# The Role of Interleukin-10 Family Members in Inflammatory Skin Diseases

## Understanding the mechanism of action of interferon lambda and interleukin-22 on human primary keratinocytes and dermal fibroblasts with a focus on healing responses in inflammatory skin diseases

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# Abstract

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**Keywords**: Interferon lambda, Interleukin-22, Interferon stimulated genes, keratinocytes, fibroblasts, Inflammation, SOCS, MAPKs

Cutaneous lupus erythematosus (CLE) is an autoimmune disease that resolves with or without permanent scars depending on the subtype. Interferons (IFNs), including the skin specific IFNλ mainly activate STAT1, which results in inflammation in CLE and may play a significant role in scar formation in chronic discoid CLE. IL-22 activates STAT3 and it is emerging as a mediator with significant impact on normal wound repair, epidermal hyperproliferation and prevention of fibrosis.

This work focussed on understanding the regulation and functional impact of IL-22 and IFNλ on skin cells. The counter-regulatory effect of IL-22 on the activities of IFNλ was assessed through downstream interferon stimulated genes (ISGs) expression in healthy and CLE keratinocytes. Cell proliferation and gap closure were investigated in skin resident cells using cell trace dye and scratch assay. Dermal fibroblasts were assessed for the presence of IFNλR1 and IL-22R1, downstream activities of the receptors.

Results showed that IL-22 accelerated “scratch” closure in keratinocytes while IFNλ caused a delay in closure. IL-22 significantly downregulated IFNλ-induced chemokines expression in healthy, but not CLE keratinocytes. Reduced IL-22R1 expression and “STAT3 signature genes” was observed in

CLE keratinocytes. A key finding of this project is that dermal fibroblasts respond to both IFNλ and IL-22.

This work shows that IL-22 can reduce the damaging effect of IFNs in inflamed skin and also identifies dermal fibroblasts as important cells in skin immune responses. In conclusion, IL-10 family members can have both beneficial and destructive effects on the skin organ depending on the micro milieu and cell-type involved. Manipulating the balance of IL-10 family members in the skin may offer new therapeutic approach for both psoriasis and CLE.

# Declaration

- I declare that this work is my own work and that where the work of others is used, this has been duly acknowledged. The content of this thesis has not been submitted for the award of PhD degree anywhere else.
- Chapter 5 of this work is based on this published work:

  IFNλ Stimulates MxA Production in Human Dermal Fibroblasts Via A MAPK-Dependent STAT1-Independent Mechanism (2015). Journal of Investigative Dermatology doi: 10.1038/jid.2015.317.

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# Dedication

- This work is dedicated to the Almighty God for his grace upon my life and for making it possible to attain this enviable height in my academic career.

- To the memory of a friend like a brother,

*Adekunle Oludare Olaifa*

*(1980-2014)*

You will forever be in my heart

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# Abbreviations

| | |
|---|---|
| ANA | Antinuclear antibody |
| ANOVA | Analysis of variance |
| APC | Allophycocyanin |
| ASK | apoptosis signal-regulating kinase |
| BCA | Bicinchoninic acid |
| $CaCl_2$ | Calcium chloride |
| cAMP | Cyclic adenosine monophosphate |
| cDNA | Complementary deoxyribonucleic acid |
| CDLE | Chronic discoid lupus erythematosus |
| CIS | Cytokine induced STAT inhibitor |
| CLE | Cutaneous lupus erythematosus |
| $cm^2$ | Centimetre square |
| DAMPs | Damage-associated molecular patterns |
| DMEM | Dulbecco's modified eagle's medium |
| DMSO | Dimethyl sulfoxide |
| $dH_2O$ | Deionised water |
| dNTP | Deoxyribonucleotide |
| dsDNA | Double stranded deoxyribonucleic acid |
| dsRNA | Double stranded ribonucleic acid |
| ECL | Enhanced chemiluminiscence |
| ECM | Extracellular matrix |
| EDTA | Ethelenediaminetetraacetic acid |
| EGF | Epidermal growth factor |
| ELISA | Enzyme-linked immunosorbent assay |

| | |
|---|---|
| EMT | Epithelial-mesenchymal transition |
| ERK | Extracelluar signal-regulated protein kinases |
| FADD | Fas-associated death domain |
| FBS | Fetal bovine serum |
| FCS | Fetal calf serum |
| FLS | Fibroblasts-like synoviocytes |
| GAPDH | Glyceraldehyde-3-phosphate dehydrogenase |
| GAS | Gamma activated sequences |
| GBP-1 | Guanylate binding protein 1 |
| G-CSF | Granulocytes-colony stimulating factor |
| g/L | Grams per litre |
| gp130 | Glycoprotein 130 |
| HBD | Human beta defensin |
| HMGB-1 | High mobility group box-1 |
| HSV | Herpes simplex virus |
| $H_2SO4$ | Sulfuric acid |
| IFI16 | Interferon inducible protein 16 |
| IFNα | Interferon alpha |
| IFNAR | Interferon alpha receptor |
| IFNλ | Interferon lambda |
| IFNλR1 | Interferon lambda receptor 1 |
| IFNγ | Interferon gamma |
| IFNGR | Interferon gamma receptor |
| IL | Interleukin |
| iNOS | Inducible nitric oxide synthase |

| | |
|---|---|
| IRF | Interferon regulatory factor |
| ISGF3 | Interferon stimulated gene factor 3 |
| ISGs | Interferon stimulated genes |
| ISRE | Interferon stimulated response element |
| JAK | Janus kinase |
| JNK | c-Jun N-terminal protein kinase |
| kDa | KiloDalton |
| KGM | Keratinocytes growth medium |
| KIR | Kinase inhibitory region |
| LE | Lupus erythematosus |
| LIF | Leukaemia inhibitory factor |
| MAPK | Mitogen activated protein kinases |
| MAP2K | Mitogen activated protein kinase kinase |
| MAP3K | Mitogen activated protein kinase kinase kinase |
| MDA5 | Melanoma differentiation-associated gene 5 |
| MFI | Mean fluorescence intensity |
| MK | MAPK-activated protein kinase |
| MLK | Mixed linage kinases |
| MNK | MAPK interacting protein kinases |
| µg | Microgram |
| µl | Microlitre |
| Mg | Milligram |
| MgCl2 | Magnesium chloride |
| mg/ml | Milligram per millilitre |
| Ml | Millilitre |

| | |
|---|---|
| Mm | Millimolar |
| mRNA | Messenger ribonucleic acid |
| MSK | Mitogen and stress-activated kinase |
| MxA | Myxovirus resistance protein A |
| NaCl | Sodium chloride |
| ng/ml | Nanogram per millilitre |
| NFAT | Nuclear factor of activated T cells |
| NFκB | Nuclear factor kappa B |
| OAS | Oligoadenylate synthetase |
| OA | Osteoarthritis |
| OSM | Oncostatin M |
| PAMPs | Pathogen-associated molecular patterns |
| PBS | Phosphate buffered saline |
| PBST | Phosphate buffered saline/Tween-20 |
| pDCs | Plasmacytoid dendritic cells |
| PE | Phycoerythrin |
| Pmol | Picomole |
| polydA:dT | Polydeoxyadenylic-deoxythymidylic |
| Poly I:C | Polyinosinic:polycytidylic acid |
| PRRs | Pattern recognition receptors |
| PVDF | Polyvinylidine fluoride |
| qRT-PCR | Quantitative real-time polymerase chain reaction |
| RA | Rheumatoid arthritis |
| RIG-I | Retinoic acid-inducible gene-I |
| RIP | Receptor-interacting protein-1 |

| | |
|---|---|
| RLR | RIG-I-like receptors |
| RNA | Ribonucleic acid |
| SCLE | Subacute cutaneous lupus erythematosus |
| SDS | Sodium dodecyl sulphate |
| SDS-PAGE | SDS-polyacrylamide gel electrophoresis |
| SH | Src homology |
| SLE | Systemic lupus erythematosus |
| SOCS | Silencer of cytokine signalling |
| SSA | Sjogren's syndrome A |
| SSB | Sjogren's syndrome B |
| ssRNA | Single stranded ribonucleic acid |
| STAT | Signal transducer and activator of transcription |
| TE | Trypsin/EDTA |
| TGFβ | Tumour growth factor |
| Th | T helper |
| TIR | Toll/interleukin-1 receptor |
| TLR | Toll-like receptor |
| TNFα | Tumour necrosis factor alpha |
| TRADD | TNFR-associated death domain |
| U/µl | Unit per microliter |
| U/ml | Unit per millilitre |
| VEGF | Vascular endothelial growth factor |

# CHAPTER 1: INTRODUCTION

# 1 Introduction

**Inflammation-associated cutaneous scarring is an important health concern, which is surprisingly neglected worldwide. Inflammatory skin diseases, such as cutaneous lupus erythematosus (CLE) can result in scarring or non-scarring outcome even in the same patient.**

## 1.1 **The Human Skin**

The skin is one of the largest organs in the body, with a surface area of 180 $cm^2$, making up 16% of body weight. The skin is structured in a way which makes it function effectively as a physical barrier by regulating inward and outward flow of water and electrolytes, while also providing protection against entry of microorganisms, toxic agents, ultraviolet radiation and mechanical insults (Bos and Kapsenberg, 1986). The skin is a dynamic organ with the outer layer constantly shed and being replaced by the basal keratinocytes, which move up to the outer surface. The human skin is typically divided into three major layers (Figure 1.1):

