
Article:
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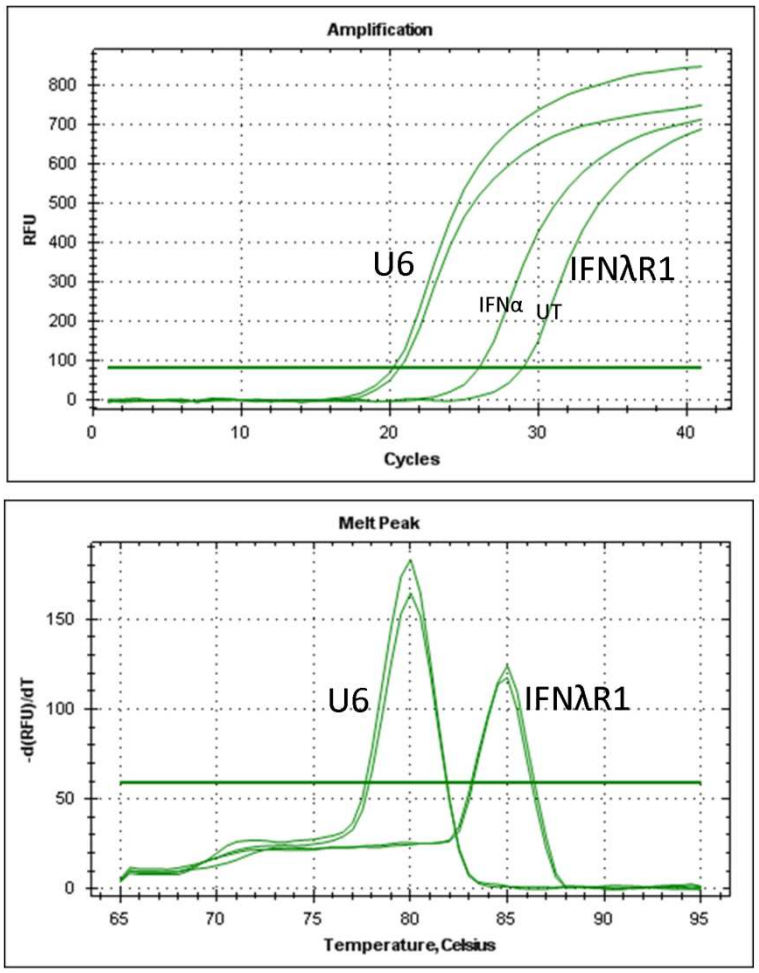

Figure S1: Human dermal fibroblasts express IFNλR1

Cultured human dermal fibroblasts were either untreated or treated with 10 ng/ml IFNα for 4 hours in serum-free medium. mRNA expression was measured by qRT-PCR. The result shows the amplification curve for IFNλR1 and U6snRNA expression, and the melting curve for both genes in a representative experiment. UT: Untreated.

