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Effect of SOCS1 overexpression on RPE cell activation by proinflammatory cytokines.

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HIGHLIGHTS

- SOCS1 mRNA overexpression in RPE cells prevents IFNγ-induced SOCS1 mRNA increase.
- SOCS1 overexpression prevents IFNγ-mediated STAT1 phosphorylation.
- SOCS1 overexpression in RPE cells inhibits IFNγ-induced decrease of IL-8 secretion.
- SOCS1 overexpression in RPE cells prevents IFNγ-induced MHC II and CD54 expression.
- SOCS1 overexpression in RPE cells does not block TNFα- or IL-17 induced IL-8 secretion.

ABSTRACT

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The purpose of this study was to investigate the in vitro effect of Suppressor Of Cytokine Signaling 1 (SOCS1) overexpression in retinal pigment epithelium (RPE) cells on their activation by pro-inflammatory cytokines IFNγ, TNFα and IL-17.

Retinal pigment epithelium cells (ARPE-19) were stably transfected with the control plasmid pIRES2-AcGFP1 or the plasmid pSOCS1-IRES2-AcGFP1. They were stimulated by IFNγ (150 ng/ml), TNFα (30 ng/ml) or IL-17 (100 ng/ml). The levels of SOCS1 mRNA were measured by real-time PCR. Signal Transducer and Activator of Transcription 1 (STAT1) phosphorylation and IκBα expression were analysed by western Blot (WB). IL-8 secretion was analysed by ELISA and expression of MHCII molecules and ICAM-1 / CD54 by flow cytometry.

Our data show that SOCS1 mRNA overexpression in RPE cells prevents IFNγ-induced SOCS1 mRNA increase and IFNγ-mediated STAT1 phosphorylation. Moreover, SOCS1 overexpression in RPE cells inhibits IFNγ-induced decrease of IL-8 secretion and prevents IFNγ-induced MHC II and ICAM1 /CD54 upregulation. However, SOCS1 overexpression does not affect TNFα-induced IκBα degradation nor block TNFα-induced or IL-17-induced IL-8 secretion. On the contrary, IL-17-induced secretion is increased by SOCS1 overexpression.

In conclusion, SOCS1 overexpression in RPE cells inhibits some IFNγ-mediated responses that lead to uveitis development. This notion raises the possibility that SOCS1 overexpression could be a novel target for treating non-infectious uveitis. However, some proinflammatory effects of TNFα and IL-17 stimulation on RPE are not blocked by SOCS1 overexpression.

KEYWORDS: RPE, SOCS1, UVEITIS, IFNΓ,IL-17, IL-8

INTRODUCTION

The retinal pigment epithelium (RPE) is a monolayer of cells in the outer retina situated between the neuroretina and the choroid [1]. It is a part of the blood retinal barrier (BRB) that

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limits the access of blood components to the retina. The BRB consists of an outer part – tight junctions of the retinal pigment epithelium (RPE) and an inner part - tight junctions of retinal endothelial cells enhanced by extensions of Muller cells and astrocytes [2]. It is worth noticing that RPE has been well documented to play an important role in the maintenance of the ocular immune microenvironment as well as in the pathogenesis of uveitis [1]. In normal conditions, the eye is isolated from the rest of the body by the blood retinal barrier (BRB). In uveitis conditions, the BRB is broken down, BRB cells are activated and express molecules implicated in recruitment and stimulation of inflammatory cells.

The major activators of BRB cells during experimental autoimmune uveitis (EAU) are the proinflammatory cytokines produced by Th1 and Th17 autoreactive lymphocytes. Th1 lymphocytes secrete interferon gamma (IFNγ) as well as Tumor Necrosis Factor alpha (TNFα) and Th17 cells secrete TNFα, IL17, IL21 and IL22. IFNγ, TNFα, IL-17 and IL-22 can modulate RPE cell function, leading to outer BRB rupture [1]. IFNγ stimulation of RPE cells results in upregulation of CD54/ICAM-1 expression, induction of MHC class II (MHCII), CD40 expression and proinflammatory cytokines secretion: IL-8, IL-6, MCP-1, GM-CSF, M-CSF, RANTES.