- Epidermis
- Dermis and,
- Subcutis or hypodermis

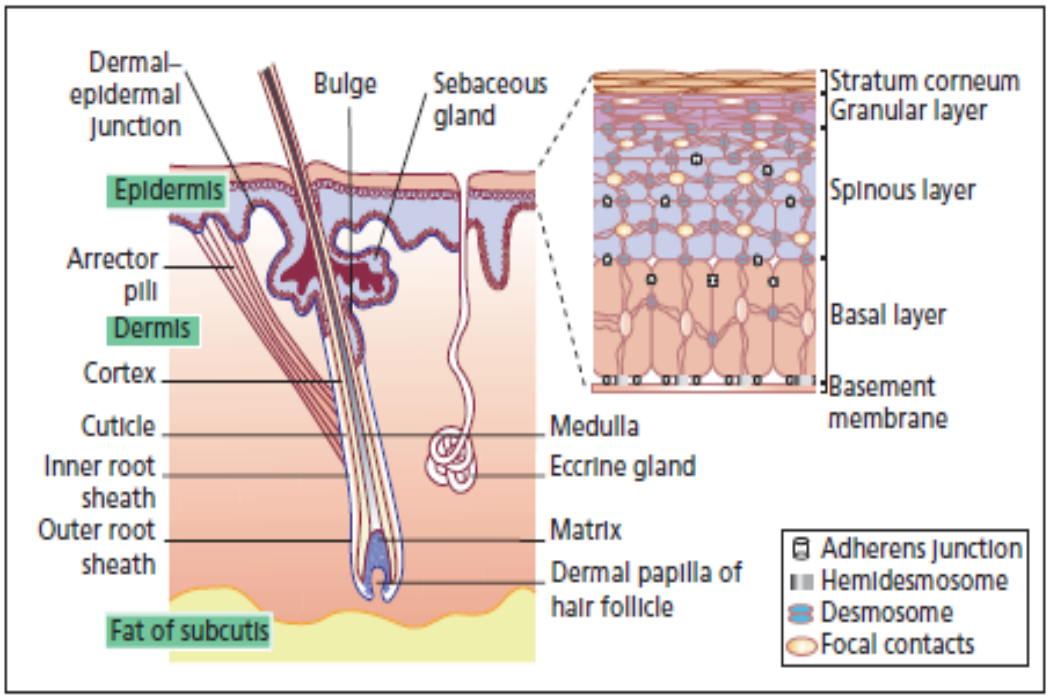



**Figure 1.1: Structure of the human skin (McGrath, 2008)**

The epidermis is the outermost layer of the skin. It is composed of a stratified, keratinised epithelium, and contributes to the physical, chemical and immunological barrier functions of the skin. The basement membrane, which links the epidermis with the dermis, is a multilayered structure, which forms the dermo-epidermal junction. The dermal compartment provides structural support to the skin. It is the region of supportive connective tissue found between the epidermis and the subcutis or hypodermis, a layer of fat deposit and loose connective tissue (Wolff, 2008). Table 1.1 shows the functions of different layers of the skin.

**Table 1.1: Functions of the skin**

| **Function** | **Layer responsible** |
|---|---|
| Acts as sensory organ | Epidermis, dermis and hypodermis |
| Protects against UV irradiation | Epidermis |
| Temperature regulation | Epidermis, dermis and hypodermis |
| Fights against microbial invasion | Epidermis, dermis |
| Permeability barrier (prevents loss of moisture) | Epidermis |
| Actively participates in immunological surveillance | Epidermis |
| Regeneration and wound repair | Epidermis, dermis |
| Physical appearance | Epidermis, dermis and hypodermis |
| Skin firmness and extracellular matrix synthesis | Mainly dermis |

### *1.1.1 The Epidermis*

The epidermis is a stratified epithelium varying in thickness from around 0.4 mm on the eyelid to around 0.8-1.5 mm on the palms of the hand and soles of the feet (Bensouilah, 2007; Wolff, 2008). The epidermis is without blood vessels and is largely composed of keratinocytes, which make up about 90% to 95% of the cell population. The other cell populations present in the epidermis are: Melanocytes, which donate pigment to keratinocytes, dendritic antigen-presenting cell population, termed Langerhans cells (LCs), which have immunological roles and Merkel cells, which are slow-adapting type I mechanoreceptors resident in areas of high tactile sensitivity (McGrath, 2008; Wolff, 2008). The epidermis is organised into four layers depending on proliferative ability and the maturity of keratin expressed by the keratinocytes. These layers are: (1) the *Stratum basale* above the basement membrane*,* (2) *Stratum spinosum,* (3) *Stratum granulosum,* and the outermost *(4) Stratum corneum.* Of note, the *Stratum granulosum* expresses molecules, such as antimicrobial peptides (AMP), including cathelicidin (CAMP/LL-37), B-defensins, lipocalin 2, psoriasin (S100A7), calgranulin A (S100A8) and calgranulin B (S100A9), which are involved in innate immunity ((Morizane and Gallo, 2012; Sorensen et al., 2003)*.* The cells in the *Stratum corneum* are referred to as corneocytes; they have lost their nuclei but have a cross-linked protein membrane structure called cornified envelope, containing many layers of neutral lipids (Lowes et al., 2014)*.*

### *1.1.2 The Dermis*

The dermis is made up of fibrous, filamentous and cellular connective tissue elements that accommodate vascular and nerve networks. The dermis provides elasticity and tensile strength to the skin, with thickness ranging from 0.6 to 3 mm. It functions in protecting the body against mechanical injury and helps in thermal regulation (Bensouilah, 2007). The dermis consists of many resident cells, such as fibroblasts, endothelial cells, macrophages, mast cell and probably some tissue resident conventional and non-conventional T cells, as well as transient circulating cells of the immune system (Glennie et al., 2015; Wolff, 2008; Zhou et al., 2015). Skin appendages, including sweat glands, hair follicles and sebaceous glands are also located in the dermis.

### *1.1.3 Collagen*

Collagens are a family of closely related, but genetically distinct proteins made up of specific amino acids, such as glycine, proline, hydroxyproline and arginine (van der Rest and Garrone, 1991; Vuorio and de Crombrugghe, 1990). Collagen is the major component of the connective tissue and it is the most abundant protein in mammals, constituting 25 to 35% of total body protein content (Szoka et al., 2015). Collagens are the main components of the ECM in the skin, synthesised predominantly by fibroblasts, with types I and III as the major subtypes expressed by these cells (Olsen et al., 1989). The amount of collagen deposited by fibroblasts is regulated by the rate of collagen biosynthesis and collagen catabolism (Chen and Raghunath, 2009). It was initially thought that all types of collagen were secreted by fibroblasts

alone; however, it is now well known that numerous epithelial cells make certain types of collagen (Lodish H, 2000). Type I collagen is the most abundant type in the dermis and it is responsible for skin tensile strength. On the other hand, type IV collagen is highly cross-linked and forms a network; hence, it maintains the mechanical stability of the basement membrane. Reduced expression of this collagen through the activity of MMP2 has been linked with wrinkles development (Contet-Audonneau et al., 1999). Mutation in the *COL3A1* gene has been linked with atrophic skin, spontaneous visceral rupture and easy bruising (Sharma et al., 2009). In addition, collagen III suppression increased myofibroblasts differentiation and scar disposition in cutaneous wound healing (Al-Qattan et al., 2015; Volk et al., 2011). Type VII collagen, expressed by both keratinocytes and dermal fibroblasts is the main component of the anchoring fibrils, the attachment structures present in the basement membrane of the skin and the mucous membranes (Chen et al., 1994). The anchoring fibrils are important for the stability of the cutaneous basement membrane zone. Therefore, structural abnormality or lack of the anchoring fibrils results in cutaneous fragility and sub-basal lamina blister formation as observed in dystrophic form of epidermolysis bullosa (EB) (Uitto and Christiano, 1992).

### *1.1.4 Wound healing and Scarring*

Tissue injury results in the activation and signalling of cytokines and growth factors, involving, endothelial, epithelial, mesenchymal and immune cells in order to initiate wound repair. Wound repair or healing is a stepwise process characterised by cell proliferation, inflammation, remodelling of extracellular

matrix, cell invasion and migration, formation of new blood vessels and regulation of blood coagulation (Dauer et al., 2005). Signal transducer and activator of transcription (STAT) 3, a transcription factor, has been implicated in wound healing due to its role as a key molecule linking many signalling pathways involved in wound healing such as EGFR, IL-6, IL-22 and hepatocyte-growth factor (HGF) signalling (Li et al., 2003). Fibrosis (scarring) occurs as a result of epithelial injury, activation and formation of subepithelial myofibroblasts foci and excessive deposition of extracellular matrix. It is an unwanted outcome of wound healing. A major difference identified between normal wound healing and healing with abnormal scar formation is the amount of collagen deposition and the arrangement of collagen. Whereas there is reduced collagen deposition and a 'basket weave' collagen arrangement during normal wound healing, increased collagen deposition is prominent in abnormal scar-forming wound healing process and the collagen has abnormal parallel arrangement (Occleston et al., 2010). Abnormal organisation of type I collagen-rich ECM during remodelling phase of wound healing is the hallmark of scar formation (Mah et al., 2014). Normal scar formation during wound healing is also characterised by increased protease activity and reduced amount and activity of protease inhibitors. On the contrary, wound repair with abnormal scar formation is characterised by decreased protease activity and increased presence and activity of protease inhibitors (Occleston et al., 2010). The process in which epithelial cells undergo phenotypic changes resulting in their loss of epithelial characteristics, and acquisition of mesenchymal phenotype referred to as epithelial to mesenchymal transition (EMT) has been implicated in the development of

fibrotic tissue (Liang et al., 2013). Fibrosis presents with high number of trans-differentiated fibroblasts referred to as myofibroblasts. Myofibroblasts are identified by overexpression of α-smooth muscle actin (α-SMA) which has been linked with aberrant synthesis of matrix in pulmonary fibrosis (Scotton and Chambers, 2007). Fibroblasts phenotype and the depth of wound have been identified as important factors in scar formation (Cuevas et al., 2007; Glim et al., 2013). During scar formation, adnexal structures such as hair follicles, normally present in the dermis are destroyed. Transforming growth factor (TGF)- β1 has been identified as a key molecule in the development of fibrous tissue due to its ability to attract fibroblasts, stimulate their proliferation and by inducing EMT either through Smad or non-Smad signalling (Xu et al., 2009). Scarring as a consequence of an inflammatory disease mostly occurs in hair follicle rich body areas and involves the pilosebaceous unit. The hair follicle harbours an important stem cell compartment and presents an immunoprivileged site. Mechanisms of atrophic scarring, frequent in acne and chronic discoid LE, are poorly understood. With regard to organ fibrosis, it has been highlighted that although diverse diseases can initiate fibrosis, the biochemical and cellular tissue responses that lead to the final outcome are largely stereotypical. This may also apply to the skin and different scarring outcome as, for example, seen in LE may indicate that the inflammation-to-scarring pathway has been arrested in different phases. Cytokines, growth factors and lipid mediators derived from surrounding tissue cells (keratinocytes, endothelium), infiltrating monocyte/ macrophages, lymphocytes, and innate lymphoid cells are important regulators of myofibroblast differentiation and activation. IL-10

family members and in particular IL-22 are key molecules in skin healing processes (Lim and Savan, 2014). The pro-healing but anti-fibrotic properties of IL-22 are beginning to be recognised. It has been suggested that fibroblasts from CDLE patients have reduced *in vitro* proliferation as compared to those from non-scarring subtypes of CLE following UVA irradiation (Nyberg et al., 2000). However, the mechanism involved in tissue scarring in CDLE is still not well understood. Hypertrophic scars presents with firm papules, nodules or plaques while atrophic scars are thin depressed plaques. Atrophic scar is the type mainly seen in CDLE and it is characterised by reduced collagen expression, epidermal atrophy, hemosiderin deposition in the dermis and extravasation of erythrocytes (Sharma et al., 2009)

