitutive expression in B cells. Third, although IFN-γ is the major inducer of MHC II genes in macrophages, they can be triggered by GM-CSF or other molecules that are not affected by SfA (Sebastián et al., 2008). The mechanism through which GM-CSF activates MHC II genes is quite similar to that of IFN-γ. CIITA induction is necessary in both cases, but the GM-CSF signaling pathway is mediated by STAT5 rather than of STAT1 (Sebastián et al., 2008). This observation explains the specificity of SfA for blocking the IFN-γ, but not the GM-CSF dependent induction of MHC II genes. Finally, the expression of various genes mediated by IFN-γ in macrophages was not inhibited by SfA.

IFN-γ regulates the expression of more than 400 genes in macrophages. Analysis of the transcription kinetics indicated that some of these genes have a rapid transcription response to IFN-γ that is followed by a decay and return to basal amounts several hours later (Boehm et al., 1997). After interaction with the corresponding receptor, IFN-γ induces a rapid pathway that does not require protein synthesis involving JAK-STAT phosphorylation and STAT1 binding to a specific sequence in the promoters of several genes (Bach et al., 1997). However, in other IFN-γ-dependent gene expressions, protein synthesis contributes to regulating the size and amount of the transcriptional response. In the presence of cycloheximide, some genes show an increase in transcription in response to IFN-γ whereas others are totally dependent (Celada et al., 1989). This observation supports the notion that IFN-γ induces the expression of transcription factors needed for the expression of these late-induced genes. In fact, this is the case for the induction of *CIIta,* which requires the synthesis of IRF1 before binding to the *CIIta* promoter and starting transcription (Muhlethaler-Mottet et al., 1998). This sequence of events would explain the lag period necessary to induce *CIIta*.

We found that IFN-γ induced IRF1 and that the binding of this transcription factor to the *CIIta* promoter was not modified by treatment with SfA. By contrast, the kinetics of STAT1 phosphorylation at tyrosine 701 or serine 727 during the critical first 2 h was not altered by SfA. Moreover, the ectopic expression of SOCS-1, the classic deactivator of kinases JAK1 and JAK2, has been shown to inhibit the induction of CIITA and MHC II by IFN-γ (Valledor et al., 2008). However, SOCS-1 does not mediate the effect of SfA because there was no modification of *Socs-1* expression.

These observations support the assertion that CypA is not necessary during the early steps of *CIIta* induction. Given the presence of a GAS box in the *CIIta* promoter, we propose that induction occurs in a two-step process, with both requiring STAT1: in an early step, STAT1 induces IRF1, which is then synthesized and bound to the *CIIta* promoter; then, in a late step, STAT1 starts the transcription of *CIIta* (Fig. S2B). Interestingly, STAT1 phosphorylation started to decrease after 2 h, probably due to the action of SOCS proteins. It has been established that STAT1 binds the *CIIta* promoter in a cooperative manner with USF-1 (Muhlethaler-Mottet et al., 1998); therefore, although STAT1 phosphorylation was decreased, it may have been sufficient to bind to the GAS box