The IFNγ proinflammatory cascade is mediated by the Janus kinase/signal transducer and activator of transcription (JAK/STAT) pathway [3]. As a result of a series of phosphorylations initiated by JAK, STAT dimerises and migrates into the cell nucleus to activate transcription of various target genes such as proinflammatory cytokines, adhesion molecules (ICAM-1/ CD54) and MHCII. Nevertheless, also negative regulators of JAK/STAT pathway are transcripted. They are called Suppressors Of Cytokine Signaling (SOCS), a family which consist of eight members, SOCS1-7 and CIS [3]. It has been shown that SOCS1, SOCS2, and SOCS3 mRNAs are induced in response to IFNγ stimulation[4]. Moreover, SOCS1 and SOCS3 but not SOCS2 inhibited the tyrosine phosphorylation and nuclear translocation of STAT1 in response to IFNγ stimulation in HeLa and MCF-7-derived stable cell lines expressing SOCS1, SOCS2, and SOCS3 proteins. Furthermore, SOCS1 exhibited a much stronger inhibition of the activation of STAT1 than SOCS3. These results suggest that SOCS1 and SOCS3 but not SOCS2 are inhibitors of IFN-mediated Janus-activated kinase/STAT signaling pathways [5].

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SOCS family members are inhibiting the IFNγ proinflammatory cascade by different mechanisms [6]. First, they bind to JAKs, inhibiting cytokine receptor phosphorylation and STAT activation. Secondly, SOCS protein can bind to P-Tyr residues on receptor chains, preventing JAK interaction with receptor chains or STAT recruitment. A third mechanism is the competitive inhibition of Grb2 recruitment by SOCS and blockade of MAPK pathway. Finally, SOCS can also induce ubiquitination and subsequent proteasome degradation of JAKs and receptor.

SOCS immunosuppressive profile is consistent with the findings that SOCS1 local overexpression is protecting target organs in experimental models of autoimmune diabetes and pulmonary inflammation [7]. Since the latter diseases are mediated through multiple cytokines, those data strongly suggest that SOCS1 can interfere not only with IFNγ signaling. Yet, some studies show that in addition to its action on the JAK/STAT pathway, SOCS1 can inhibit other signaling pathways, including that of Nuclear Factor Kappa B (NFkB) used by TNFα [6].This would have great relevance for the possible therapeutical possibilities of SOCS1 overexpression in the treatment of non-infectious uveitis. In this study, we have thus tested the hypothesis that overexpression of SOCS1 in RPE cells can modulate their activation by the pro-inflammatory cytokines IFNγ, TNFα and IL-17. We found that SOCS1 overexpression in RPE cells prevents IFNγ-mediated STAT1 phosphorylation and prevents MHCII and ICAM-1 upregulation. However, it does not affect TNFα-induced IκBα degradation, nor TNFα /IL-17 -induced IL-8 secretion.

MATERIALS and METHODS

Plasmid construction and clone selection.

American retinal pigment epithelium type 19 (ARPE-19) is a spontaneously arising human RPE cell line obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). ARPE cells were stably transfected using lipofection by Fugene (Roche Diagnostics) with the plasmid pSOCS1-IRES2-AcGFP1 or the control plasmid pIRES2-AcGFP1 (Clontech). The plasmid containing the SOCS1 gene (pSOCS1-IRES2-AcGFP1) was constructed in our laboratory by inserting the SOCS1 sequence from plasmid pORF5-hSOCS1v24 (InvivoGen) into multiple cloning site of pIRES2-AcGFP1. Several cell clones containing plasmid pSOCS1-IRES2-AcGFP1 (SOCS1 clone) or control plasmid pIRES2-AcGFP1 (CTRL clone) were tested by flow cytometry (FACS) for green

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fluorescence. The highest expressing ones were selected and further tested by quantitative reverse transcription PCR analysis (qRT-PCR) for SOCS1 gene expression.

Cell culture and treatment

ARPE-19 cells as well as CTRL clone and SOCS1 clone were cultured in a 1:1 mixture of Dulbecco's modified Eagle's medium (DMEM; Invitrogen, Merelbeke, Belgium) and Ham's F12 with 2.5 mM L-glutamine (Invitrogen), supplemented with 10% fetal bovine serum (FBS), 100 IU/ml penicillin and 100 μg/ml streptomycin in a 5% CO2 and 95% humidity incubator at 37 °C. In CTRL and SOCS1 clone cultures, 1mg/mL Gentamycin 418 (InvivoGen) was added in order to maintain antibiotics induced selection. ARPE-19 cells, SOCS1 clone or CTRL clone were seeded at 5×10^5 cells/well in 6-well plates (*Fig. 1*, *Fig. 2C*) or at 2.5 $x10^5$ cells/well/ml (*Fig. 2B, Fig. 3B, Fig. 3C*). Recombinant human IFNγ, TNFα and IL-17 were purchased from Invitrogen and added to cultures as specifically indicated below. In the experiments, CTRL clone unstimulated and ARPE-19 cells unstimulated were used as controls.