## 1.2 **Lupus Erythematosus**

Lupus erythematosus (LE) is a complex autoimmune disease with both systemic and cutaneous manifestations. The systemic disease (SLE) can be life threatening with conditions, such as nephritis, arthritis, serositis, hematologic and central nervous system involvement. The skin is the main target organ in LE and there is cutaneous manifestation in 85 to 90% of patients with SLE (Wittmann, 2011). Cutaneous manifestations can present on their own without signs of systemic involvement. In both cases, skin symptoms can significantly affect the quality of life of patients (Klein et al., 2011; Wenzel et al., 2010). The pathogenesis of LE is multifactorial and it is believed to be a result of concerted action of genetic, environmental and immunological factors (Kuhn and Bijl, 2008; Kuhn et al., 2006). Regarding

cutaneous flares, research findings suggest a strong involvement of UV irradiation as a trigger factor (Kuhn et al., 2006; Meller et al., 2005) but infections, drugs and trauma are also described to initiate LE symptoms (Wenzel et al., 2010). The immunological mediators triggered in LE mimic the host's response to viral infection which results in the production of interferons (IFNs) (types I, II and III) and IFN stimulated genes (ISGs), such as CXCL9, CXCL10, myxovirus protein A (MxA), guanylate binding protein-1 (GBP-1) and oligoadenylate synthetase (OAS) (Arrue et al., 2007; Kim et al., 2009; Naschberger et al., 2010; Wenzel et al., 2005a; Wu et al., 2011; Zahn et al., 2010). Some other proinflammatory cytokines, such as interleukin (IL)-18, High mobility group box (HMGB)-1, IL-1β and tumour necrosis factor alpha (TNFα) have also been implicated in pathogenesis of LE (Aringer and Smolen, 2012; Ohl and Tenbrock, 2011; Popovic et al., 2005; Wang et al., 2008).

### *1.2.1 Systemic Lupus Erythematosus (SLE)*

The pathogenesis of SLE is often associated with production of autoantibodies, formation of immune complexes and complement activation resulting in inflammation (Herrmann et al., 2000; Munoz et al., 2010; Ohl and Tenbrock, 2011). Genetic aberration in SLE has been highlighted in studies; however, no single gene has been identified as the most important but rather several genes, such as *TREX1*, *IRF5*, ITGAM, *STAT4*, gene coding for C1q and many genes on the IFN pathway were implicated in the risk of developing SLE (Munoz et al., 2010; Rhodes and Vyse, 2008). Defective or delayed clearance of apoptotic cells, which may result in secondary necrosis,

is discussed as the main source of autoantigens in SLE. The accumulation of cellular remnants may lead to a breakdown in immunological tolerance resulting in autoimmune inflammation (Herrmann et al., 2000). Autoantibodies and nucleic acids from dying cells form immune complexes, which amplify immune activation, such as plasmacytoid dendritic cells (pDCs) activation resulting in production of type I IFNs and other inflammatory molecules (Lovgren et al., 2004).

### *1.2.2 Cutaneous Lupus Erythematosus (CLE)*

The morphology and distribution of skin lesion in CLE is highly diverse and this has led to its categorisation into three main subtypes based on clinical features, laboratory abnormalities, extent and duration of skin lesion and histological changes. They are:

1. Chronic discoid CLE (=CDLE)
2. Subacute CLE (= SCLE)
3. Acute CLE (=ACLE)

## 1.3 **Scarring and non-scarring CLE**

CDLE mostly presents with a scarring outcome whereas skin lesions in the context of SCLE and ACLE heal without scarring.

### *1.3.1 Scarring CLE*

CDLE is the most common form of CLE and it is characterised by indurated, erythematous plaques which may be disseminated over head, neck, trunk, arms and leg or localised to one area of the skin, particularly face and

capillitium, which is the case in about 80% of people with CDLE. The hallmark of this type of CLE is that it heals with significant permanent scarring and scarring alopecia. Transition of CDLE to SLE is rare (Kuhn et al., 2000; Wenzel et al., 2010).

### *1.3.2 Non-scarring CLE*

SCLE presents with annular papulosquamous erythematous plaques of different morphological forms. Patients suffering from this subtype of CLE are often highly photosensitive and show presence of anti-Ro/SSA and anti-La/SSB autoantibodies, which belong to the group of antinuclear autoantibodies (ANA). Skin lesions typically heal without scarring but there may be post inflammatory changes in pigmentation. Histological examination shows features of so-called interface dermatitis, which is also seen in discoid CLE (Wenzel et al., 2010; Yu et al., 2013). Interface dermatitis describes specific morphological pattern of basal epidermal layer, characterised by vacuolar changes (liquefaction), high levels of keratinocytes apoptosis and infiltration of dermo-epithelial junction by CD8+ lymphocytes (Wenzel and Tuting, 2008).

ACLE typically presents centro-facial, disseminated erythema referred to as malar rash or butterfly rash. ACLE is mostly found in the context of SLE. Patients show positive ANA and up to 90% of the patients have high anti-dsDNA autoantibodies (Crowson and Magro, 2001).

## 1.4 **Pathogenesis of CLE**

The pathogenesis of CLE remains poorly understood; however, various concepts have been postulated for the initial trigger and the progression of the disease. There is evidence for the involvement of autoantibody generation, ultraviolet irradiation (UV) and cytokines/chemokines dysregulation in the pathogenesis of CLE. Immunological, genetic and genome-wide studies have confirmed the important role of the IFN pathway/ISGs in the pathogenesis of CLE and maintenance of the chronic state of the disease (Dey-Rao and Sinha, 2015; Dey-Rao et al., 2014; Yu et al., 2013). The clearance hypothesis, which has been suggested as the main trigger of CLE is briefly described below.

### *1.4.1 UV irradiation and defective clearance in CLE*

UV irradiation is an important trigger for CLE. UV-induced keratinocyte apoptosis can result in the exposure of normally intracellular, nuclear antigens on the surface of keratinocytes (e.g. “in apoptotic blebs”), which may be of relevance in CLE pathogenesis (Herrmann et al., 2000; Kuhn et al., 2006). ANA targeting these antigens are found in varying levels in CLE depending on the subtype, with SCLE patients having high levels of anti-Ro/SSA and anti-La/SSB, which are associated with photosensitivity (Sontheimer et al., 1982; Wenzel et al., 2000a; Wenzel et al., 2000b). Increased apoptosis and defective clearance of apoptotic debris have been reported in LE patients. The defective clearance of these apoptotic cells has been attributed to many factors, the best described of which is deficiency in the complement factor, C1q (Zhang and Dong, 2007). Impaired macrophage

function due to the presence of autoantibodies that are specific for class A scavenger receptors has also been implicated in the delayed or defective clearance of apoptotic cells (Chen et al., 2011). Important ISGs, such as MxA, GBP-1, CXCL9 and CXCL10, involved in the pathogenesis of CLE have be shown to be highly upregulated in UV-induced cutaneous lesion, suggesting that UV acts as a trigger of IFN response in LE skin lesions (Naschberger et al., 2010). This could possibly be in the context of nucleic acids found outside of the cell and thus, initiating an anti-viral type immune response. Induction of chemokines, such as CXCL9, CXCL10 and CXCL11 in response to either nucleic acids or IFNs leads to recruitment of pathogenic IFNγ-producing CXCR3+ T cells and plasmacytoid dendritic cells (pDCs), which produce high amount IFNα to the skin compartment. This results in exacerbation and maintenance of disease state. TNFα and IL-18 are other cytokines that play important roles in increased keratinocytes apoptosis seen in CLE. A schematic for the described factors believed to be involved in CLE pathogenesis is shown in Figure 1.2.

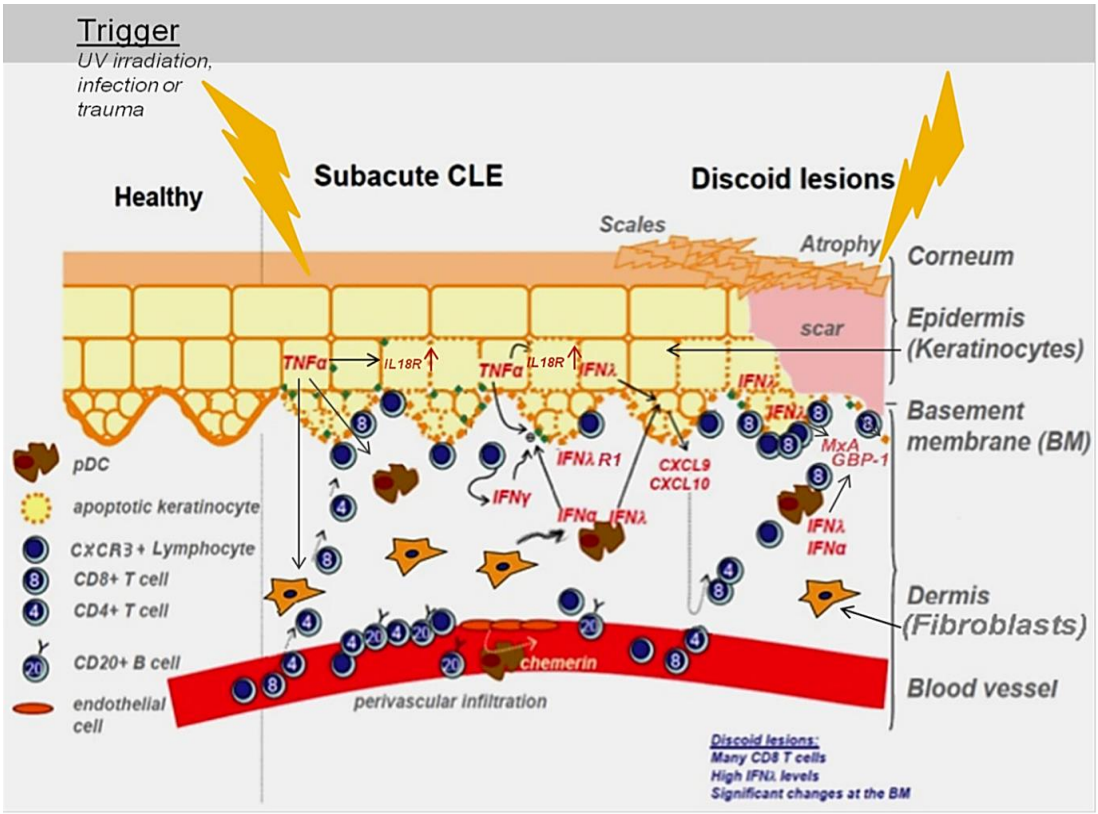



**Figure 1.2: Cytokine involvement in the pathogenesis of CLE**

CLE is characterised by interface dermatitis. Upon external trigger (UV, infection etc) resulting in damage and apoptosis (secondary necrosis), keratinocytes produce IFNλ that induces IFN stimulated proteins including MxA, GBP-1 and the chemokines CXCL9/10. These chemokines recruit IFNγ-producing CXCR3+ T cells (cytotoxic cells) and marginally pDCs. Chemerin as well as anaphylatoxins have been described to attract pDCs from peripheral blood into the dermo-epidermal junction of the skin. pDCs are particularly competent IFN producers. High levels of IL-18 and increased expression of IL-18 receptor in keratinocytes contributes to rapid apoptosis through increased TNFα. CLE, cutaneous lupus erythematosus; FB, fibroblasts; KC, keratinocytes; pDC, plasmacytoid dendritic cell; Tc, T cell; UV, ultraviolet light. Adapted from (Wittmann, 2011).