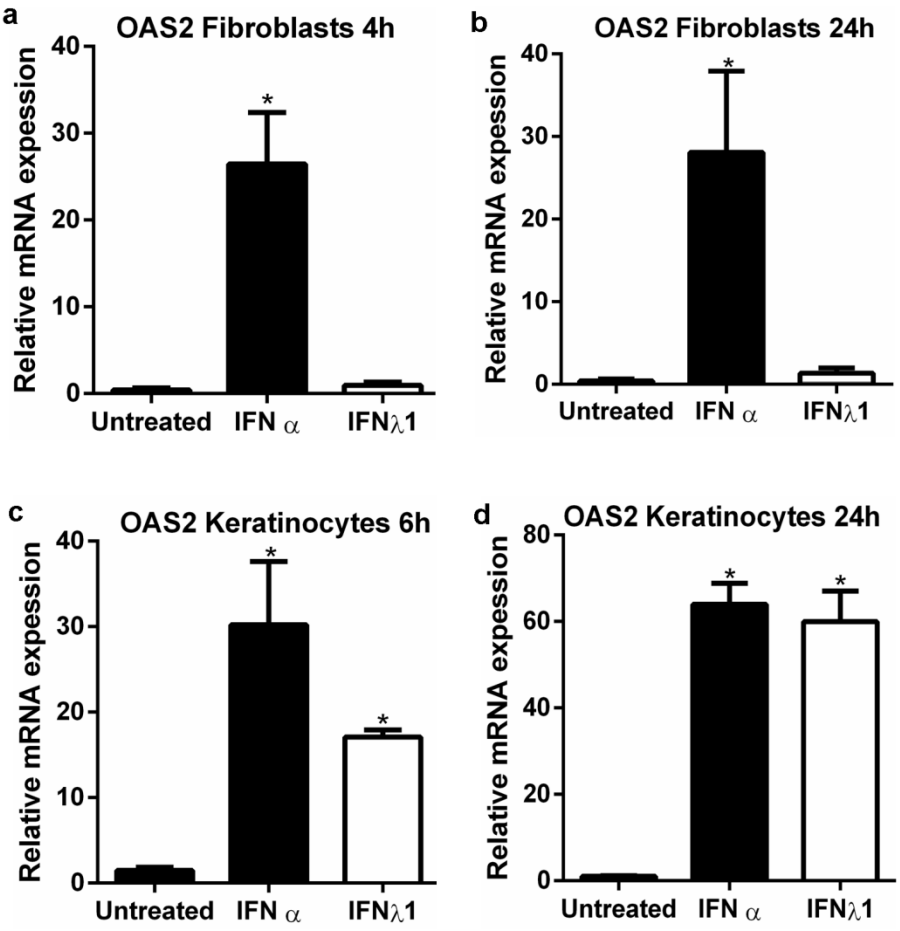

Figure S2: IFNλ1 induces OAS2 in human keratinocytes but not fibroblasts

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. Expression levels in fibroblasts: (a) OAS2, 4 hours (b) OAS2, 24 hours. Expression levels in keratinocytes: (c) OAS2, 6 hours (d) OAS2, 24 hours. For statistical analysis, Mann Whitney U test was used. n=3, values represent mean ± SEM. *$p<0.05$, **$p<0.01$.

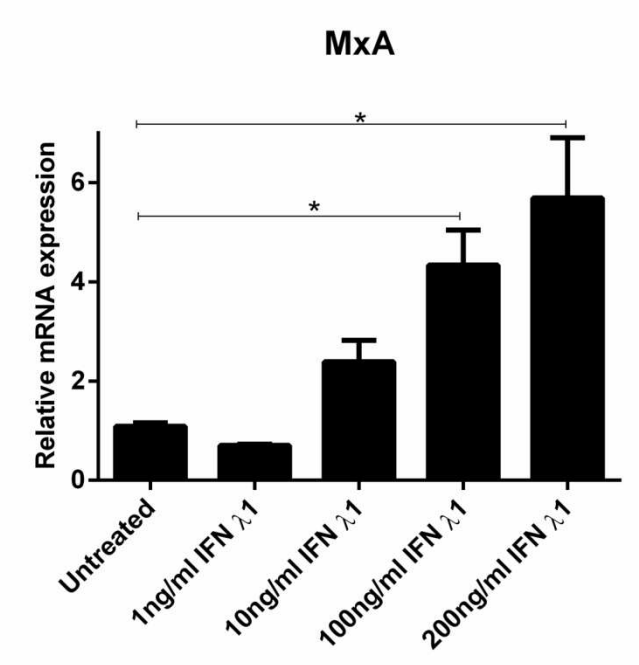

Figure S3: IFNλ1 signalling dose-dependently induces MxA expression in human dermal fibroblasts

Cultured dermal fibroblasts were either untreated or treated with varying concentrations of IFNλ1 for 4 hours. MxA expression was measured by qRT-PCR. For statistical analysis, Mann Whitney U test was used. n=3, values represent mean ± SEM. *p<0.05, **p<0.01.

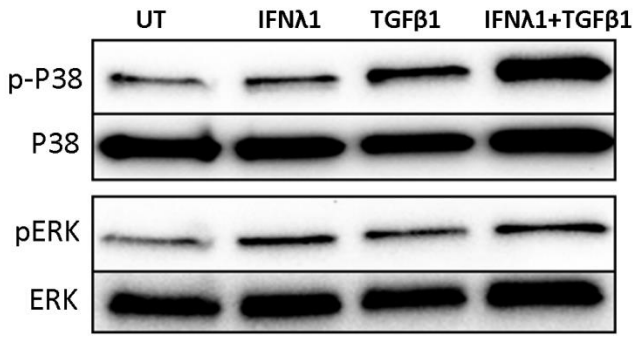

Figure S4: TGFβ and IFNλ1 have synergistic effect on p38 activation

Cultured dermal fibroblasts were either untreated or treated with 100 ng/ml IFNλ1 or 5 ng/ml TGFβ1 or both together for 30 minutes. Cells were lysed and expression of phosphorylated p38 and ERK was analysed by Western blotting. A Representative figure from three independent experiments is shown. UT: Untreated.

