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IFNλ stimulates MxA production in human dermal fibroblasts via a MAPK-dependent STAT1-independent mechanism

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Abbreviations

GBP-1, guanulate binding protein 1; IFN, interferon; MxA, myxovirus protein A; MAPK, mitogen activated protein kinase; STAT1, signal transducer and activator of transcription.

Abstract

Interferon lambda (IFNλ) is important for epidermal defence against viruses. It is produced by, and acts on, keratinocytes, whereas fibroblasts were previously considered to be unresponsive to this type III IFN.

Herein we report novel findings revealing cell type-specific differences in IFNλ signalling and function in skin resident cells. In dermal fibroblasts, IFNλ induces the expression of MxA, a potent antiviral factor, but not other interferon signature genes as it does in primary keratinocytes. In contrast to its effect on keratinocytes, IFNλ does not phosphorylate STAT1 in fibroblasts, but instead activates MAPK. Accordingly, inhibition of MAPK activation (p38 and p42/44) blocks expression of MxA protein in fibroblasts but not in keratinocytes. Functionally, IFNλ inhibited proliferation in keratinocytes but not in fibroblasts. Moreover, IFNλ upregulated the expression of TGFβ1-induced collagens in fibroblasts. Taken together, our findings identify primary human dermal fibroblasts as responder cells to IFNλ. Our study shows cutaneous cell type-specific IFN signalling and suggests that IFNλ, whilst important for epidermal anti-viral competence, may also have a regulatory role in the dermal compartment balancing type I IFN-induced inhibition of tissue repair processes.

Introduction

Fibroblasts and keratinocytes are the main tissue cell types found in the dermis and epidermis respectively. While many recent studies have identified the immunological role of keratinocytes, the role of dermal fibroblasts in host defence and inflammatory diseases are only beginning to emerge. Furthermore, dermal fibroblasts synthesise extracellular matrix including collagens (Xu *et al.*, 2009) and thus play an important role in maintaining skin structure and wound healing. Interferon lambda (IFNλ) is a type III IFN comprising four members, IFNλ1 (IL-29), IFNλ2 (IL-28A), IFNλ3 (IL-28B) and IFNλ4 (Kotenko *et al.*, 2003; Prokunina-Olsson *et al.*, 2013; Sheppard *et al.*, 2003). IFNλ belongs to the IL-10 family of cytokines along with IL-10, IL-19, IL-20, IL-22, IL-24 and IL-26. IFNλ uses the heterologous receptor complex comprising IFNλR1 (also known as IL-28Rα1) and the ubiquitously expressed IL-10 receptor 2 (IL-10Rβ) which is also used by IL-10, IL-22 and IL-26 as a second receptor subunit (Kotenko *et al.*, 2003; Sheppard *et al.*, 2003). IFNλR1 is the signalling subunit and so its presence determines cellular responsiveness. Although IFNλ is structurally related to IL-10, it resembles type I IFNs in its antiviral and antiproliferative functions (Miknis *et al.*, 2010). They both signal mainly through the recruitment and activation of members of the janus family of kinases, JAK1/TYK2 leading to the phosphorylation of signal transducer and activator of transcription (STAT) 1/2 and subsequently to induction of a set of downstream genes known as IFN-stimulated genes (ISGs) such as Myxovirus protein A (MxA), Guanylate binding protein 1 (GBP-1), 2,5-oligoadenylate synthetase (2,5-OAS) and IFN inducible protein 16 (IFI16) with potent antiviral activities (Ank *et al.*, 2008; Sommereyns *et al.*, 2008). Beyond JAK/STAT activation, type I IFNs have also been found to activate MAPK (p38 and ERK (p42/p44) MAPK) which allows for full activation of downstream ISGs (Nguyen *et al.*, 2000; Uddin *et al.*, 2000); and possibly downstream phosphorylation of STAT1 on serine 727 (Goh *et al.*, 1999).

Type I IFNs signal through membrane associated IFNAR1 and IFNAR2 (Lutfalla *et al.*, 1995). While the two subunits of IFNAR are present on all nucleated cell types, IFNλR1 is selectively expressed and functional in certain cell types only (Dickensheets *et al.*, 2013;

Kotenko *et al.*, 2003; Witte *et al.*, 2009; Zahn *et al.*, 2010). Witte et al. described the presence of IL-28Rα1 mRNA expression in human dermal fibroblasts. However, it was observed that these cells were unresponsive to IFNλ, and this was attributed to the very low expression level of the receptor in these cells (Lasfar *et al.*, 2011; Witte *et al.*, 2009).