## 1.5 Cytokines and signalling pathways linked to CLE pathogenesis

### *1.5.1 Interferon (IFN) signalling and induction of ISGs*

IFNs belong to the class 2 cytokines family and were initially known for their anti-viral activities. However, it is now clear that they play a major role in the pathogenesis of a large number of conditions, including autoimmune diseases, such as LE, rheumatoid arthritis (RA) and lichen planus (Vilcek, 2006). To date, there are three types of IFNs known namely; Type I IFNs (IFN-α, -β, -ε, -κ, -ω), type II (IFNγ), type III (IFN-λ1, IFN-λ2, IFN-λ3, IFN-λ4). The classification is partly due to their use of different types of receptors to mediate signal transduction (Table 1.2). The type III (lambda) IFNs, which were discovered recently by two independent groups, are also referred to as IL-29, IL-28A and IL-28B for the first 3 respectively (Kotenko et al., 2003; Sheppard et al., 2003). Type I IFNs signal through the membrane-associated IFNα receptor (IFNAR), consisting of IFNAR1 and IFNAR2 subunits (Lutfalla et al., 1995; Novick et al., 1994) while IFNγ signals through membrane-associated IFNγ receptor (IFNγR), consisting of IFNGR1 and IFNGR2 subunits (Soh et al., 1994). Type III IFNs (IFNλ) signal through heterodimeric IFN receptor complex comprising IFNλR1, selectively expressed and functional in certain types of cells (Kotenko et al., 2003) and the ubiquitously expressed IL-10R2, which is a subunit of the receptors for IL-10, IL-22 and IL-26 (Kotenko et al., 2003; Sheppard et al., 2003). IFNλ is functionally similar to type 1 IFNs but structurally similar to the IL-10 family of cytokines (Miknis et al., 2010). In fact, IFNλ has only 20% homology to type I IFN; however, they are classified as IFN because they share intracellular signalling pathways (George et al., 2012). The binding of types I and III IFNs

to their receptors results in conformational changes which allows access to two member of the cytoplasmic Janus tyrosine kinases family, JAK1 and Tyk2. This leads to subsequent phosphorylation of members of the signal transducers and activator of transcription (STAT) family of transcription factors, mainly STAT1 and STAT2, which form heterotrimer with IFN regulatory factor 9 (IRF9) resulting in a heterotrimeric complex referred to as IFN-stimulated gene factor 3 (ISGF3). This complex translocates to the nucleus where it binds to IFN stimulated response element (ISRE) leading to activation of IFN stimulated genes (ISGs) (Li et al., 1996). The only type II IFN, IFNγ, binds to IFNGR (IFNGR1 and IFNGR2) resulting in access to JAK1 and JAK2 with subsequent phosphorylation of STAT1 homodimer referred to as gamma activated factor (GAF), which translocates to the nucleus to bind to gamma activated sequences (GAS) at the promoter region of downstream genes or ISGs. These IFNs induce the expression of genes, such as myxovirus resistant protein A (MxA), CXCL9, CXCL10, 2, 5-Oligoadenylate synthetase (OAS), guanylate binding protein 1 (GBP-1) and IFN-inducible protein 16 (IFI16). The signalling pathways are described in Figure 1.3.

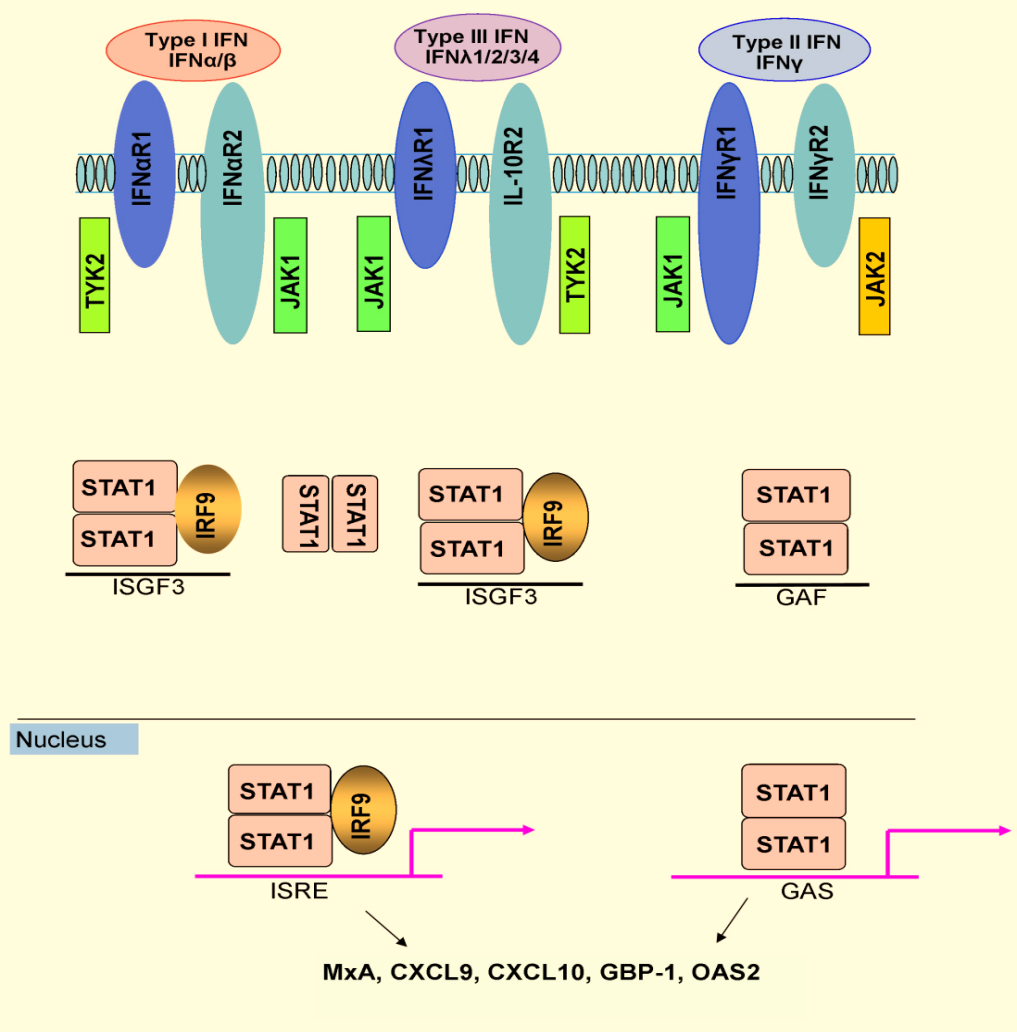



**Figure 1.3: IFN signalling via JAK/STAT pathway**

Types I and III IFNs bind to their respective receptor complexes; resulting in recruitment of JAK1 and TYK2 to the intracellular compartment of these receptors. They activate similar downstream signalling pathways and induce overlapping set of genes mainly through the activation of IFN stimulated gene factor 3 (ISGF3) which binds to IFN stimulated response element (ISRE) at the promoter of these downstream genes. They can also use STAT1 homodimer. Type II IFN (IFNγ) recruits JAK1 and JAK2, and activates STAT1 homodimer, which binds to gamma associated sequences (GAS) to induce some overlapping set of genes as type I and III IFNs but also distinct set of IFN stimulated genes (ISGs). Adapted from (Heim, 2012)

**Table 1.2: IFNs Classification**

| Types | Sub-types | Main sources | Receptor Complex | Functions | Signalling pathways / Transcription factors |
|---|---|---|---|---|---|
| Type I | IFNα (13 isoforms of this subtype), IFNβ, IFNε, IFNκ, IFNω | pDCs, fibroblasts, all nucleated cells | IFNAR1 IFNAR2 | Antiviral, antitumour, cytotoxicity, antiproliferative, proapoptotic | JAK1 TYK2 STAT1 STAT2 STAT3 STAT4 STAT5 NFκB MAPK p53 |
| Type II | IFNγ | Natural killer (NK) cells, T cells, macrophages | IFNGR1 IFNGR2 | Antibacterial, antiviral, antitumour, Th1 response, immune modulation | JAK1 JAK2 STAT1 STAT3 STAT5 |
| Type III | IFNλ1 (IL-29), IFNλ2 (IL-28A), IFNλ (IL-28B), IFNλ4 | Keratinocytes, pDCs, hepatocytes | IFNλR1 (Mainly epithelial cells and hepatocytes) IL-10R2 | Antiviral, antitumour, antiviral, antiproliferative, proapoptotic | JAK1 TYK2 STAT1 STAT2 STAT3 STAT5 |

### *1.5.2 Production of IFNs*

IFNs are produced as a result of the interaction between pathogen-associated molecular patterns (PAMPs) or damage-associated molecular patterns (DAMPs), such as viral or self-RNA or DNA and pattern recognition receptors (PRRs). Toll-like receptors (TLRs) are the best characterised of these PRRs. The endosomal TLRs: 3, 7,8 and 9 recognise nucleic acids. TLR3 recognises double stranded RNA (dsRNA), TLR7/8 recognise single stranded RNA (ssRNA) and TLR9 recognises unmethylated DNA with CpG motif (double stranded DNA, dsDNA). Another important pathway leading to induction of IFN is the cytosolic pathway comprising what is called the retinoic acid-inducible gene-I (RIG-I) like receptors (RLRs). This group consists of cytosolic RNA helicases, such as RIG-I and melanoma differentiation-associated gene 5 (MDA5) (Yoneyama et al., 2004). Activation of TLRs and RLRs results in signalling cascades involving nuclear factor kappa B (NFkB) and IFN regulatory factors, such as IRF3 and IRF7. IRF7 is expressed at low levels in cells but strongly activated on stimulation with type I IFN while IRF3 is constitutively expressed in most cells. IRF3 and NFkB act downstream of TLR3 and other cytosolic receptors to induce the production of IFNβ and IFNλ1; while TLR7/8 and TLR9 activate IRF7 and NFkB resulting in the production of IFNα, IFNλ2 and λ3 (Heim, 2012). Immune complexes containing DNA or RNA are recognized as key inducer of IFN production in LE conditions (Farkas et al., 2001; Zahn et al., 2011). DAMPS, such as cathelicidin (LL37) and high mobility group box (HMGB)-1, which are released as a result of assault on cells may act as adjuvant for the recognition of ‘self-nucleic’ acids resulting in activation of the immune

system leading to proinflammatory cytokines production. LL-37, an antimicrobial peptide binds to and protects 'self-nucleic' acids from degradation, allowing access to TLR7 and TLR9 resulting in triggering of IFNα production in pDCs (Eloranta et al., 2013; Lande et al., 2007). TLR3 is highly upregulated in LE lesions and its ligation has been identified as a main trigger for type III IFN expression in human primary keratinocytes (Zahn et al., 2010). While TLR3 agonist is extremely important in the pathogenesis of CLE, the endogenous source of dsRNA is still not well defined. However, a possible source could be long non-coding RNA (lnRNA).