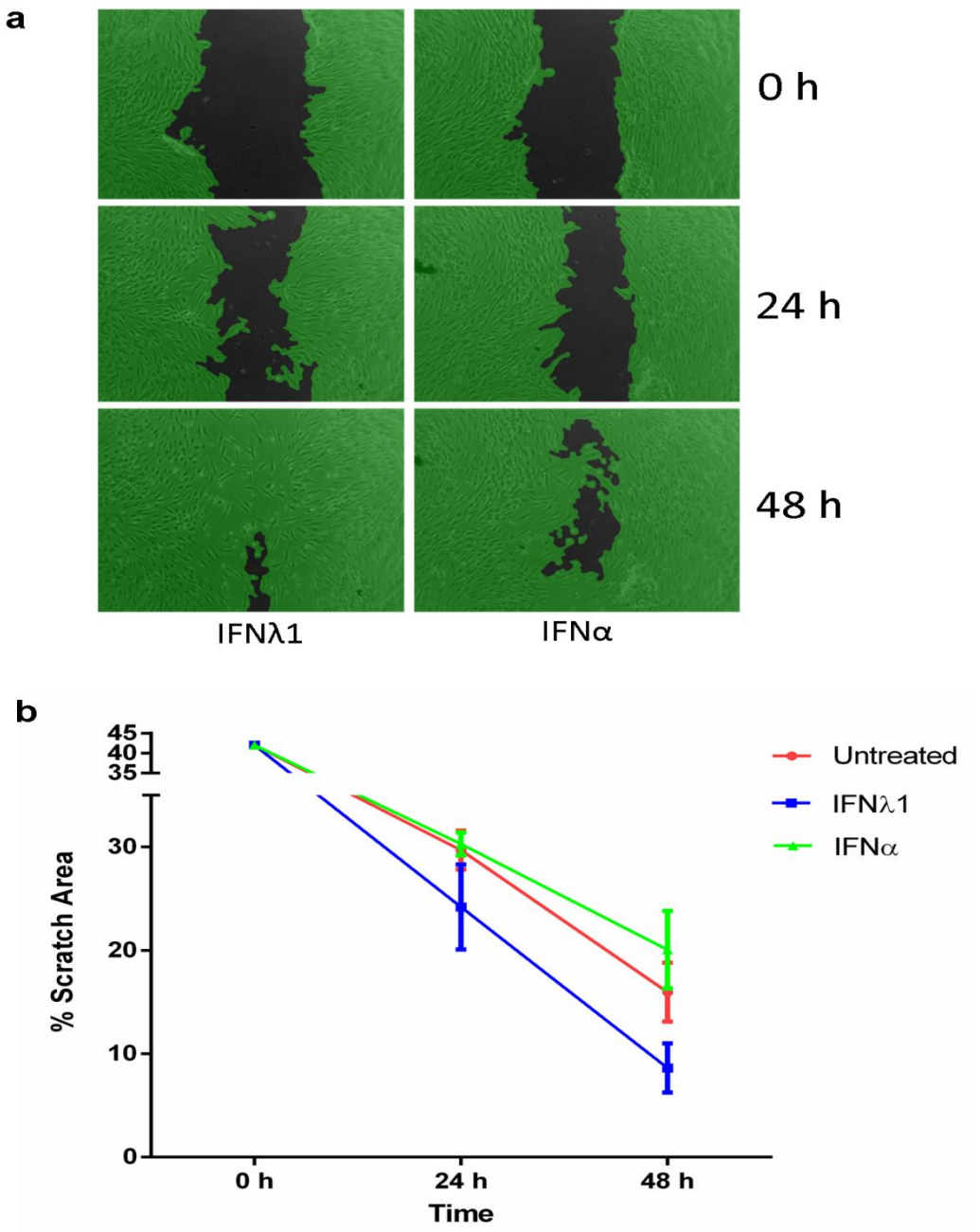

Figure S5: Differential effects of IFNλ1 and IFNα on dermal fibroblasts monolayer culture gap closure

Monolayer culture of human dermal fibroblasts in 24-well plate was allowed to grow to 95% confluence and scratched with p200 (yellow) pipette tip. Cells were either untreated or treated with IFNλ1 or IFNα for 48 hours in DMEM without serum. Images were taken at 0, 24 and 48 hours with Nikon camera under x4 objective. Images are representatives of three independent experiments using three different donors. n=3; values represent mean ± SEM.