Real-time PCR

ARPE-19 cells, CTRL clone or SOCS1 clone were seeded at 5×10^5 cells/well in 6-well plates. One day later, IFNγ (150 ng/ml), TNFα (30 ng/ml) or IL-17 (100 ng/ml) were added to the cultures for 24 hours. Cells were collected for qRT-PCR analysis of SOCS1 gene expression. β-Actin was used as a non-modulated reference gene. The mRNA extraction and isolation were carried out using the automated MagNA Pure LC Instrument system (LightCycler® RNA Master) and the MagNAPure LC mRNA Isolation Kit II, following manufacturer's instructions (Roche Applied Science, Vilvoorde, Belgium). A one step real-time quantitative RT-PCR technique using the RNA Master Hybridization Probes Kit (Roche Applied Science) was used to quantify mRNA expression using specific primes and fluorescent probes for human SOCS1 (Primer and probe SOCS1 human, Applied Biosystems SOCS1 TaqMan) and β-actin (Primer and probe B-actine human, Applied Biosystems, TaqMan Gene Expression Assays).Data are presented as normalized expression of SOCS1 versus β-actin ($2^{-(CtSOCS1-Ct\beta actin)}$).

Western blot analysis

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ARPE-19 cells, CTRL clone and SOCS1 clone were cultured and stimulated with IFNγ (150ng/ml), TNFα (30ng/ml) or IL-17 (100 ng/ml). STAT1 phosphorylation and IκBα expression were analysed by Western blot, 5 and 30 minutes after stimulation. The WB were performed as described by Makhoul M. et al. [8]. Briefly, specific primary antibodies used for immunodetection were anti-phospho-STAT and anti- total STAT (Cell Signalling) and anti-IκBα (Santa Cruz).

IL-8 ELISA

IL-8 secretion in culture supernatants of ARPE-19 cells, CTRL clone and SOCS1 clone was analysed by ELISA (HU IL-8 Cytoset, Invitrogen) following manufacturer's instructions. Cells were seeded at 2.5 x10^5 cells/well/ml and stimulated for 24 hours with IFNγ (150 ng/ml) or TNFα (30 ng/ml) or IL-17 (100 ng/ml).

Flow cytometry

CTRL clone and SOCS1 clone were plated at 5x10^5 cells/well/2ml in complete medium and left overnight for adhesion. Then, the complete medium was changed and the cells were stimulated for another 48h with IFNγ (150 ng/ml) or TNFα (30 ng/ml) or IL-17 (100 ng/ml). The cells were then recovered and washed by Phosphate Buffered Saline (PBS) containing sodium azide 0.1% and diluted in 100µL of PBS-azide 0.1%. The expression of MHCII (MCHII BD mouse anti-human HLA-DR PE-conjugated) and CD54 (ICAM-1)(mouse anti-human BD CD54 PE-conjugated) molecules was quantified by flow cytometry using mouse anti-human specific antibodies comparatively to control isotypes (isotype control mouse IgG2a PE κ, BD). Labelled cells were analysed with a FACS Calibur cytometer and the Cell Quest Software (Becton-Dickinson). CTRL clone and SOCS1 clone were also controlled for the presence of FL1-H signal coming from Aequorea coerulescenes green fluorescent protein (AcGFP1).

Statistical analysis

The statistical significance between samples was established using the unpaired t test with Welch's correction or Kruskal-Wallis test when 3 independent groups where compared. Data are expressed as means +/- SEM.

RESULTS

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SOCS1 mRNA is constitutively expressed in SOCS1 clone.

We first tested the levels of SOCS1 mRNA in ARPE-19 cells, CTRL clone and SOCS1 clone using qRT-PCR. As shown in *Fig 1A*, SOCS1 mRNA levels were nearly undetectable in unstimulated ARPE-19 cells and in CTRL clone. In contrast, SOCS1 clone expressed constitutively large amounts of SOCS1 mRNA, significantly higher than in CTRL clone or in ARPE-19 cells. Similar high and dose-related expression of SOCS1 mRNA can be obtained in ARPE-19 cells by INFɣ stimulation. These data first confirmed that SOCS1 mRNA overexpression was achieved in SOCS1 clone as compared to CTRL clone and second, defined the experimental conditions for an inducible expression of SOCS1 in ARPE-19 cells. Those clones and culture conditions were thus used for further experiments.