### *1.5.3 Type I IFNs*

The most extensively studied of the type I IFNs are IFNα and β. Type I IFNs are produced in varying amount by almost all cell types. pDCs are considered to be the main producers of type I IFNs and these cells have been shown to accumulate in skin lesions of LE patients (Farkas et al., 2001). The IFNAR is ubiquitously expressed; hence, most nucleated cells are responsive to type I IFNs (IFNα/β) (Table 1.2). IFN inducible genes (ISGs) also known as IFN signature genes; have been shown to be highly expressed in lesional skin of CLE patients. For instance, strong expression of MxA has been linked with local production of type I IFNs (Wenzel et al., 2005a). CLE skin biopsies revealed that pDCs are concentrated along the dermo-epidermal junction and within perivascular dermal filtrates; this was shown to correlate with the expression levels of MxA, granzyme B as well as high amount of inflammatory infiltrate (Vermi et al., 2009). The role of type I IFNs in enhancing Th1-biased immune response and local T-lymphocytes

inflammation is well documented (Klimpel et al., 1990; Wenzel et al., 2005a). Type I IFNs induce cytokines and chemokines, such as CXCL9/10, which recruit T-cells expressing the chemokine receptor, CXCR3 into the tissue. Ligand binding of CXCL9/10 to its corresponding receptor, CXCR3 on T-cells positive for the receptor has been associated with increased tissue damage in CLE (Amoura et al., 2003; Wenzel et al., 2005a).

### *1.5.4 Type II IFNs*

IFNγ is the only type II IFN known and it is produced mainly by T-cells and natural killer (NK) cells. IFNγ is one of the most effective molecules at inducing proinflammatory responses in keratinocytes (Albanesi et al., 1998). IFNγ-producing CXCR3+ T-lymphocytes have been implicated in the pathogenesis of CLE. It is believed that these cells are recruited to the dermo-epidermal junction by IFN inducible proteins, such as CXCL9/10 where they produce IFNγ, thereby contributing to the IFN profile in CLE. Our work (Alase, 2013) suggests that the presence of IFNγ in the epidermal compartment of the skin may contribute to increased production of IFNλ and also enhance keratinocytes responsiveness to IFNλ by upregulating IFNλ receptor expression. Activation of keratinocytes by IFNγ also results in the upregulation of CD54, TLRs, MHC class I and II expression, production of cytokines/chemokines, such as TNFα, CXCL10, metalloproteases, such as MMP-1, and production of GBP-1 which has been shown to be highly upregulated in cutaneous lesions in CLE and is linked with reduced angiogenesis (Kajita et al., 2015; Naschberger et al., 2010).

### *1.5.5 Type III IFNs*

Type III IFNs were originally known to be produced by only keratinocytes and hepatocytes; however, our own results point to fibroblasts as an additional source (Alase, 2013) and pDCs have also been described to produce this class of IFNs. Another subset of human DCs, XCR1+ DCs, derived from CD34+ progenitor cells are recently reported to produce large amount of IFNλ (Balan et al., 2014; Tomasello et al., 2014). Whereas all nucleated cells are responsive to IFNα; only limited cell types, such as epithelial cells (keratinocytes), hepatocytes and pDCs respond to IFNλ (Dickensheets et al., 2013; Witte et al., 2009; Yin et al., 2012). Stimulation of keratinocytes with IFNλ, results in the production CXCL9 and CXCL10; which are chemoattractant for IFNγ-producing CXCR3+ T lymphocytes (Wenzel et al., 2005a). It has been shown that the expression of these chemokines and other ISGs, such as MxA correlates with the distribution of CXCR3+ T-cells in cutaneous lesions in CLE (Wenzel et al., 2005a; Wenzel et al., 2007). The overexpression of MxA is attributed mainly to the presence of pDCs in the skin lesion and high expression of IFNα (Wenzel et al., 2005a). However, it is now known that IFNλ induces MxA in keratinocytes which may contribute to the high expression of MxA in LE lesion (Alase, 2013).

### *1.5.6 IFNs, ISGs and scarring*

IFNs are known as antipoliferative and pro-apoptotic molecules. Hence, high levels of IFNs in CLE may suggest reduced angiogenesis, proliferation, increased apoptosis and consequently, dysregulation of repair mechanism. A combination of these factors may explain the permanent scarring observed in

CDLE patients. GBP-1, an ISG, has been shown to be highly expressed in lesional skin of CLE patients. Interestingly, high expression of GBP-1 has been linked with reduced angiogenesis in certain cancers (Naschberger et al., 2008). IFI16 protein, another ISG, is associated with cellular senescence in dermal fibroblasts and endothelial cells. This protein has been shown to induce growth arrest in human dermal fibroblasts by negatively regulating the *hTERT* gene (Song et al., 2010). The destruction of the stem cell niche in the bulge region of the hair follicle by cytotoxic T lymphocytes and CXCR3+ T lymphocytes containing cytotoxic granzyme B+ granules may play a major role in scarring DCLE; as these cells were found in skin biopsies from scarring CDLE lesions (Wenzel et al., 2005b; Wenzel et al., 2007).

### *1.5.7 TNFα in LE inflammation*

TNFα plays an important pathogenic role in many inflammatory skin diseases as evidenced by the success of TNFα inhibition in a range of conditions, including psoriasis and inflammatory bowel diseases. TNFα is produced by many cell types, including keratinocytes and signals through the membrane-bound TNF receptor (TNFR) made up of TNFR1 and TNFR2. The binding of TNF to TNFR1 results in the interaction with TNFR-associated death domain (TRADD). TRADD serves as a platform for recruitment of downstream mediators, including Fas-associated death domain (FADD), receptor-interacting protein-1 (RIP-1) and TNF-receptor associated factor-2 (TRAF-2). TRAF-2 is involved in the recruitment of transcription factors, such as NF-kB and JNK while FADD activates the apoptotic pathway (Postal and Appenzeller, 2011; Yu et al., 2013). TNFα is significantly increased in CLE

lesion (Popovic et al., 2005; Wittmann, 2011). Elevated TNFα production is associated with UV irradiation (Casciola-Rosen and Rosen, 1997); increased TNFα production has been shown to correlate with high membrane expression of Ro (SS-A) and La (SS-B) antigen in human keratinocytes (Dorner et al., 1995). High levels of TNFα have been reported in sera and skin lesions of SCLE patients. IL-18 induced apoptosis in keratinocytes from CLE patients has been attributed to high autocrine TNFα production by these cells (Wang et al., 2008). Another evidence implicating TNFα in the pathogenesis of CLE is the polymorphism observed at -308A TNFα promoter region (Werth et al., 2000). Many studies have shown increased serum level of TNFα in patients with active SLE (Aringer et al., 2002; Nordstrom and Eriksson, 2012) while others have either observed no significant change or that the increased serum level was present in inactive stage of the disease, which suggests a role for TNFα in the remission of the disease (Rana et al., 2012). Despite its expression in LE, cases of drug-induced lupus with increased formation of ANA have been reported in patients receiving anti-TNFα therapy (Moustou et al., 2009; Williams and Cohen, 2011). Increased titres of anti-dsDNA antibodies have also been found in rheumatoid arthritis patients receiving anti-TNFα therapy. *In vitro* studies have shown that TNFα is capable of downregulating IFNα production in pDCs (Palucka et al., 2005) and IFNλ in human dermal fibroblasts (Alase, 2013). We also observed that TNFα acts as negative regulator of IFNλ response in human primary keratinocytes. As observed in SLE patients, TNFα may act as a repressor of uncontrolled or abnormal production of IFNs in LE (Lee et al., 2009; Palucka

et al., 2005). According to available reports, TNFα may be acting as both pro and anti-inflammatory cytokine in the context of CLE.

## 1.6 Epidermal hyperproliferation and tissue repair

### *1.6.1 Psoriasis: Brief overview*

Psoriasis (psoriasis vulgaris) is an immune-mediated, chronic inflammatory skin disease affecting about 2 to 3% of people worldwide (Hao, 2014; Lowes et al., 2014). Psoriasis was initially thought to be an inflammatory disease of the skin resulting from just dysfunctional hyperproliferative epidermal keratinocytes (Raut et al., 2013). Psoriasis also affects the nails and the joints and has recently been discussed as an independent risk factor for other conditions, including coronary heart disease, depression and metabolic syndrome (Nickoloff et al., 2007). Histological pictures of psoriatic lesions show thickened epidermis (acanthosis), parakeratosis, hypogranulosis, presence of infiltrating T cells in the dermis, dilated blood vessels in the papillary dermis and presence of neutrophils and CD8+ T cells in the epidermis (Fujita, 2013). Many factors, such as injury, trauma (Koebner effect), medication, infection and Toll-like receptor 7 (TLR7) agonist, imiquimod have been identified as possible triggers for psoriasis. The pathogenesis of psoriasis is complex involving both innate and adaptive immune responses. Cytokines produced by activated T-cells and dendritic cells, such as TNF-α, IFNγ, IL-17 and IL-22 are capable of inducing inflammatory molecules and altering differentiation pathway in keratinocytes (Griffiths and Barker, 2007; Nograles et al., 2008). Despite the important role played by T helper 1 (Th1) cells in the pathogenesis of psoriasis, it is now

clear that Th17 cells play a key role in the disease and these findings are supported by clinical studies targeting IL-17 (e.g. biologics, such as secukinumab) (Fujita, 2013). IL-22 is one of the main T-cell derived mediators involved in skin pathology in psoriasis and IL-22 impacts on the production of CCL2, induction of genes involved in cell-cycle progression, such as cyclin D1 and the expression of β-defensins (HBD-2 and HBD-3) (Kubo, 2013; Lew et al., 2004). Th17, Th22 and innate lymphoid cells type 3 (ILC3) have been identified as the main producers of IL-22 in inflamed skin (Duhen et al., 2009; Eyerich et al., 2009; Trifari et al., 2009). In addition to induction of antimicrobial peptides, IL-22 triggers regenerative and proliferative programme in epithelial cells; hence, its role in epidermal hyperproliferation (Eyerich et al., 2009; Tohyama et al., 2009). STAT3 is the main transcription factor activated by IL-22 and aberrant activation of STAT3 has been reported in epidermis of psoriatic lesions (Lew et al., 2004; Sestito et al., 2011).