IFNλ is the main IFN produced by keratinocytes and production of varying amounts of this cytokine has been reported also in other cell types (Wolk *et al.*, 2013; Yin *et al.*, 2012; Zahn *et al.*, 2010). Keratinocytes are susceptible to IFNλ1 and this cytokine substantially contributes to the antiviral competence of the human epidermis (Wolk *et al.*, 2013). Apart from antiviral properties, both types I and III IFNs are known for their pro-apoptotic and anti-proliferative activities (Abushahba *et al.*, 2010; Maher *et al.*, 2008; Steen and Gamero, 2010). These activities have been linked to the ability of IFNs to phosphorylate STAT1 (Zitzmann *et al.*, 2006). High expression of ISGs such as CXCL9, CXCL10, MxA and GBP-1 has been reported in interface dermatitis conditions such as cutaneous lupus erythematosus (CLE) and here increased expression is linked with disease severity (Naschberger *et al.*, 2010; Wenzel *et al.*, 2005).

In this study, we sought to understand if human dermal fibroblasts are responsive to IFNλ1 through the activation of STAT1 and subsequent downstream induction of ISGs. We also wanted to understand the role of this cytokine in maintenance of skin integrity with regard to collagen expression and proliferation of skin resident cells.

Results

IFNλR1 is expressed by human dermal fibroblasts

In order to confirm the expression of IFNλR1 in fibroblasts and to investigate its regulation, fibroblasts were stimulated for 4 or 16 hours for analysis of mRNA and protein expression, respectively. mRNA expression was measured by qPCR and protein expression was measured by Western blotting and flow cytometry. We found that IFNλR1 was expressed in dermal fibroblasts and was upregulated in the presence of IFNα (Figure 1a-e and S1).

IFNλ induces significant expression of MxA in human dermal fibroblasts

To investigate if IFNλR1 is functional in human dermal fibroblasts, cells were treated with rhIFNλ or rhIFNα (positive control) for 4 or 24 hours. mRNA expression of ISGs was quantified using qRT-PCR. We found significant expression of MxA but not GBP-1 (Figure 2a-d) or OAS2 (Figure S2) after 4 and 24 hours of IFNλ1 treatment. As expected, when compared to IFNλ1, IFNα induced significantly higher expression of MxA and GBP-1 after 4 hours of treatment (Figure 2a-d). However, while the expression of MxA remained high after 24 hours of treatment with IFNα, GBP-1 expression dropped significantly over this time period (Figure 2b/d). In fibroblasts, IFNα appeared to induce higher upregulation of MxA as compared to GBP-1 (Figure 2a-d). Dose-dependent effect of IFNλ1 on MxA expression in dermal fibroblasts was also carried out after 4 hours of treatment with different concentrations of IFNλ1 (Figure S3).

IFNα and IFNλ induce ISG expression in human primary keratinocytes

We compared our findings on ISGs expression in fibroblasts with the expression pattern in human primary keratinocytes. The responsiveness of keratinocytes to both type I and III IFNs is well documented (Bachmann *et al.*, 2013; Zahn *et al.*, 2011; Zahn *et al.*, 2010). We found that both IFNα and IFNλ1 induced significant mRNA expression of MxA, OAS2 and GBP-1 with a higher induction of MxA as compared to OAS2 and GBP-1 (Figure 2e-h and

Figure S2). Interestingly, we observed a different time kinetic for the ISG response to IFNα as compared to IFNλ1. IFNα showed a strong upregulation of MxA and GBP-1 at 6 hours, while IFNλ1 stimulation resulted in a strong increase in MxA and GBP-1 expression between 6 and 24 hours. This suggests that IFNλ1 stimulation may result in more delayed and/or longer lasting effects (Figure 2e-h) as previously observed in hepatocytes (Bolen *et al.*, 2014).