SOCS1 mRNA overexpression in RPE cells prevents IFNγ-induced SOCS1 mRNA increase.

We thereafter investigated the influence of IFNγ stimulation on SOCS1 mRNA levels in CTRL and SOCS1 clones comparatively to ARPE-19 cells. The data from *Fig 1B* show an IFNγ-induced increase of SOCS1 mRNA levels in CTRL clone as in ARPE-19 cells, but not in the SOCS1 clone where the constitutive overexpression of SOCS1 prevented this inducing effect.

SOCS1 overexpression prevents IFNγ-mediated STAT1 phosphorylation.

Next, we investigated the effect of SOCS1 stable overexpression on IFNγ-mediated intracellular signal transduction. One of the first steps of IFNγ signal transduction is STAT1 phosphorylation. We therefore tested by Western Blot the presence of phosphorylated STAT1 (P-STAT1) in ARPE-19 cells, CTRL clone and SOCS1 clone stimulated or not by IFNγ. As shown in *Fig 2A*, in absence of IFNγ stimulation, P-STAT1 was undetectable, neither in ARPE-19 cells nor in CTRL clone or in SOCS1 clone. After 5 and 30 minutes of stimulation with IFNγ, P-STAT1 was detected in ARPE-19 cells, in CTRL clone and in SOCS1 clone but here at a largely lesser extent. These data thus demonstrate that SOCS1 stable overexpression prevents STAT1 phosphorylation induced by IFNγ stimulation.

SOCS1 overexpression in RPE cells inhibits IFNγ-induced decrease of IL-8 secretion.

IL-8 production in RPE cells was shown to play an important role in different inflammatory retinal diseases. We thus measured by ELISA the level of IL-8 secretion in CTRL clone and SOCS1

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clone. As shown in *Fig 2B*, IL-8 secretion was strongly inhibited by IFNγ stimulation in CTRL clone but unchanged in SOCS1 clone.

SOCS1 overexpression in RPE cells prevents IFNγ-induced MHC II and ICAM-1 (CD54) expression.

In addition to IL-8 production, MHCII and ICAM-1 (CD54) membrane expression are classically used as markers of RPE cell activation. We therefore assessed by flow cytometry the effect of the SOCS1 overexpression on the expression of ICAM-1 (CD54) and MHC II molecules. As illustrated in *Fig 2C*, CTRL clone and SOCS1 clone expressed constitutively the same level of ICAM-1 (CD54) but no MHC II. IFNγ stimulation increased CD54 expression and induced MHCII expression in CTRL clone but not in SOCS1 clone. Isotype control was used showing no labeling.

SOCS1 overexpression does not affect TNFα-induced IκBα degradation.

TNFα is another important cytokine involved in RPE cell activation during inflammatory retinal disease and has been shown to be modulated by SOCS family members [3,6]. We thus investigated if TNFα-mediated response may be affected by SOCS1 overexpression. TNFα stimulation leads to the phosphorylation and degradation of IκBα in cytoplasm which allows the release of the transcription factor NFκB and its translocation to the nucleus. We therefore tested by Western Blot the expression of IκBα in ARPE-19 cells, CTRL clone and SOCS1 clone either unstimulated or after TNFα stimulation. The data from *Fig 3A* show that IκBα was similarly degraded in TNFα-stimulated ARPE-19 cells, CTRL clone and SOCS1 clone.

SOCS1 overexpression in RPE cells does not block TNFα-induced IL-8 secretion.

Results from *Fig 3B* show that, as expected, stimulation with TNFα increased very strongly the basal secretion of IL-8 in CTRL clone and SOCS1 clone. Thus, the stable SOCS1 overexpression did not prevent the TNFα-induced IL-8 secretion. However, simultaneous stimulation with TNFα and IFNγ inhibited the TNFα-induced IL-8 secretion in CTRL clone but not in SOCS1 clone.

SOCS1 overexpression does not block IL-17 induced IL-8 secretion.