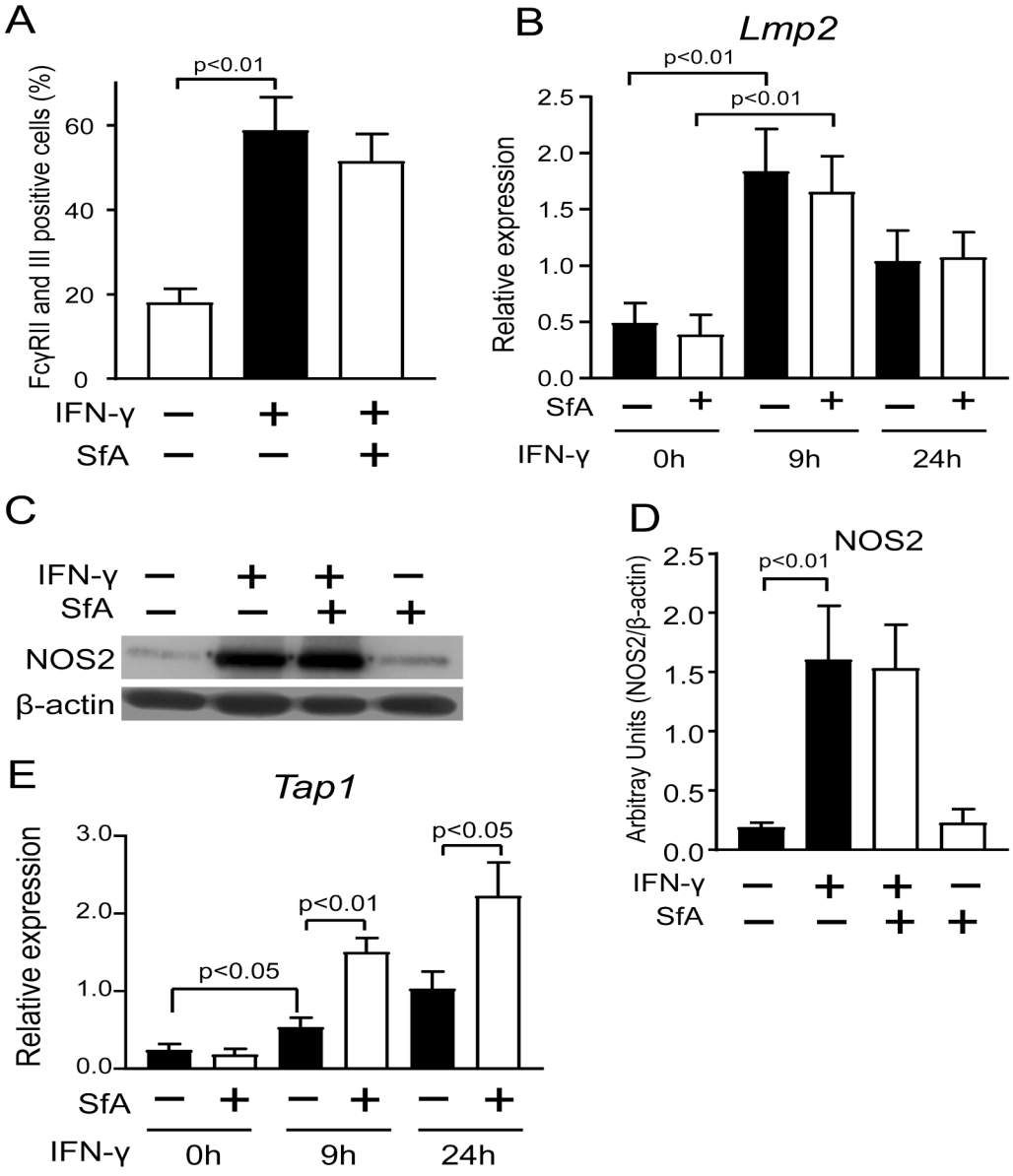


Fig. 5. SfA did not inhibit the expression of several IFN-γ-dependent genes. (A) Macrophages were incubated for 1 h with medium or SfA and then IFN-γ was added for 16 h. The expression of FcγRII and III on the macrophage surface was then analyzed by flow cytometry. (B) Macrophages were treated as in A for the indicated times, and the expression of *Lmp2* was determined by RT-PCR. (C) Macrophages were treated as in (A), lysed, and then had NOS2 detected by Western blotting. β-actin was used as a loading control and for transfer. (D) Quantification of the experiment in C. (E) Macrophages were treated as in A for the indicated times, and the expression of *Tap1* was determined. Each graph shows the mean ± SD of three independent experiments.

(Fig. 6D). Of note, while STAT1 remained phosphorylated for at least 12 h when cells were treated with SfA, the phosphorylation of this transcription factor disappeared after 2 h (Fig. 6C). Also, the binding of STAT1 to the *CIIta* promoter, together with both IRF1 and USF1, is required for transcription to occur (Muhlethaler-Mottet et al., 1998). Our results suggest that maintaining STAT1 phosphorylation at later times is critical for inducing the transcription of *CIIta* and that SfA inhibits the binding of STAT1 at 4 h, thereby explaining the lack of *CIIta* induction.