IFNλ activates p38 and ERK MAPKs but not STAT1 (Tyr701) in human dermal fibroblasts

Having established that dermal fibroblasts respond to IFNλ1, we were interested in understanding their mechanism of action. To achieve this, dermal fibroblasts were treated with either IFNλ1 or IFNα for different time points and protein phosphorylation was determined using western blotting. As expected, IFNα-induced STAT1 phosphorylation with maximal phosphorylation detected after 30 minutes of stimulation (Figure 3a). However, we failed to observe any STAT1 phosphorylation in IFNλ1 treated cells in any of the time points investigated. Various groups have reported that phosphorylation of p38 may be necessary for full activation of STAT1 dependent genes (Goh *et al.*, 1999; Platanias, 2003; Uddin *et al.*, 2000). We therefore investigated the ability of IFNλ1 to induce the phosphorylation of p38 in dermal fibroblasts and clearly found activation of this MAPK (Figure 3a). We confirmed that IFNα treatment resulted in p38 phosphorylation in addition to STAT1 phosphorylation (Figure 3a). Furthermore, we found that both IFNα and IFNλ1 induced phosphorylation of ERK, but not the Akt (p60) (data not shown) pathway in dermal fibroblasts. . As expected, we found that both IFNα and IFNλ1 induced STAT1 phosphorylation in primary keratinocytes (Figure 3b). However, we did not observe clear IFNλ dependent activation of either the p38 or ERK pathway upon treatment with IFNs (Figure 3b).

P38 and ERK inhibitors attenuate IFNλ1-induced MxA protein expression

We sought to understand if the activation of P38 and ERK was responsible for the induction of MxA expression in dermal fibroblasts. To investigate this, fibroblasts were pretreated with p38 (10 μM SB203580) or ERK inhibitor (30 μM PD98059 or 10 μM U0126) for 1 hour before treatment with IFNλ1 or IFNα. mRNA and protein expression were measured by qRT-PCR and Western blotting respectively. Interestingly, our results showed that the expression of MxA was not regulated at the mRNA level by the MAPK inhibitors (Figure 4a/b). However, the presence of either the p38 or ERK inhibitor abrogated IFNλ1-induced MxA protein production (Figure 4c). We did not observe a decrease in IFNα-induced MxA protein production in fibroblasts in the presence of SB203580, PD98059 (not shown) or U0126 (Figure 4d). We also analysed whether MAPKs played a role in IFNλ1-induced MxA protein expression in human primary keratinocytes. We failed to find a significant effect of p38 or ERK inhibition on IFNλ1 and IFNα-induced MxA protein production in keratinocytes (Figure 4e).

IFNλ enhances collagen expression in human dermal fibroblasts

As conditions with high type I IFN expression can be linked to impaired healing responses, we investigated whether IFNλ1 had any influence on collagen expression. Fibroblasts were treated with IFNλ1, IFNα, TGFβ1 or a combination of IFN and TGFβ1. TGFβ1 was used as a control, given its well described stimulation of fibroblast proliferation and collagen synthesis. We measured *Col1A1*, *Col3A1*, *Col4A2* and *Col7A1* using qRT-PCR. There was no difference in collagen expression after 6 hours of stimulation with these cytokines (data not shown). However, after 24 hours of treatment, TGFβ1 significantly induced the expression of these collagens. Combined treatment of cells with IFNλ1 and TGFβ1 resulted in a significantly higher expression of these collagens (except for *Col3A1)* than with either treatment alone (Figure 5a-d). To investigate which of the MAPK pathways may be responsible for the synergy, we treated cultured dermal fibroblasts with either IFNλ1 or TGFβ1 or both together for 30 minutes and checked for the phosphorylation of p38 and ERK by Western blotting. We found that both IFNλ1 and TGFβ1 activated the p38 and ERK

pathways independently. Interestingly, we observed a clear synergistic effect between IFNλ1 and TGFβ1 on the activation of the p38 pathway. The effect is less clear with the ERK pathway (Figure S4). This suggests that the p38 pathway may be responsible for the synergistic effect observed on collagen expression.

IFNλ has potent anti-proliferative activity in primary keratinocytes but not in dermal fibroblasts

The anti-proliferative effect of IFNλ1 on HaCaT keratinocyte cell lines has been described (Maher *et al.*, 2008). We aimed to compare the effect of both IFNλ1 and IFNα on primary skin cells. Keratinocytes and fibroblasts were stained with cell tracer dye and subsequently stimulated with either IFNλ or IFNα for 72 hours. As expected, both IFNs clearly reduced keratinocyte proliferation (Figure 6a/b). By contrast, we observed that IFNα but not IFNλ1 inhibited dermal fibroblast proliferation (Figure 6c/d). TGFβ1, known to induce fibroblast proliferation, was used as positive control (Figure 6c/d).