IL-8 secretion was significantly increased by IL-17 stimulation in ARPE-19 cells, CTRL clone and SOCS1 clone. As shown in *Fig 3C,* SOCS1 overexpression does not prevent the induction of IL-8 secretion by IL-17 stimulation in RPE cells. Moreover, the fold increase of IL-8 secretion was significantly greater in SOCS1 clone than in CTRL clone or ARPE-19 cells ($p=0{,}004$).

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DISCUSSION

During noninfectious uveitis, retinal pigment epithelium (RPE) cells undergo multiple stimulation by different cytokines secreted by autoreactive lymphocytes. Among all of them, IFNγ, IL-17 and TNFα appear to play a central role in RPE activation, a required step for inflammatory cell entrance into the eye and uveitis development.

The actual treatment of noninfectious uveitis in humans is based on the use of nonspecific immunosuppressive drugs that globally block the immune system [9]. More recently, with the development of biological drugs, a more specific approach has been proposed, and different cytokines can now be directly targeted in the whole organism [9]. A further step could be to act more locally in the eye, or even, more specifically by limiting the effect of the cytokine in cells involved in disease development, here the retina [10]. In this context, earlier works have explored the possibility to specifically block the NF-κB signaling pathway [11]. In this work, we have investigated if the IFNγ pathway could be similarly targeted by using SOCS1, a classical inhibitor of IFNγ signaling.

Our data first demonstrate how SOCS1 overexpression in RPE cells affects their response to proinflammatory cytokines involved in uveitis. We show that IFNγ stimulation in RPE cells increases SOCS1 mRNA expression, which is prevented by SOCS1 mRNA overexpression in RPE cells. SOCS1 overexpression also prevents the IFNγ-mediated STAT1 phosphorylation. These data are consistent with experiments on HeLa and MCF-7 cell lines showing that SOCS1 and SOCS3 are inhibitors of IFN-mediated Janus-activated kinase/STAT signaling pathways [5].

Furthermore, we demonstrate for the first time that SOCS1 overexpression in RPE cells inhibits the IFNγ-induced decrease of IL-8 secretion. Our results confirm the baseline secretion of IL-8 that has been described by other groups in human adult RPE cells as well as in the ARPE-19 cell line. Our data showing that IFNγ stimulation of RPE cells induces a decrease of IL-8 secretion are compatible with the described IFNγ-induced decrease of IL-8 gene expression in ARPE-19 [12]. However, in other cell types, IFNγ stimulation can have different effect on IL-8 secretion: either a decrease in IL-8 secretion like in endothelial cells [13], or no effect like in human bronchial epithelial cells or even an increase in IL-8 gene expression like in human monocytic cells. Such a variety of IL-8 responses to IFNγ stimulation in different cell types may explain the discrepancy

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between in vitro experiments and clinical data. On one hand, it has been shown that the IL-8 level increases in serum of patients with Bechet disease as well as in serum and intraocular fluid of patients with intermediate uveitis [14], but on the other hand, patients with autoimmune noninfectious uveitis presented a 3.4 fold decrease in IL-8 gene expression.

We also found that SOCS1 overexpression in RPE cells prevents the IFNγ-induced MHC II and ICAM-1/ CD54 expression. Such an IFNγ-induced MHC II and ICAM-1/CD54 expression was also observed in human bronchial epithelial cells [15]. Both MHC II and ICAM-1/CD54 expression play an important role in the recruitment and activation of immunocompetent cells. ICAM-1/CD54 expression was shown to play a crucial role in passing leukocytes through the blood-retinal barrier during EAU and ICAM-1/CD54 expression was increased on human RPE cells in the presence of TNFα, IFNγ, and IL-1β. A high expression of MHC class II has been demonstrated to be required for uveitogenic lymphocytes activation [16].

Current evidence suggests that SOCS1 blocks in vivo multiple cytokine-mediated diseases. For example, SOCS1 was shown to ameliorate bleomycin-induced pulmonary inflammation, pulmonary fibrosis and mortality, mainly through its suppressive effect on TNFα secretion [7]. Furthermore, the group of Egwuagu demonstrated in mice that the forced transgenic retinal expression of SOCS1 protects them from developing severe experimental autoimmune uveitis [10]. Their model mimics a human non-infectious uveitis and implicates the activation of RPE cells by multiple cytokines including IL-17 and TNFα. However, in our study, we observe that SOCS1 overexpression in ARPE-19 cells does not affect the TNFα-induced IκBα degradation. Moreover, our results indicate that the SOCS1 overexpression in RPE cells does not block the TNFα-induced IL-8 secretion and neutralizes the inhibitory effect of IFNγ stimulation on the TNFα-induced IL-8 secretion.