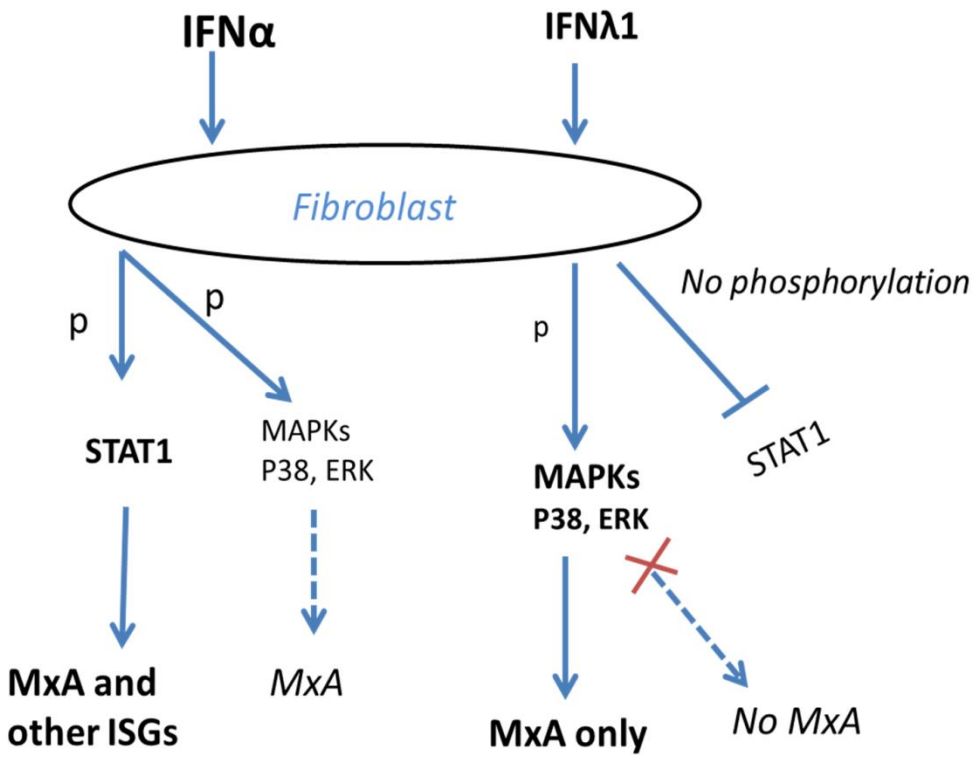

Figure S6: Proposed schematics of IFNα and IFNλ1 signalling in human dermal fibroblasts

Stimulation of fibroblasts with IFNα mainly leads to phosphorylation of STAT1 and to a lesser extent MAPKs (p38 and ERK). This results in the production of MxA and other ISGs. Blocking the MAPKs pathways, may lead to slight reduction in MxA production and possibly of other ISGs too. By contrast, stimulation of fibroblasts with IFNλ results in phosphorylation of mainly the MAPKs pathway with no phosphorylation of STAT1. The phosphorylation of p38 and ERK leads to significant production of MxA but not other ISGs. Blocking these MAPKs pathways results in attenuation of MxA production.