### *1.6.2 Interleukin 22 (IL-22)*

IL-22 is a member of the IL-10 family of cytokines; consisting of IL-10, IL-19, IL-20, IL-22, IL-24, IL-26 and IFNλ (I, II and III). IL-22 exerts its activities by binding to its heterodimeric receptor complex comprising IL-22R1 and IL-10R2, and inducing the activation of STAT3 (Wolk et al., 2005; Xie et al., 2000). Canonical IL-22 signalling pathway is shown in Figure 1.4. Activation of STAT1, STAT5 and MAPK pathways has also been reported (Lejeune et al., 2002). The cellular effect of IL-22 is thought to be restricted to hepatocytes and epithelial cells; an observation, which correlates with tissue

restricted expression of the IL-22R1 (Wolk et al., 2004; Wolk et al., 2005). Although IL-10R2 protein is ubiquitously expressed, cellular responsiveness to IL-22 is determined by the expression of IL-22R1. Leukocytes and cells of hematopoietic origin do not express IL-22R1 and are therefore non-responsive to IL-22. There is a soluble IL-22R2 referred to as IL-22 binding protein (BP), a natural antagonist, which acts as a decoy receptor and has been shown to prevent the binding of IL-22 to its receptor *in vitro* (Wolk et al., 2006) and has a binding affinity to IL-22 up to ten times higher than that of IL-22 to IL-22R1 (Wolk et al., 2007). IL-22 is produced mainly by Th17, Th22, NK22 and ILC3s (Kumar et al., 2013). High number of CD90, CCR6, CD127 and RORγt-expressing ILC3s has been reported at mucosal surfaces during infections (Van Maele et al., 2014). These cells produce large amount of IL-22 in response to IL-23 stimulation (Longman et al., 2014; Van Maele et al., 2014). IL-22 is associated with increased cell proliferation, reduced apoptosis and reduced fibrosis in liver cells. The ability of IL-22 to act as survival factor, by activating the anti-apoptotic and pro-survival pathway in hepatocytes has been reported (Radaeva et al., 2004). IL-22 promotes proliferation, migration and the production of matrix metalloproteinase (MMP)-9 in human keratinocytes, fibroblasts-like synoviocytes and gastric cancer cells through the activation of the PI3K/AKT/mTOR signalling pathway (Ji et al., 2014; Mitra et al., 2012b). IL-22 has both inflammatory/pathological and immunomodulatory roles. As a pathological agent, it is involved in active induction and recruitment of chemokines and cytokines, such as CXCL1, CXCL2, CXCL5, IL-6, IL8 and TNFα to the site of inflammation (Cobleigh and Robek, 2013). IL-22 is also involved in the recruitment of neutrophils and

MMP production. Immunoregulatory activity of IL-22 includes: induction of IL-10 and TGFβ in responsive cells (Lim and Savan, 2014). IL-22 is a unique cytokine in that it lacks autocrine and paracrine activities but rather functions as a transmitter of information from leukocytic to non-leukocytic cells. IL-22 has protective role in both humans and mouse. It enhances the proliferative ability of intestinal epithelial cells and maintains integrity of epithelial barriers by preventing dissemination of pathogenic bacteria (Zenewicz and Flavell, 2011). During hepatitis infection, IL-22 prevents liver damage by activities of IFNs and IFN-producing cells. This suggests that IL-22 is capable of counteracting or limiting the destructive effects of these cytokines (Zenewicz et al., 2007). IL-22 has been shown to be protective against fibrosis in various organs of the body (Muhl et al., 2013) and it is involved in liver cells regeneration (Brand et al., 2007). Decreased levels of IL-22 protein have been reported in the serum of SLE patients (Pan et al., 2009); however, few studies have also linked IL-22 to the pathogenesis of SLE. Yu et al. reported a relationship between genetic variation in IL-22 copy number and the risk of SLE (Yu et al., 2011). This report however requires further studies to confirm the mechanism of action of IL-22 in the pathogenesis of SLE. The “repair promoting” role of IL-22 on epithelial cells makes it a promising therapeutic molecule for conditions with abnormal scarring (Eyerich et al., 2009).

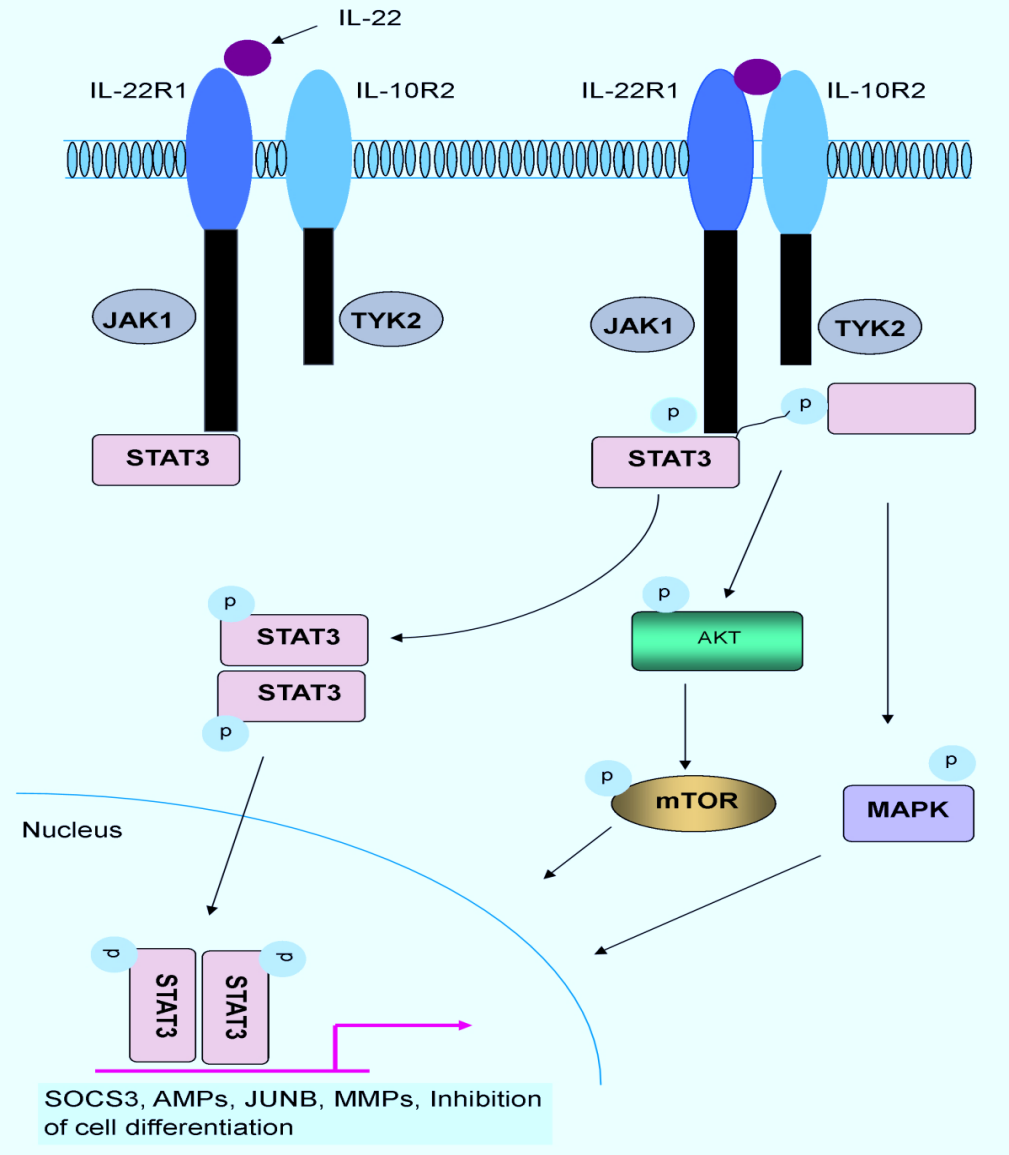



**Figure 1.4: IL-22 signalling pathway**

IL-22 binds to its heterodimeric receptor complex comprising IL-22R1 and IL-10R2. This binding results in phosphorylation Jak1 and Tyk2, leading to the activation of STAT3 and to a lesser extent the AKT/mTOR pathway. Activation of MAPK pathway (MEK1/2, ERK1/2, JNK and p38) has also been described (Pan et al., 2013). Downstream effects of IL-22 include transcription of genes such as SOCS3, MMPs, and AMPs that are involved in inflammation, proliferation, migration and angiogenesis. Adapted from (Sabat et al., 2014).