We have next investigated a potential role of SOCS1 overexpression on IL-17 mediated RPE cell activation. The literature data concerning the role of IL-17 on activation of retinal pigment epithelium are inconsistent. One group showed that IL-17 significantly enhanced chemokine and IL-6 production [17], whereas another group has shown that RPE respond to IL-17 by increasing their levels of SOCS1 and SOCS3 proteins resulting in a limited production of proinflammatory cytokines and chemokines and an increased amount of suppressive cytokines, such as LIF [18].

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Altogether, these data suggest a potential role of SOCS1 in modulating IL-17 activation of RPE cells.

However, unexpectedly, we could not find evidence in our study that SOCS1 overexpression in RPE cells affects negatively their response to IL-17 stimulation. The present data demonstrate that IL-17 stimulation increases the IL-8 secretion in RPE cells which is consistent with the described IL-17-induced IL-8 secretion in adherent cells like fibroblasts, keratinocytes, epithelial and endothelial cells [19]. Moreover, we demonstrate that SOCS1 overexpression does actually not block this IL-17 induced IL-8 secretion but even maybe increases it. This is however surprising considering that in SOCS-Tg rat and mice, stimulation of retinal cells by IL-17 decreases levels of IL1b and RANTES in comparison to wild types littermates [10].

CONCLUSIONS

In conclusion, our results indicate that SOCS1 overexpression in RPE cells inhibits some IFNγ-mediated responses that lead to uveitis development. This notion raises the possibility that SOCS1 overexpression could be a novel target for treating non-infectious uveitis. However, some proinflammatory effects of TNFα and IL-17 stimulation in RPE are not blocked by SOCS1 overexpression.

The authors declare no financial conflicts of interests.

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FIGURE LEGENDS

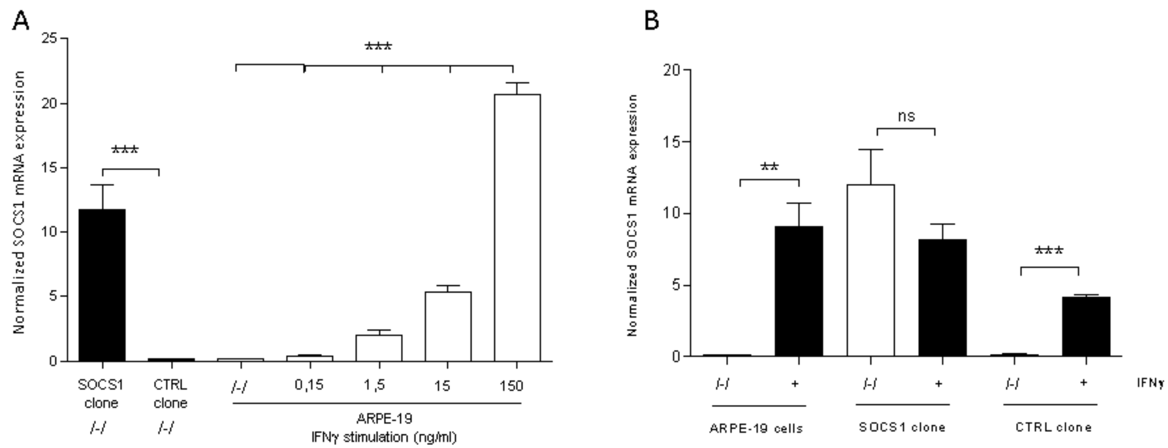

Fig 1. SOCS1 mRNA expression. SOCS1 mRNA overexpression in RPE cells prevents IFNγ-induced SOCS1mRNA increase. ARPE-19 cells, SOCS1 clone or CTRL clone were seeded at 5 × 105 cells/well in 6-well plates. One day later, IFNγ was added to the cultures for 24 hours, (A) IFNγ (at 150 or 15 or 1.5 or 0.15 ng/ml or none /-/) was added only to the ARPE-19 cell cultures. (B) IFNγ (at 150 ng/ml). Cells were collected for quantitative reverse transcription PCR

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analysis of SOCS1 gene expression. /-/ unstimulated, /+/ IFNγ stimulated. Experiment was performed (A) three times in duplicates, n=3 (B) three times in triplicates, n=3. ***$p < 0.001$; **$p < 0.005$; ns: not significant (compared with unstimulated condition), mean ± SEM .