The transient transcriptional response to IFN-γ is due to the disappearance of STAT1 from the nucleus as a system of transcription control. After IFN-γ treatment, localization of tyrosine phosphorylated STAT1 in the nuclei of cells was maximal at 20–30 min and remained for 2–2.5 h. The disappearance of STAT1 from the nucleus was not due to protein degradation, but rather, it correlated with tyrosine dephosphorylation (Haspel et al., 1996). The protein inhibitor of activated STAT (PIAS) proteins, namely PIAS1, PIAS3, PIASx, and PIASy, function as E3-type ligases to help small ubiquitin-like modifier (SUMO) protein binding to target proteins. The SUMOylation site at lysine 703 is in close proximity to tyrosine 701 of STAT1, and this SUMOylation dephosphorylates STAT1 (Zimnik et al., 2009). Following the release of STAT1, DNA becomes available detectable by Crm1. Crm1 is an exportin that binds to a consensus nuclear export signal in the DNA binding domain of STAT1 to remove it from the nucleus. This domain becomes available to Crm1 when nuclear STAT1 is unbound from DNA (Reich, 2013).

The IFN-γ receptor is also internalized with the ligand and degraded inside macrophages while the receptor is recycled to the cell surface (Celada and Schreiber, 1987). If the amount of extracellular IFN-γ is insufficient, however, the receptor cannot be engaged. This explains why STAT1 becomes phosphorylated during the first hours of IFN-γ treatment and induces a limited transcription. The puzzling question is how the second wave of STAT1 phosphorylation occurs that directly or indirectly involves CypA (Graphical abstract). The fact that JNK is implicated in the induction of *CIIta* by IFN-γ (Valledor et al., 2008), and that CypA is necessary to induce the late phosphorylation of STAT1 (Fig. 6C), implicates a connection between these molecules. Moreover, CypA has been reported to activate MAPK, including JNK (Jin et al., 2004), and is known to be secreted by macrophages in response to inflammatory stimuli and reactive oxygen species (Satoh et al., 2009). These species are induced by IFN-γ treatment. Interestingly, CypA also