### *1.6.3 Transforming growth factor β (TGFβ) in tissue repair*

TGFβ was initially described as a secreted polypeptide with the ability to induce fibroblasts growth and collagen production (Roberts et al., 1986). TGFβ is a multifunctional cytokine that is involved in the regulation of essential cellular functions, such as apoptosis, extracellular matrix production, proliferation, angiogenesis and epithelial-mesenchymal transition (EMT) (ten Dijke and Hill, 2004). There are three isoforms of TGFβ identified in mammalian tissue: TGFβ1, TGFβ2 and TGFβ3 and they all share the same receptor (Kubiczkova et al., 2012). However, TGFβ1 is the most abundant and ubiquitously expressed isoform. TGFβ exerts its biological effect by binding to and bringing together its receptors, type I (TβRI) and type II (TβRII) receptor serine/threonine kinases on cell surface (Takekawa et al., 2011). Binding of TGFβ to its receptor, results in the phosphorylation of TβRII, which in turn activates TβRI. Activation of TβRI leads to propagation of signal through the phosphorylation of R-Smad, mainly Smad2 and Smad3, which are cytoplasmic transcription factors (Shi and Massague, 2003). These phosphorylated Smad proteins undergo heterotrimerization and form a stable heterometric complex with a common partner, Smad4 (Shi and Massague, 2003). Cellular responses to TGFβ are dependent on the cell type and physiological conditions (Kubiczkova et al., 2012). All the three isoforms (TGFβ1, 2, 3) have been described as inhibitors of proliferation in most cell types and are known to induce apoptosis in epithelial cells. However, in mesenchymal cells, such as fibroblasts, they induce proliferation and production of extracellular matrix, and they also induce fibrotic response in many tissues *in vivo* (Leask and Abraham, 2004). One of the major functions

of TGFβ is the regulation of cell cycle progression by modulating the transcription of cell cycle regulators (Kubiczkova et al., 2012). TGFβ plays a key role in wound healing and scar formation; some of which include: regulating the production of proteases, protease inhibitors, chemokines, extracellular matrix (ECM) and proliferation of stem cells (Wang et al., 2007). Foetal wound that heals without scar formation has been shown to express reduced and transient amount of TGFβ isoforms and their receptor in comparison to adult wounds (Cowin et al., 2001).

## 1.7 Some regulatory molecules and signalling pathways involved in inflammation

### *1.7.1 Silencer of cytokine signalling (SOCS)*

Suppressor of cytokine signalling (SOCS) proteins are intracellular molecules, which regulate the responses of immune and tissue cells to cytokines. SOCS were initially discovered as inhibitory regulators of the cytokine-induced JAK-STAT pathway of cytokine receptor signalling. SOCS proteins are unstable and are encoded as early response genes. Most of these proteins are induced by cytokine; hence, they act as negative regulators of cytokine signalling. The SOCS proteins are not highly expressed in unstimulated cells or tissues, but are encoded by highly inducible genes, which are rapidly transcribed upon exposure to cytokines, often in a phosphorylated STAT-dependent manner (Alexander, 2002).

#### *1.7.1.1 SOCS1 and SOCS3*

SOCS1 and SOCS3 are the best characterised members of the SOCS protein family. SOCS1 is a natural antagonist of the STAT1 signalling pathway and it is inducible by IFNs as a negative feedback mechanism (Tomasello et al., 2014). SOCS3 carries out its inhibitory function by first binding to the receptor and then interacts with phosphorylated activation loop and catalytic pocket through the src homology (SH) domain and kinase inhibitory region (KIR) respectively (Yoshimura et al., 2007). A similar mechanism of interaction between SOCS1 and JAK2 is proposed for the inhibition of IFNγ signalling. However, another mechanism involving KIR binding to the surface of JHI rather than catalytic pocket and inhibiting phosphate transfer has been reported (Babon et al., 2012). SOCS3 is able to directly inhibit the catalytic domain of JAK1, JAK2 and TYK2 but not JAK3 (Inagaki-Ohara et al., 2013). SOCS3 acts mainly as regulator of STAT3 activation in response to cytokines using glycoprotein 130 (gp130) and other related receptors. gp130 is a component of the receptor complex for cytokines belonging to the IL-6 family, such as IL-6, IL-27, oncostatin M (OSM), leukaemia inhibitory factor (LIF) and IL-11 (Carow and Rottenberg, 2014; Yoshimura et al., 2007). SOCS3 is also capable of inhibiting STAT3 activation in response to cytokines, growth factors and hormones in a gp130 independent manner. SOCS3 also interacts with other receptors that do not activate STAT3 (Carow and Rottenberg, 2014). Despite the ability of SOCS3 to bind and inhibit JAK catalytic activity, its preference for binding to gp130 receptor has been demonstrated in mice (Bergamin et al., 2006). STAT3 activation is essential in Th17 differentiation; however, SOCS3 has been

identified as a major negative modulator of IL-23-mediated STAT3 phosphorylation during Th17 generation (Chen et al., 2006; Milner et al., 2008). High expression of SOCS3 has been reported in synovial fibroblasts of rheumatoid arthritis (RA) patients and skin of patients with psoriasis (Madonna et al., 2012; Shouda et al., 2001). Although SOCS3 is well known as a negative regulator of STAT3 signalling, its role in the regulation of STAT1 pathway has also been reported. SOCS3 deficient cells showed increased expression of STAT1 signature genes in response to IL-6 (Croker et al., 2003). Herpes simplex virus (HSV) induced SOCS3 expression has been shown to suppress type I IFN production and responses (Yokota et al., 2005). SOCS3 also acts as negative regulator of IFNβ response in macrophages (Carow and Rottenberg, 2014).

### *1.7.2 Mitogen activated protein kinases (MAPK)*

There are fourteen (14) MAPKs described so far in mammalian cells; however, three (3) main families- extracellular signal-regulated kinases (ERK; ERK 1 and 2), the p38 MAPKs (α, β, δ and γ) and the c-Jun NH2-terminal kinases (JNK; JNK1, JNK2 and JNK3) have been studied extensively (Arthur and Ley, 2013; Chang and Karin, 2001). The MAPK pathway is one of the most evolutionary conserved, playing key roles in both innate and adaptive immunity (Zhang and Dong, 2005). The pathway was initially described as proteins activated by growth factors (Krzyzowska et al., 2010) and they control fundamental cellular processes, such as proliferation, cellular survival, apoptosis, metabolism, mitosis and differentiation. MAPK signalling cascade is organised into three tiers: MAPK kinase kinase (MAP3K), MAPK kinase

(MAP2K) and MAPK, which are sequentially activated in order to achieve downstream gene expression (Meister et al., 2013) as shown in Figure 1.5.

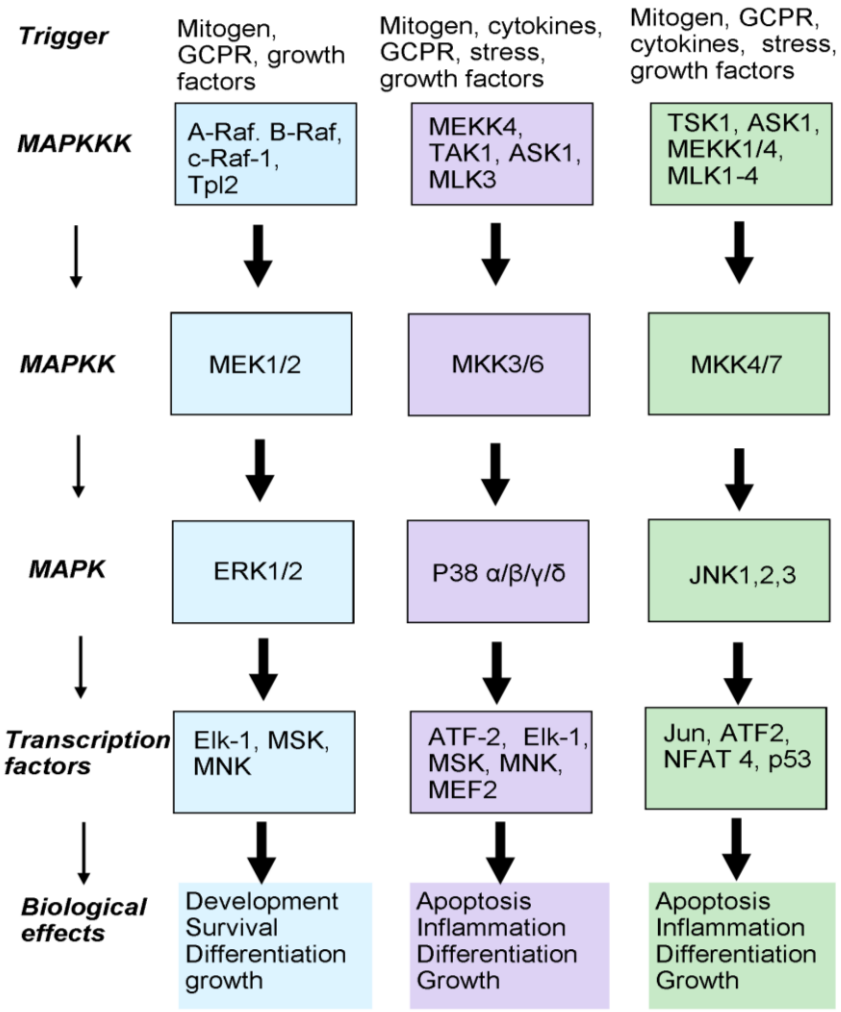



**Figure 1.5: MAPK cascades**

There are three main mammalian MAPKKK–MAPKK–MAPK protein kinase cascades. The ERK pathway is commonly activated by growth factors while the JNK and p38 pathways are activated by environmental stress, including osmotic shock, ionizing radiation. Many nuclear transcription factors, such as MNK, MEFS and NFAT4 are substrate for MAPKs. The activation of these transcription factors results in nuclear translocation and subsequent biological effects, such as proliferation, apoptosis, inflammation and growth. Adapted from (Morrison, 2012; Roberts and Der, 2007).

#### *1.7.2.1 Extracellular signal-regulated protein kinase (ERK)*

ERK1/2 are ubiquitously expressed proteins that form part of the Ras-Raf-MEK-ERK signalling cascade with important roles in cellular processes, such as cell survival, cell adhesion, cell migration, transcription, cell proliferation, differentiation and cell cycle progression (Roskoski, 2012a). The MAP3K component of ERK signalling cascade is the Raf serine/threonine kinase (c-Raf-1, A-Raf and B-Raf), which is activated by Ras-GTP. Raf kinases phosphorylate and activate MEK1 and MEK2. Activation of these dual-specificity protein kinases results in the downstream phosphorylation of tyrosine and threonine in ERK1/2, their only known substrate (Roskoski, 2010). While it is known that Raf kinase and MEK1/2 have restricted substrate specificity, activation of ERK1/2 results in the phosphorylation and activation of over 200 cytoplasmic and nuclear substrates (Roskoski, 2012b). It is well known that the activation of ERK1/2 results in inhibition of apoptosis; particularly in response to: TNF, Fas ligand, osmotic stress, hydrogen peroxide and radiation (Lu and Xu, 2006). Inhibition of ERK1/2 has been shown to promote apoptosis; however, the mechanism by which ERK1/2 inhibit apoptosis is somewhat complicated and depend on cell type. One mechanism by which ERK promotes cells survival is by enhancing the activity of anti-apoptotic molecules, such as Mcl-1, a member of the Bcl-2 family (Domina et al., 2004). ERK1/2 is also capable of regulating the activities of anti- and pro-apoptotic transcription factor, such as cAMP response element binding protein (CREB) (Bonni et al., 1999). Although ERK1/2 are well known as pro-survival factor, they can also function in a pro-apoptotic manner. It has been suggested that ERK1/2 activation is involved in neurodegeneration and

inhibition of ERK1/2 pathway attenuates apoptosis and G2-M arrest induced by mitogen or UV (Lu and Xu, 2006).