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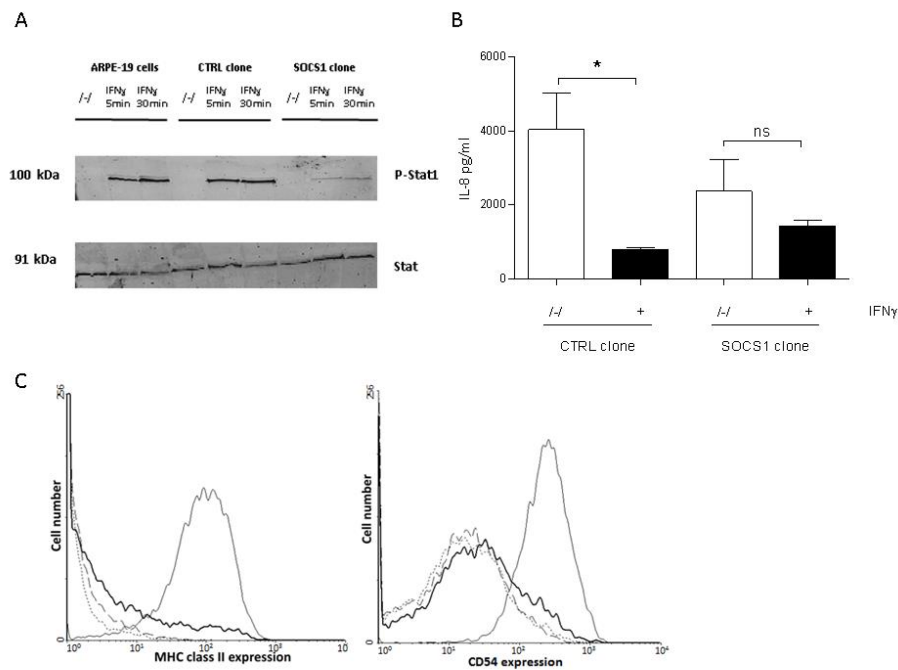

Fig 2. SOCS1 overexpression in RPE cells affects IFNγ-induced responses. (A) SOCS1 overexpression prevents IFNγ-mediated STAT 1 phosphorylation. ARPE-19 cells, SOCS1 clone and CTRL clone cultures were stimulated with IFNγ (at 150ng/ml). STAT1 phosphorylation and total STAT expression were analysed by Western Blot, 5 and 30 minutes after stimulation. /-/ unstimulated. Data are from one representative experiment out of three, n=3. ***(B) SOCS1 overexpression in RPE cells inhibits IFNγ-induced decrease of IL-8 secretion.*** SOCS1 clone and CTRL clone were seeded at 2.5 x10^5 cells/well/ml and stimulated for 24 hours with IFNγ (at 150 ng/ml). IL-8 secretion in culture supernatants was analysed by ELISA. /-/ unstimulated. Experiment was performed three times in triplicates, n=3. *p=0.011, ns: not significant, mean ± SEM. ***(C) SOCS1 overexpression in RPE cells prevents IFNγ-induced MHC II and CD54 expression.*** CTRL clone and SOCS1 clone were plated at 5×10^5 cells/well in 6-well plates. One day later, IFNγ (at 150 ng/ml or none /-/) was added to the SOCS1 clone and CTRL clone cell cultures for 48 hours. Membrane expression of CD54 and MCHII molecules was monitored by flow cytometry. Data are representative of three independent experiments, n=3. **–** (bold) SOCS1 clone stimulated with IFNγ; - CTRL clone stimulated with IFNγ ; … CTRL clone unstimulated; .-.-. SOCS1 clone unstimulated.