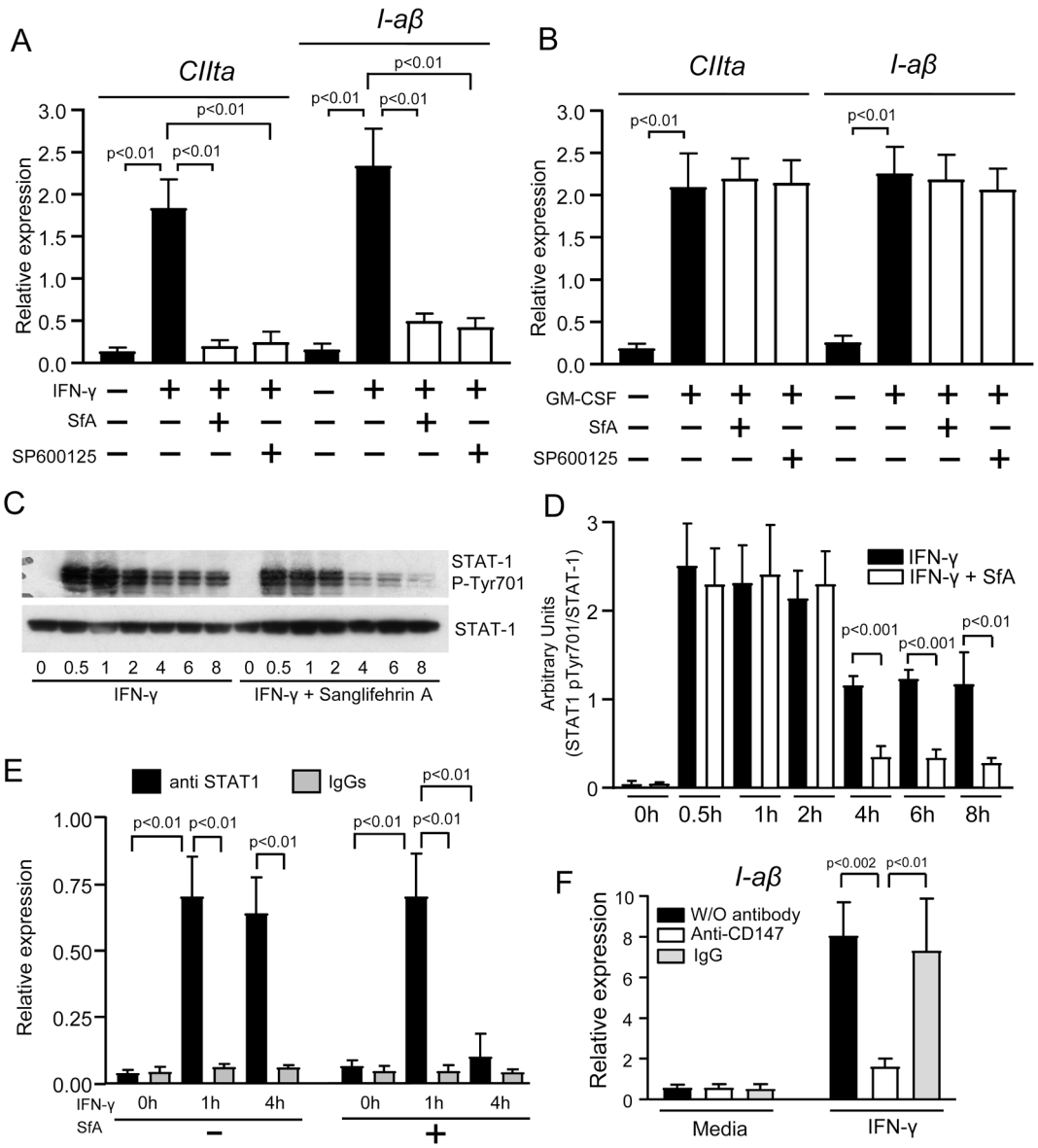


Fig. 6. SfA inhibited IFN-γ-induced late STAT1 phosphorylation that blocked *CIIta*.

upregulates the expression of metalloproteinase 9 and the adhesion of monocytes/macrophages by interacting with CD147 and signaling through JNK (Yang et al., 2008).

Previous observations in our lab and in the present study have demonstrated the requirement of JNK activation for CIITA to be induced after IFN-γ stimulation (Valledor et al., 2008). Moreover, in the present study, we show that JNK behaves in a similar way to CypA and that it is not essential for the induction of *CIIta* after stimulation by GM-CSF. Furthermore, JNK activation after IFN-γ occurs after 2 h of induction (Valledor et al., 2008). One could therefore hypothesize that JNK is also required for the late phosphorylation of STAT1, which is itself supported by previous observations that STAT1 is phosphorylated by activated JNK (Gorina et al., 2011; Wei et al., 2014).

In conclusion, we showed that IFN-γ-dependent induction of CIITA promoter IV required for MHC II transcription in macrophages involves a two-step process. During the first 2 h of IFN-γ treatment, STAT1 is phosphorylated and activates the induction of the transcription factor IRF1. Later, a second step is required in which the CypA induced by IFN-γ is released to the media and interacts with CD147 to activate JNK, which then phosphorylates STAT1, and in turn, binds to the *CIIta* promoter to induce transcription. Based on our findings, CypA and JNK act in a coordinated manner to sustain STAT1 phosphorylation and induce CIITA and MHC II molecules, thereby maintaining the inflammatory response after stimulation by IFN-γ.