We also investigated the effects of IFNλ1 and IFNα on "gap" closure in monolayer cultures of dermal fibroblasts. We observed that IFNλ1 improved gap closure in fibroblasts while IFNα delayed gap closure after 48 hours of stimulation in comparison to the untreated cells (Figure S5).

Discussion

Type I and III, innate IFNs were initially identified for their potent antiviral activities (MacMicking, 2012; Sadler and Williams, 2008). In addition, IFNs also have immunoregulatory, anti-proliferative and pro-apoptotic properties; they have been identified as potential anticancer drugs (Abushahba *et al.*, 2010; Witte *et al.*, 2010). However, they are also important mediators in the pathogenesis of autoimmune diseases such as cutaneous lupus erythematosus (Meyer, 2009; Ronnblom and Eloranta, 2013). MxA is a highly inducible ISG and one of the most potent anti-viral factors capable of blocking early replication events of different RNA and DNA viruses (Sadler and Williams, 2008).

Type III IFN is the most recently described group of IFNs. Expression of their specific receptor was thought to be restricted mainly to epithelial cells (keratinocytes) and hepatocytes. There is, however, emerging evidence that IFNλR1 is also present and functional on hematopoietic cells (Dai *et al.*, 2009; Liu *et al.*, 2011; Mennechet and Uze, 2006; Yin *et al.*, 2012). There are reports identifying MAPKs as regulators of IFN downstream activities (Gough *et al.*, 2008; Uddin *et al.*, 2000; van Boxel-Dezaire *et al.*, 2006) and the importance of different (non JAK) kinases activated in response to IFNs has been speculated before to contribute to cell-type and IFN subtype specific responses (van Boxel-Dezaire *et al.*, 2006). The main novelty of our findings is that we show a distinct signalling and thus also functional response of primary human fibroblasts to IFNλ which is different from the response of these cells to type I IFN and which is clearly different from the response of epidermal cells. Indeed, keratinocytes show a similar signalling and functional response to both type I and type III IFNs which is characterised by a STAT1-dependent upregulation of a broad range of ISGs. Dermal fibroblasts were considered unresponsive to IFNλ (Abushahba *et al.*, 2010). We here show that human fibroblasts indeed respond to IFNλ1 treatment with significant expression of MxA mRNA and protein. In line with our findings, a recent study has reported the responsiveness of CMV infected foreskin fibroblast to IFNλ3 (Egli *et al.*, 2014). It is well documented that types I and III IFNs induce similar sets of ISGs in many cell types (Ank *et al.*, 2008; Sommereyns *et al.*, 2008). However, in fibroblasts, we failed to observe significant expression of GBP-1 (Fig. 2) or other ISGs upon

IFNλ1 treatment. The inability of IFNλ1 to activate STAT1 (Tyr701) in fibroblasts was unexpected as this is the known canonical pathway for the induction of downstream ISG genes. However, our findings on IFNλ1 induced activation of both p38 and ERK suggest that the MAPK pathway may be an alternative for the induction of MxA by IFNλ in fibroblasts. It has been suggested that IFNα-induced phosphorylation of MAPKs and STAT1 are independent of each other; however, they work in tandem to ensure full activation of ISGs (Li *et al.*, 2004). It is interesting that the inhibition of p38 and ERK in IFNα treated dermal fibroblasts did not result in significant downregulation of MxA protein expression; although we observed a slight reduction in MxA production by primary keratinocytes following inhibition of ERK but not p38. Activation of PI3K-AKT and Raf-MEK-ERK pathways by IFNλ1 and IFNα in HepG2.2.15 cell lines has been reported (Chai *et al.*, 2011), but we could not observe AKT pathway activation in our experiments. Our proposed mechanism of IFNα and IFNλ1 signalling in dermal fibroblasts is shown in Figure S6. To our surprise, we failed to observe any significant regulatory effects of the MAPK inhibitors at the transcriptional level suggesting that p38 and ERK inhibition results in posttransciptional regulation of MxA. Mechanisms of mRNA translation of ISGs have been reviewed (Joshi *et al.*, 2010). Both the p38 and p42/44 MAPKs can control the activation of eIF4E and other substrates of MAPK interacting protein kinases 1 and 2 (Mnk1 and 2) which influence translation initiation (Joshi *et al.*, 2010). Posttransriptional regulation of TNFα gene expression in the presence of p38 inhibitor has been described (Clark *et al.*, 2003). Future experiments will show the underlying mechanism of the posttranscriptional regulation observed for MxA in dermal fibroblasts. In this study, we have confirmed previous report that human dermal fibroblasts express IFNλR1 (Witte *et al.*, 2009) and that IFNα can upregulate its expression. The ability of IFNα to enhance the expression levels of IFNλR1 is evidence for a cross-talk between type I and type III IFNs (Duong *et al.*, 2014).