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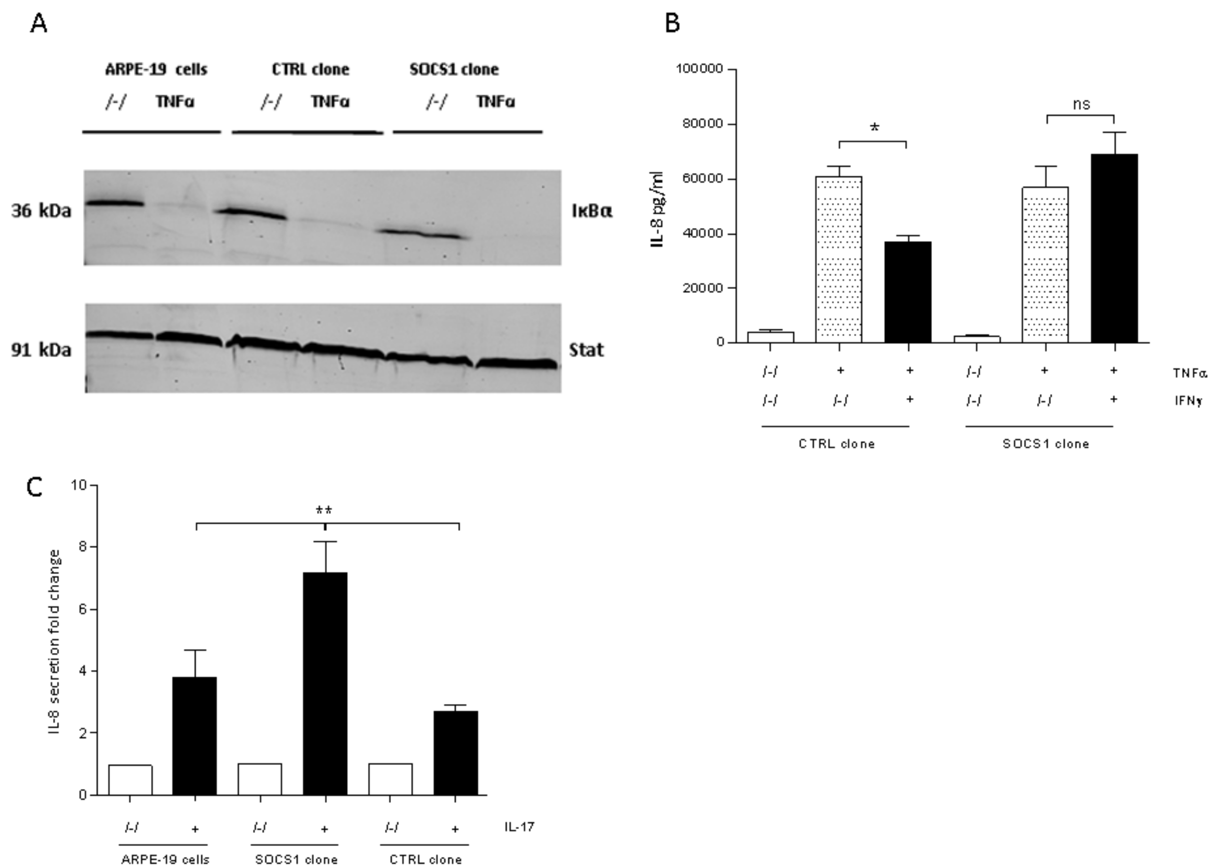

Fig 3. SOCS1 overexpression in RPE cells does not affect TNFα- nor IL-17-induced responses. (A) SOCS1 overexpression does not affect TNFα-induced IκBα degradation. ARPE-19 cells, SOCS1 clone and CTRL clone cultures were stimulated with *TNFα* (at 30 ng/ml). IκBα expression was analysed by the Western Blot 30 minutes after stimulation. /-/ unstimulated. Data are representative of three independent experiments, n=3. ***(B) SOCS1 overexpression in RPE cells does not block TNFα-induced IL-8 secretion.*** SOCS1 clone and CTRL clone were seeded at 2.5 $x10^5$ cells/well/ml and stimulated for 24 hours with TNFα (at 30 ng/ml) or TNFα (at 30 ng/ml) and IFNγ (at 150 ng/ml). IL-8 secretion in culture supernatants was analysed by ELISA. /-/ unstimulated. Data are representative of three independent experiments, n=3. * p=0.012, ns: not significant, mean ± SEM. ***(C) SOCS1 overexpression does not block IL-17-induced IL-8 secretion.*** ARPE-19 cells, SOCS1 clone and CTRL clone were seeded at 2.5 $x10^5$ cells/well/ml and stimulated for 24 hours with IL-17 (at 100 ng/ml). IL-8 secretion in culture supernatants was

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analysed by ELISA. /-/ unstimulated. Experiment was performed three times in triplicates, n=3. **p = 0.004 (IL-17 stimulation compared with unstimulated condition for ARPE-19 cells, SOCS1 clone, clone CTRL respectively), mean ± SEM .