Our description on the role of cyclophilin A for MHC class II expression may have a clinical application. An excess of MHC class II molecules is associated with autoimmune diseases (Trowsdale and Knight, 2013), and cyclophilin A may be a new therapeutic target for treatment of these diseases. At the present time, the growing number of CypA inhibitors have been proposed as treatment for infectious (Liao et al., 2021) and liver (Naoumov, 2014) diseases.

Author contributions

JT, CF, EST, TV, PG, and AFE are responsible for the study concept, experiments, and data analysis. JLL and AC are responsible for the study design, study management, and manuscript writing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence

the work reported in this paper.

Acknowledgments

This work was supported by grants to JLL and AC from the Ministerio de Ciencia, Innovación y Universidades (no. BFU2017-85353) and to JT from the Formacion del Profesorado Universitario (no. AP2012-02327). We thank Novartis (Basel, Switzerland) for kindly providing sanglifehrin A, cyclosporin A, and rapamycin, and we thank the Debiopharm Group (Lausanne, Switzerland) for kindly providing alisporivir.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.imbio.2021.152114.

References

Bach, E.A., Aguet, M., Schreiber, R.D., 1997. The IFN gamma receptor: a paradigm for cytokine receptor signaling. Annu. Rev. Immunol. 15, 563–591.
Benoist, C., Mathis, D., 1990. Regulation of major histocompatibility complex class-II genes: X, Y and other letters of the alphabet. Annu. Rev. Immunol. 8 (1), 681–715.
Boehm, U., Klamp, T., Groot, M., Howard, J.C., 1997. Cellular responses to interferon-gamma. Annu. Rev. Immunol. 15, 749–795.
Burdo, T.H., Walker, J., Williams, K.C., 2015. Macrophage polarization in AIDS: dynamic interface between anti-viral and anti-inflammatory macrophages during acute and chronic infection. J. Clin. Cell Immunol. 6.
Casals, C., Barrachina, M., Serra, M., Lloberas, J., Celada, A., 2007. Lipopolysaccharide up-regulates MHC class II expression on dendritic cells through an AP-1 enhancer without affecting the levels of CIITA. J. Immunol. 178 (10), 6307–6315.
Castoldi, A., Naffah de Souza, C., Camara, N.O., Moraes-Vieira, P.M., 2015. The macrophage switch in obesity development. Front. Immunol. 6, 637.
Celada, A., Gray, P.W., Rinderknecht, E., Schreiber, R.D., 1984. Evidence for a gamma-interferon receptor that regulates macrophage tumoricidal activity. J. Exp. Med. 160, 55–74.
Celada, A., Klemsz, M.J., Maki, R.A., 1989. Interferon-gamma activates multiple pathways to regulate the expression of the genes for major histocompatibility class II I-A beta, tumor necrosis factor and complement component C3 in mouse macrophages. Eur. J. Immunol. 19, 1103–1109.
Celada, A., McKercher, S., Maki, R.A., 1993. Repression of major histocompatibility complex IA expression by glucocorticoids: the glucocorticoid receptor inhibits the DNA binding of the X box DNA binding protein. J. Exp. Med. 177, 691–698.
Celada, A., Schreiber, R.D., 1987. Internalization and degradation of receptor-bound interferon-gamma by murine macrophages. Demonstration of receptor recycling. J. Immunol. 139, 147–153.
Cullell-Young, M., Barrachina, M., Lopez-Lopez, C., Gonalons, E., Lloberas, J., Soler, C., Celada, A., 2001. From transcription to cell surface expression, the induction of MHC class II I-A alpha by interferon-gamma in macrophages is regulated at different levels. Immunogenetics 53, 136–144.
Chen, X.P., Losman, J.A., Rothman, P., 2000. SOCS proteins, regulators of intracellular signaling. Immunity 13 (3), 287–290.
Gonalons, E., Barrachina, M., Garcia-Sanz, J.A., Celada, A., 1998. Translational control of MHC class II I-A molecules by IFN-gamma. J. Immunol. 161, 1837–1843.