Supplementary Materials and Methods

Cell Isolation, Culture and Ethics

Keratinocytes and dermal fibroblasts were derived from skin tissue from healthy volunteers undergoing cosmetic surgery. Patients gave their written informed consent; informed consent, tissue handling and data management were performed under the approval and guidance of Ethical Tissue Bradford. Skin samples were transferred into universal tubes containing transport medium (Dulbecco's modified eagle's medium (DMEM; Lonza, Slough, UK) containing 0.5 mg/ml streptomycin and 500 U/ml penicillin and 2.5 µg/ml amphotericin B (Gibco/life technologies, Paisley, UK) prior to processing for cell isolation. Cells were isolated as follows. Skin samples were stripped of fat using scissors and were cut up into approximately 1.0 cm^2. Cut skin samples were washed well in PBS, submerged (epidermis side up) in 0.25% Trypsin (Gibco/life technologies) at 4°C overnight and subsequently heated at 37°C for 30 minutes to remove epidermal sheets. The epidermis was incubated for 5 minutes at 37°C in a water bath in 0.05% Trypsin/EDTA (TE; Gibco/life technologies), then vortexed for 1 minute. Trypsinized cell suspension was carefully aspirated and transferred into serum-containing media to neutralise TE and subsequently centrifuged for 5 minutes at 1000rpm to pellet cells. The cell pellet was resuspended in Keratinocytes growth medium-2 (KGM2; Promocell, Heidelberg, Germany) mixed with DMEM containing 10% fetal bovine serum (FBS; PAA/GE healthcare, Little Chalfont, UK) in ratio two to one (KGM-2/DMEM; ratio 2:1) and cells were seeded in a T75 culture flask. This culture medium was replaced with KGM alone after 2 days and keratinocytes were cultured to ~80 % confluency at 37°C and 5% CO_2 before passage. Human dermal fibroblasts were isolated from the dermis 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. The flask was incubated at 37°C for 1 to 2 weeks with medium change every 2 to 3 days until fibroblasts explanted. Fibroblasts were trypsinized and cultured in DMEM containing 4.5 g/L glucose and L-Glutamine (Lonza) and

supplemented with 10% FBS (PAA), 0.1 mg/ml streptomycin and 100 U/ml penicillin. Culture medium was changed every 2- 3 days.

Flow cytometric analysis of IL-28Ra (IFNλR1)

Fibroblasts were washed in ice-cold PBS. For intracellular staining, cells were fixed in fixation buffer (Biolegend, Cambridge Bioscience Ltd, Cambridge, UK) for 15 minutes at room temperature and permeabilized by washing the cells three times in permeabilization buffer (Biolegend). Cells were stained with either 10 µg/ml PE-conjugated anti-IFNλR1 antibody (20 µl per test) or PE-conjugated isotype control in permeabilization buffer for 20 minutes at room temperature. Keratinocytes were resuspended in permeabilization buffer and centrifuged at 800 x g for a total of 2 washes. Cells were resuspended in cell staining buffer (Biolegend,) ready for analysis. For flow cytometry, we used BD LSRFortessa (BD Biosciences, UK). Data collected were analysed using FlowJo software (Tree star Inc., USA)

Agarose gel electrophoresis

qPCR products were run at 40 mA for 30 minutes on 2% agarose gel. Agarose powder was dissolved in Tris, Boric acid and EDTA (TBE) buffer. 1 µl ethidium bromide was added to the solution. TBE buffer was poured into the running tank and qPCR products containing loading dye (30% (v/v) glycerol, 0.25% (w/v) bromophenol blue, 0.25% (w/v) xylene cyanol) were run at 40 mA for 30 minutes. Image was taken under ultraviolet (UV) light.

Cell trace proliferation assay

Human primary keratinocytes and dermal fibroblasts were harvested and resuspended in KGM or serum-free DMEM respectively. Cells were then stained 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 incubated in a water bath at 37^{o}C for 30 minutes to allow the cells to take up the dye. 10 ml KGM or DMEM containing 10% FBS was then added to the keratinocytes or fibroblasts respectively and cells were incubated in water bath at 37^{o}C for

10 minutes in order to remove any unbound dye. Cells were centrifuged at 350 x g for 5 minutes. Keratinocytes were resuspended in KGM while dermal fibroblasts were resuspended in DMEM containing 1% serum. 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. Cells were trypsinized and analysed after 72 hours using flow cytometry. 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).

Scratch assay

Human dermal fibroblasts were seeded in 24-well plates and allowed to grow to about 95% confluency. Cells were scratched vertically at the centre of each well with p200 (yellow) pipette tip. Cells were washed twice with pre-warmed PBS to remove cell debris and were subsequently covered with 500 µl DMEM without serum. Cells were then either untreated or treated with 100 ng/ml IFNλ1 or 10 ng/ml IFNα and were allowed to grow for 48 hours while monitoring gap closure. Images were taken using a Nikon camera on 4x objective at 0, 24 and 48 hours after the scratch.