TGFβ1 is well known to induce collagen expression in fibroblasts. Types I, III, IV and VII collagens are the main ECM components, basement membrane zone and the anchoring fibrils at the dermal-epidermal junction (Tiedtke, 2007). We found that IFNλ1 potentiated the

TGFβ-associated induction of collagen I, but not collagen type III, expression. IFNγ, a type II IFN exhibited an inhibitory effect on TGFβ1-induced ECM or collagen deposition in primary human lung (Eickelberg *et al.*, 2001) and in human foreskin fibroblasts (Ghosh *et al.*, 2001). Of interest, keloid-derived dermal fibroblasts seem non-responsive to this IFNγ-dependent type I collagen regulation (Hasegawa *et al.*, 2003). Excessive secretion of type I collagen is linked with hypertrophic scar formation, while a sufficient amount of type III collagen may prevent scar formation (Oliveira *et al.*, 2009). The net effect of IFNλ1 on cutaneous repair and healing responses is not clear yet and needs to be further investigated. The ability of IFNλ1 to upregulate the expression of type IV (*Col4A2*) and VII collagens (*Col7A1*) suggests that is may support basement membrane integrity (Nystrom *et al.*, 2013).

Our proliferation assay results in primary keratinocytes are consistent with the roles of IFNα and IFNλ1 as anti-proliferative agents. Our results show that IFNλ1 does not have an inhibitory effect on dermal fibroblasts' proliferation and this may be due to its inability to induce STAT1 phosphorylation in these cells. In addition, the anti-proliferative ability of GBP-1 on intestinal epithelial cells has been described (Capaldo *et al.*, 2012) and IFNλ1 failed to induce GBP-1 expression in dermal fibroblasts.

This study has shown that IFNλR1 is active in dermal fibroblasts through the activation of p38 and ERK pathways. We have shown that MxA induction by IFNλ1 occurs through a STAT1-independent pathway and this may also explain the role of this cytokine in enhancement of collagen expression and lack of anti-proliferative activity. Of note, IFNλ1 is highly expressed in both the scarring and the non-scarring subtype of CLE. Our data suggest that IFNλ1 may not be a key molecule in the reduced proliferation seen in dermal fibroblasts from discoid CLE patients (Nyberg *et al.*, 2000).

Egli et al have shown that in CMV treated foreskin derived fibroblast cell lines, IFNλ exerts an inhibitory action on IFNα induced antiviral activity (Egli *et al.*, 2014). However, IFNα also enhances fibroblasts susceptibitlity to IFNλ. It is thus possible that IFNλ, while important for the epidermal antiviral competence, may exert important balancing and regulatory functions

in the dermal compartment. One of the effects could be to counteract the type I IFN induced impairment of repair mechanisms.

Materials and Methods

Cytokine and antibodies

Recombinant human (rh) IFNλ1 (100 ng/ml) was purchased from ebioscience (Hatfield, UK); rhIFNα2a (10 ng/ml; Merck Millipore, Merck Serono Ltd, Middlesex, UK), rhTGFβ1 (5 ng/ml; Peprotech EC Ltd, London, UK). Anti-human (rabbit phospho p38, mouse phospho ERK, mouse ERK, rabbit p38, rabbit phospho STAT1 and rabbit STAT1) antibodies were purchased from Cell signalling Technology (Leiden, The Netherlands); anti-human (rabbit MxA, rabbit IFNλR1 and mouse GAPDH) antibodies were purchased from Abcam (Cambridge, UK) and Santa Cruz (Insight Biotechnology Ltd, Middlesex, UK) respectively. Horseradish peroxidase (HRP) conjugated donkey anti-rabbit and donkey anti-mouse secondary antibodies were from Santa Cruz. PD98059, U0126 (MEK/ERK inhibitors) and SB203580 (p38 inhibitor) were from Cell Signalling Technology.