Gorina, R., Font-Nieves, M., Marquez-Kisinousky, L., Santalucia, T., Planas, A.M., 2011. Astrocyte TLR4 activation induces a proinflammatory environment through the interplay between MyD88-dependent NFkappaB signaling, MAPK, and Jak1/Stat1 pathways. Glia 59, 242–255.
Guerrini, V., Gennaro, M.L., 2019. Foam cells: one size doesn't fit all. Trends Immunol. 40 (12), 1163–1179.
Haspel, R.L., Salditt-Georgieff, M., Darnell, J.E., 1996. The rapid inactivation of nuclear tyrosine phosphorylated Stat1 depends upon a protein tyrosine phosphatase. EMBO J. 15 (22), 6262–6268.
Hellemans, J., Mortier, G., De Paepe, A., Speleman, F., Vandesompele, J., 2007. qBase relative quantification framework and software for management and automated analysis of real-time quantitative PCR data. Genome Biol. 8, R19.
Herrero, C., Marques, L., Lloberas, J., Celada, A., 2001. IFN-gamma-dependent transcription of MHC class II IA is impaired in macrophages from aged mice. J. Clin. Invest. 107, 485–493.
Holtz, R., Choi, J.C., Petroff, M.G., Piskurich, J.F., Murphy, S.P., 2003. Class II transactivator (CIITA) promoter methylation does not correlate with silencing of CIITA transcription in trophoblasts. Biol. Reprod. 69, 915–924.
Jin, Z.-G., Lungu, A.O., Xie, L., Wang, M., Wong, C., Berk, B.C., 2004. Cyclophilin A is a proinflammatory cytokine that activates endothelial cells. Arterioscler. Thromb. Vasc. Biol. 24 (7), 1186–1191.
Kim, K.J., Kanellopoulos-Langevin, C., Merwin, R.M., Sachs, D.H., Asofsky, R., 1979. Establishment and characterization of BALB/c lymphoma lines with B cell properties. J. Immunol. 122, 549–554.
Kobayashi, K.S., van den Elsen, P.J., 2012. NLRC5: a key regulator of MHC class I-dependent immune responses. Nature Rev Immunol 12 (12), 813–820.
Kramer, O.H., Knauer, S.K., Greiner, G., Jandt, E., Reichardt, S., Guhrs, K.-H., Stauber, R. H., Bohmer, F.D., Heinzel, T., 2009. A phosphorylation-acetylation switch regulates STAT1 signaling. Genes Dev. 23 (2), 223–235.
LeibundGut-Landmann, S., Waldburger, J.-M., Krawczyk, M., Otten, L., Suter, T., Fontana, A., Acha-Orbea, H., Reith, W., 2004. Mini-review: specificity and expression of CIITA, the master regulator of MHC class II genes. Eur. J. Immunol. 34 (6), 1513–1525.
Liao, Y., Luo, D., Peng, K., Zeng, Y., 2021. Cyclophilin A: a key player for etiological agent infection. Appl. Microbiol. Biotechnol. 105 (4), 1365–1377.
Mach, B., Steimle, V., Martinez-Soria, E., Reith, W., 1996. Regulation of MHC class II genes: lessons from a disease. Annu. Rev. Immunol. 14 (1), 301–331.
Mantovani, A., Biswas, S.K., Galdiero, M.R., Sica, A., Locati, M., 2013. Macrophage plasticity and polarization in tissue repair and remodelling. J. Pathol. 229, 176–185.
Masternak, K., Muhlethaler-Mottet, A., Villard, J., Zufferey, M., Steimle, V., Reith, W., 2000. CIITA is a transcriptional coactivator that is recruited to MHC class II promoters by multiple synergistic interactions with an enhanceosome complex. Genes Dev. 14, 1156–1166.
McGonagle, D., Sharif, K., O'Regan, A., Bridgewood, C., 2020. The role of cytokines including interleukin-6 in COVID-19 induced pneumonia and macrophage activation syndrome-like disease. Autoimmun. Rev. 19 (6), 102537. https://doi.org/10.1016/j.autrev.2020.102537.
Meraz, M.A., White, J.M., Sheehan, K.C.F., Bach, E.A., Rodig, S.J., Dighe, A.S., Kaplan, D. H., Riley, J.K., Greenlund, A.C., Campbell, D., Carver-Moore, K., DuBois, R.N., Clark, R., Aguet, M., Schreiber, R.D., 1996. Targeted disruption of the Stat1 gene in mice reveals unexpected physiologic specificity in the JAK-STAT signaling pathway. Cell 84 (3), 431–442.
Morris, A.C., Beresford, G.W., Mooney, M.R., Boss, J.M., 2002. Kinetics of a gamma interferon response: expression and assembly of CIITA promoter IV and inhibition by methylation. Mol. Cell. Biol. 22 (13), 4781–4791.
Muhlethaler-Mottet, A., Di Berardino, W., Otten, L.A., Mach, B., 1998. Activation of the MHC class II transactivator CIITA by interferon-gamma requires cooperative interaction between Stat1 and USF-1. Immunity 8, 157–166.