Cell Isolation, Culture and Ethics

Keratinocytes and dermal fibroblasts were derived from anonymised healthy volunteers undergoing cosmetic surgery with informed consent, local research ethics approval (Ethical Tissue, University of Bradford). Skin samples were processed on the day of collection. Fibroblasts were isolated by placing the trypsinized dermis in a T75 culture flask; ensuring the upper layer just below the epidermis adhered firmly to the flask. 10 ml DMEM containing 10% FBS was carefully added to the flask so as not to allow the dermis to float. Fibroblasts explanted after 5 to 7 days. Full details in supplementary information.

Cell Stimulation

Keratinocytes or fibroblasts were seeded into 24-well or 6-well and allowed to grow to ~ 80% confluency. For keratinocytes, culture medium was replaced with KGM without human epidermal growth factor and hydrocortisone (KGM -/-) prior to cell treatment with cytokines. Dermal fibroblasts were starved of serum for 24 hour before treatment in serum-free DMEM. For inhibition experiments, cells were pre-treated for 1 hour with 10 μM SB203580 (Cell

signalling), 30 μM PD98059 or 10 μM U0126 (Cell signalling) before treatment with IFNα or IFNλ1. Controls included untreated cells and cells treated with dimethylsulphoxide vehicle (DMSO), SB203580 or PD98059/U0126 alone for 24 hours.

Quantitative RT-PCR

MxA, *GBP-1*, *OAS2*, *IFI-16*, *CXCL9*, *IFNλR1*, *Col1A1*, *Col3A1*, *Col4A2* and *Col7A1* QuantiTect primer assays were obtained from Qiagen (Hilden, Germany) while U6 primer (forward – 5' CTCGCTTCGGCAGCACA 3'; reverse-5' AACGCTTCACGAATTTGC 3') was purchased from Sigma Aldrich, UK. The following parameters were used: Initial heat activation, 95°C for 5 minutes; denaturation, 95°C for 10 seconds; combined annealing and elongation, 60°C for 30 seconds for a 40 cycle run. Data were analysed using the delta-delta ct method. mRNA expression of each gene of interest was normalised to U6 SnRNA housekeeping gene.

Agarose gel electrophoresis

qPCR products were run at 40 mA for 30 minutes on 2% agarose gel. Image was taken under ultraviolet (UV) light. Full details in supplementary information.

Western blotting

Cells were lysed with CelLytic M lysis buffer (Sigma-Aldrich) containing protease inhibitor cocktail (Roche Applied Bioscience, Rotkreuz, Switzerland) and phosphatase inhibitor (Thermo Scientific). 30 μg of total protein was separated on any kDa mini protean gel (BioRad) and proteins were blotted onto 0.2 μm PVDF trans-blot pack (Bio-Rad). Membranes were probed with rabbit anti-human pSTAT1 (1:1000), STAT1 (1:1000), phospho p38 (1:1000), mouse anti-human pERK (1:2000), p38 (1:1000) or GAPDH (1:3000) in TBST containing 5% bovine serum albumin (BSA) overnight at 4°C. Rabbit anti-human MxA (1:1000), IFNλR1 (1:1000) and GAPDH were used in 5% milk PBST overnight at 4°C. Donkey anti-rabbit and donkey anti-mouse HRP-conjugated secondary antibodies were used

at 1:5000 and 1:3000 respectively for 1 hour at room temperature. For a repeat Western, membranes were stripped, blocked and re-probed with primary antibody.

Cell trace proliferation assay

Human primary keratinocytes and dermal fibroblasts were stained in KGM or serum-free DMEM respectively with cell trace violet (Molecular probes/Life technologies) away from the source of light (2 µl cell trace to 1 million cells in 1 ml media). Cells were plated in 6 well plates and were either treated with cytokines or untreated for 72 hours. Some cells were used for the day 0 analysis. For flow cytometric analysis, a BD LSRFortessa (BD Bioscience, Oxford, UK) machine was used. Cell proliferation was calculated using ModFit software version 3.2 (Verity Software House, Topsham, ME. USA). Full details in supplementary information.

Statistical analysis

Statistical significance was determined using Mann Whitney test or unpaired *t*-test. Analysis was performed using GraphPad Prism software (GraphPad Software Inc. La Jolla, CA, USA). All data are expressed as means ± SEM and values of $p < 0.05$ were considered significant. 'n' represents independent experiments; *p< 0.05, **p< 0.01.

Conflict of Interest

Authors state no conflict of interest

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Figure legends

1. **IFNλR1 expression and production in human dermal fibroblasts**

   Cultured human dermal fibroblasts were either untreated or treated with 10 ng/ml IFNα for 4 or 16 hours in serum-free medium for mRNA and protein expression respectively. mRNA expression was measured using qPCR and protein by Western blotting and flow cytometry. (a) qPCR products for IFNλR1 and the housekeeping gene U6snRNA on 2% agarose gel are shown as a representative experiment out of five independent experiments (b) Relative mRNA expression; n=5 (c) Western blot analysis of IFNλR1 protein (d) protein band intensity; n=3 (e) flow cytometry. For statistical analysis, Mann Whitney U test was used to compare columns. Values represent mean ± SEM. *p<0.005, **p<0.01. UT: Untreated; K: keratinocytes.

2. **ISGs expression in human skin resident cells**

   Cultured dermal fibroblasts were either untreated or treated with 10 ng/ml IFNα or 100 ng/ml IFNλ1 for 4 or 24 hours in serum-free medium. Primary keratinocytes were treated with the same concentration of cytokines for 6 or 24 hours in KGM (-/-). mRNA expression of ISGs was measured using qRT-PCR. a to d show expression levels in fibroblasts (a) MxA, 4 hours (b) MxA, 24 hours (c) GBP-1, 4 hours (d) GBP-1, 24 hours . e to h show expression levels in keratinocytes (e) MxA, 6 hours (f) MxA, 24 hours (g) GBP-1, 6 hours (h) GBP-1, 24 hours. For statistical analysis, Mann

Whitney U test was used to compare columns. n=5, values represent mean ± SEM. *p<0.005, **p<0.01.

3. IFNλ1 induced STAT1 and MAPKs phosphorylation fibroblasts and keratinocytes

Cultured human dermal fibroblasts (3a) or primary keratinocytes (3b) were either non treated or treated with 10 ng/ml IFNα or 100 ng/ml IFNλ1 for 10, 30 or 60 minutes. Total and phosphorylated p38, ERK and Stat1 along with GAPDH were detected by Western Blot. A representative out of three independent experiments with cells from different donors is depicted. UT: untreated.

4. Inhibition of MxA production by phospho- p38 and -ERK inhibitors

Cultured dermal fibroblasts or primary keratinocytes were either untreated or treated with MAPK inhibitors or IFNs for 4 and 24 hours and MxA was detected on the mRNA level and by Western blot (24 hours only). (a) MxA mRNA expression, 4 hours; n=3 (b) MxA mRNA expression, 24 hours; n=3 (c) MxA protein expression by IFNλ1 in the presence or absence of MAPK inhibitors in fibroblasts (d) MxA protein expression by IFNα in the presence or absence of MAPK inhibitors in fibroblasts (e) MxA protein expression by IFNλ1 and IFNα in human primary keratinocytes in the presence or absence of MAPK inhibitors. Figures are representative of three independent experiments. SB: SB203580 (p38 inhibitor); U: U0126 (MEK/ERK inhibitors).

5. Effect of IFNλ of collagen expression in human dermal fibroblasts

Cultured dermal fibroblasts were either untreated or treated with 10 ng/ml IFNα, 100 ng/ml IFNλ1, 5 ng/ml TGFβ1 or combination of IFNλ1 and TGFβ1 for 24 hours. mRNA expression of ISGs was measured using qRT-PCR. Depicted is *Col1A1* (a) *Col3A1* (b) *Col4A2* (c) and *Col7A1* (d) expression. For statistical analysis, Mann

Whitney U test was used to compare columns. n=5, values represent mean± SEM. *p<0.005, **p<0.01.

6. Effects of IFNs on proliferation of skin resident cells

Human dermal fibroblasts or primary keratinocytes labelled with cell tracer violet in DMEM containing 1% FBS and KGM respectively. Plated cells were either untreated or treated with cytokines. Daughter cell populations (different colours) of dividing cells were visualised by flow cytometric analysis after 72 hours of stimulation. (a) Cell tracer dependent fluorescent signal in keratinocytes at 0 hour and after 72 hours of stimulation. (b) Percentage proliferation of keratinocytes after 72 hours (c) cell tracer dependent fluorescent signal in fibroblasts at 0 hour and 72 hours after stimulation. (d) Percentage proliferation of fibroblasts after 72 hours. For statistical analysis, unpaired *t* test was used to compare columns; n=3 and values represent mean± SEM. *p<0.005, **p<0.01.