Naoumov, N.V., 2014. Cyclophilin inhibition as potential therapy for liver diseases. J. Hepatol. 61 (5), 1166–1174.
O'Keefe, G.M., Nguyen, V.T., Ping Tang, L.L., Benveniste, E.N., 2001. IFN-gamma regulation of class II transactivator promoter IV in macrophages and microglia: involvement of the suppressors of cytokine signaling-1 protein. J. Immunol. 166, 2260–2269.
Otten, L.A., Steimle, V., Bontron, S., Mach, B., 1998. Quantitative control of MHC class II expression by the transactivator CIITA. Eur. J. Immunol. 28 (2), 473–478.
Pathria, P., Louis, T.L., Varner, J.A., 2019. Targeting tumor-associated macrophages in cancer. Trends Immunol. 40 (4), 310–327.
Reich, N.C., 2013. STATs get their move on. Jak-Stat 2 (4), e27080. https://doi.org/10.4161/jkst.27080.
Reith, W., LeibundGut-Landmann, S., Waldburger, J.-M., 2005. Regulation of MHC class II gene expression by the class II transactivator. Nat. Rev. Immunol. 5 (10), 793–806.
Reith, W., Mach, B., 2001. The bare lymphocyte syndrome and the regulation of MHC expression. Annu. Rev. Immunol. 19, 331–373.
Roche, P.A., Furuta, K., 2015. The ins and outs of MHC class II-mediated antigen processing and presentation. Nat. Rev. Immunol. 15 (4), 203–216.
Sànchez-Tilló, E., Wojciechowska, M., Comalada, M., Farrera, C., Lloberas, J., Celada, A., 2006. Cyclophilin A is required for M-CSF-dependent macrophage proliferation. Eur. J. Immunol. 36 (9), 2515–2524.
Sanglier, J.J., Quesniaux, V., Fehr, T., Hofmann, H., Mahnke, M., Memmert, K., Schuler, W., Zenke, G., Gschwind, L., Maurer, C., et al., 1999. Sanglifehrins A, B, C and D, novel cyclophilin-binding compounds isolated from Streptomyces sp. A92-308110. I. Taxonomy, fermentation, isolation and biological activity. J. Antib., 52, 466-473.
Satoh, K., Nigro, P., Matoba, T., O'Dell, M.R., Cui, Z., Shi, X.i., Mohan, A., Yan, C., Abe, J.-I., Illig, K.A., Berk, B.C., 2009. Cyclophilin A enhances vascular oxidative stress and the development of angiotensin II-induced aortic aneurysms. Nat. Med. 15 (6), 649–656.
Sebastián, C., Serra, M., Yeramian, A., Serrat, N., Lloberas, J., Celada, A., 2008. Deacetylase activity is required for STAT5-dependent GM-CSF functional activity in macrophages and differentiation to dendritic cells. J. Immunol. 180 (9), 5898–5906.
Serra, M., Forcales, S.V., Pereira-Lopes, S., Lloberas, J., Celada, A., 2011. Characterization of Trex1 induction by IFN-gamma in murine macrophages. J. Immunol. 186, 2299–2308.
Serrat, N., Serra-Sarasa, M., Barrachina, M., Lloberas, J., Celada, A., 2010. The locus control region of the MHC class II promoter acts as a repressor element, the activity of which is inhibited by CIITA. Mol. Immunol. 47 (4), 825–832.
Ting, J.-Y., Trowsdale, J., 2002. Genetic control of MHC class II expression. Cell 109 (2), S21–S33.
Trowsdale, J., Knight, J.C., 2013. Major histocompatibility complex genomics and human disease. Annu. Rev. Genom. Hum. Genet. 14 (1), 301–323.
Tur, J., Pereira-Lopes, S., Vico, T., Marín, E.A., Muñoz, J.P., Hernández-Alvarez, M., Cardona, P.-J., Zorzano, A., Lloberas, J., Celada, A., 2020. Mitofusin 2 in macrophages links mitochondrial ROS production, cytokine release, phagocytosis, autophagy, and bactericidal activity. Cell Rep. 32 (8), 108079. https://doi.org/10.1016/j.celrep.2020.108079.
Turesson, C., 2004. Endothelial expression of MHC class II molecules in autoimmune disease. Curr. Pharm. Des. 10, 129–143.
Valledor, A.F., Sanchez-Tillo, E., Arpa, L., Park, J.M., Caelles, C., Lloberas, J., Celada, A., 2008. Selective roles of MAPKs during the macrophage response to IFN-gamma. J. Immunol. 180, 4523–4529.
Wang, J., Kubes, P., 2016. A reservoir of mature cavity macrophages that can rapidly invade visceral organs to affect tissue repair. Cell 165 (3), 668–678.

Wei, Z.Z., Yu, S.P., Lee, J.H., Chen, D., Taylor, T.M., Deveau, T.C., Yu, A.C.H., Wei, L., 2014. Regulatory role of the JNK-STAT1/3 signaling in neuronal differentiation of cultured mouse embryonic stem cells. Cell. Mol. Neurobiol. 34 (6), 881–893.

Wright, K.L., Ting, J.-Y., 2006. Epigenetic regulation of MHC-II and CIITA genes. Trends Immunol. 27 (9), 405–412.

Wynn, T.A., Chawla, A., Pollard, J.W., 2013. Macrophage biology in development, homeostasis and disease. Nature 496 (7446), 445–455.

Xaus, J., Cardo, M., Valledor, A.F., Soler, C., Lloberas, J., Celada, A., 1999. Interferon gamma induces the expression of p21waf-1 and arrests macrophage cell cycle, preventing induction of apoptosis. Immunity 11, 103–113.

Yang, Y., Lu, N., Zhou, J., Chen, Z.-n., Zhu, P., 2008. Cyclophilin A up-regulates MMP-9 expression and adhesion of monocytes/macrophages via CD147 signalling pathway in rheumatoid arthritis. Rheumatology 47 (9), 1299–1310.

Zhang, X., Blenis, J., Li, H., Schindler, C., Chen-Kiang, S., 1995. Requirement of serine phosphorylation for formation of STAT-promoter complexes. Science 267 (5206), 1990–1994.

Zhu, D.i., Wang, Z., Zhao, J.-J., Calimeri, T., Meng, J., Hideshima, T., Fulciniti, M., Kang, Y., Ficarro, S.B., Tai, Y.-T., Hunter, Z., McMilin, D., Tong, H., Mitsiades, C.S., Wu, C.J., Treon, S.P., Dorfman, D.M., Pinkus, G., Munshi, N.C., Tassone, P., Marto, J. A., Anderson, K.C., Carrasco, R.D., 2015. The Cyclophilin A-CD147 complex promotes the proliferation and homing of multiple myeloma cells. Nature Med. 21 (6), 572–580.

Zimnik, S., Gaestel, M., Niedenthal, R., 2009. Mutually exclusive STAT1 modifications identified by Ubc9/substrate dimerization-dependent SUMOylation. Nucl. Acids Res. 37, e30.