#### *1.7.2.2 P38 MAPK*

P38 was discovered as evolutionarily conserved protein kinase that can be activated by lipopolyssacharide (LPS) and IL-1 (Han et al., 1994; Lee et al., 1994). Activation of the p38 signalling pathway can be triggered by a variety of stimuli; hence, the receptor and downstream substrate are diverse. MAP3Ks involved in the activation of p38 signalling include: apoptosis signal-regulating kinase (ASK) 1, TAK1, MTK1 and mixed lineage kinase (MLK) 2/3 (Yang et al., 2014). Phosphorylation and activation of these kinases result in activation of MAP2Ks, such as MKK3 and MKK6 which are directly involved in the activation of p38. Transcription factors activated downstream of p38 include: Elk1, STAT1, MAPK-activated protein kinase 2/3 (MK2/3), p53 and ETS (Morrison, 2012). Activation of the p38 pathway contributes to inflammation, cytokine production, cell differentiation, cell cycle regulation, cell proliferation and apoptosis (Cuadrado and Nebreda, 2010; Yang et al., 2014). In human skin fibroblasts, activation of p38 has been shown to lead to rapid dephosphorylation of MEK1/2 resulting in apoptosis. On the contrary, inhibition of p38 prevents dephosphorylation of MEK1/2 and apoptosis (Li et al., 2003). Uncontrolled activation of p38, particularly in the absence of mitogen stimuli results in apoptosis, whereas, inhibition of this pathway confers resistance to stress-induced cell death (Takekawa et al., 2011). P38 also plays a role in mRNA stability of some cytokines, such as TNFα, IL-6 and IL-1β (Yang et al., 2014).

#### *1.7.2.3 c-Jun N-terminal Kinases (JNK)*

JNK pathway is activated by stress factors (heat, oxidative stress and DNA damage), cytokines and growth factors. The JNK stress pathway is involved in intracellular processes, such as apoptosis and cell survival (Dhanasekaran and Reddy, 2008; Morrison, 2012). JNK signalling cascade involves the Rho family of GTPases, Cdc42 and Rac (Johnson and Nakamura, 2007). JNKs are directly activated by dual-specificity kinases, MKK4 and MKK7, and their activation lead to downstream phosphorylation and activation of c-Jun and ATF2 transcription factors and Bcl family members (Krzyzowska et al., 2010; Lei and Davis, 2003).

## 1.8 **Hypothesis**

This project is placed in the overall context of improving our knowledge of CLE pathogenesis and in particular scarring versus a non-scarring outcome of CLE inflammation.

Experimental designs for this work were based on the following **hypothesis:**

IL-22 is a key molecule that strongly activates STAT3 which is actively involved in normal wound healing. IFNs on the other hand are strong activators of STAT1 signalling, which has been implicated in increased apoptosis and reduced cell proliferation. CLE, in particular CDLE is characterised by increased apoptosis and enhanced production of IFNs and ISGs. Based on the understanding of the fact that IL-22 and IFNλ share a second receptor, IL-10R2, and the contrasting roles of these cytokines with regards to proliferation, it was hypothesised that the balance between IL-22/STAT3 and IFNλ/STAT1 pathways may be very important in determining scarring and non-scarring outcomes in inflammatory skin diseases.

## 1.9 **Aims**

The specific **aim of this project** was to investigate the regulation and influence of IL-10 family members on epidermal and dermal responses which could influence the overall outcome of inflammation resolution and repair.

This aim can be sub-divided as follows:

- To understand how IFNλ and IL-22 are produced, regulated and functionally impact on dermal and epidermal cells.
- To understand the interaction between IL-22, STAT3 signature genes, IFNs and ISGs in the context of inflammatory and repair related cutaneous responses.
- To understand, if any, the counter regulatory effect of IL-22 versus IFNλ on signalling and downstream activities in skin resident cells.

# CHAPTER 2: MATERIALS AND METHODS

# 2 Materials and Methods

## 2.1 Ethics

Keratinocytes and dermal fibroblasts were isolated and cultured from skin tissue from individuals undergoing cosmetic surgery (face lift). Patients gave informed written consent and ethical approval was granted by the University of Bradford ethics committee. Skin tissue was prepared on the day of collection. CLE keratinocytes were hair-derived and provided from Hannover Medical School, Hannover, Germany. Patient derived biopsies were collected and processed in Leeds under the CONVAS ethics (REC 10/H1306/88).

## 2.2 Reagents/cytokines

Phosphate buffered saline (PBS) (Gibco/life technologies, Paisley, UK), Ethelenediaminetetraacetic acid (EDTA) (Lonza, Slough, UK), 0.25% trypsin (Gibco), trypsin/EDTA (TE) (Lonza), Trypsin neutralising solution (TNS) (Lonza), Penicillin/Streptomycin and Fetal bovine serum (FBS) (PAA/GE healthcare, Sommerset, UK), Dulbecco's modified eagle's medium (DMEM) (PAA), Keratinocytes growth medium (KGM) with supplements (Promocell, Heidelberg, Germany), CryoSF freezing medium (Promocell), Fetal calf serum (promocell), recombinant human (rh) IFNα2a (Merck/Calbiochem, Merck Serono Ltd, Middlesex, UK), rhIFNλ1 and rhIL-22 (ebioscience, Hatfield, UK), rhIFNγ and rhTNFα (Immunotools, Friesoythe, Germany), poly I:C and poly dA:dT (Invitrogen, Life Technologies), rhTGFβ1 (Peprotech, London, UK). Primers were purchased from Qiagen (Manchester, UK) and Sigma-Aldrich (Dorset, UK). Antibodies were purchased from Abcam

(Cambridge, UK), Cell signaling (Hitchin, Hertfordshire, UK), RnD systems (Abingdon, UK), Santa Cruz Biotechnology, Insight Biotechnology (Middlesex, UK) and Biolegend (London, UK).

**Table 2.1: List of primers**

| Primer | Sequence or ID | Company |
|---|---|---|
| Col1A1 | QT00037793 | Qiagen |
| Col3A1 | QT00058233 | Qiagen |
| Col4A2 | QT01329461 | Qiagen |
| Col7A1 | QT00998886 | Qiagen |
| CXCL-9 | QT00013461 | Qiagen |
| CXCL10 | QT01003065 | Qiagen |
| GBP-1 | QT00011641 | Qiagen |
| IFI16 | QT00066675 | Qiagen |
| IFNλ1 | QT00222495 | Qiagen |
| IFNλR1 | QT00034440 | Qiagen |
| IL-22 | QT00034853 | Qiagen |
| IL-22R1 | QT00018550 | Qiagen |
| IL-22R2 | QT00021539 | Qiagen |
| JUNB | QT00201341 | Qiagen |
| MxA | QT00090895 | Qiagen |
| SOCS1 | QT00202475 | Qiagen |
| SOCS3 | F-5'-AGACTTCGATTCGGGACCA-3’<br>R-5’-AACTTGCTGTGGGTGACCA-3’ | Sigma |
| VEGF-A | QT01010184 | Qiagen |
| U6 | F- 5’ CTCGCTTCGGCAGCACA 3’<br>R- 5’ AACGCTTCACGAATTTGC 3’ | Sigma |

**Table 2.2: Primary antibodies used**

| **Antibodies** | **Source** | **Clone** | **Company** |
|---|---|---|---|
| GAPDH | Mouse | Monoclonal | Santa Cruz |
| IFNλR1 (Western blotting) | Rabbit | Polyclonal | Abcam |
| IFNλR1 (PE; flow cytometry) | Mouse | Monoclonal | Biolegend |
| IL-22R1 (Western blotting) | Rabbit | Polyclonal | Abcam |
| IL-22R1 (APC; Flow cytometry) | Mouse | monoclonal | RnD |
| MxA(ab198178) | Rabbit | Polyclonal | Abcam |
| P38(#9212) | Rabbit | Polyclonal | Cell signaling |
| Phospho p38(#9311) | Rabbit | Polyclonal | Cell signaling |
| p42/44 (ERK) (#9102) | Mouse | Monoclonal | Cell signaling |
| Phospho p42/44 (ERK)(E10,#9106) | Mouse | Monoclonal | Cell signaling |
| Phospho STAT1(#9171) | Mouse | Monoclonal | Abcam |
| STAT1(#9172) | Rabbit | Polyclonal | Cell signaling |
| Phospho STAT3(#9131) | Rabbit | Polyclonal | Cell signalling |
| STAT3(#4904) | Rabbit | Polyclonal | Cell signaling |

**Table 2.3: Secondary antibodies used**

| Antibodies | Source | Company |
|---|---|---|
| Donkey anti-mouse HRP | Donkey | Santa cruz |
| Donkey anti-rabbit HRP | Donkey | Santa cruz |

## 2.3 **Cell culture**

### *2.3.1 Keratinocytes isolation*

Skin samples from cosmetic surgery were stripped of fat using scissors or razor blades and were cut up into approximately 1.0 $cm^2$ pieces. Cut skin samples were thoroughly washed in PBS. Skin was incubated overnight submerged (epidermis side up) in 0.25% Trypsin at 4°C and then heated to 37°C for 30 minutes prior to removal of the epidermis. The epidermis was removed and incubated for 5 minutes at 37°C in a water bath in 1 ml 0.05% Trypsin/EDTA (TE) and vortexed for 1 minute thereafter. The resulting trypsinised cell suspension was carefully aspirated and transferred into serum-containing media to neutralise TE. Incubation in TE was repeated twice more. Cells- containing suspension was centrifuged for 5 minutes at 1000 rpm to pellet cells. The cell pellet was resuspended in KGM-2/DMEM (ratio 2:1) and cells were seeded in T75 flask. DMEM contained 5% or 10% fetal calf serum (FCS; Promocell) or fetal bovine serum (FBS; PAA). Alternatively, cell pellet was resuspended in T75 flask coated with type IV collagen (Sigma Aldrich, Dorset, UK). Media was replaced with complete KGM two days after initial seeding and cells were fed every 2-3 days until subconfluent.

### *2.3.2 Fibroblasts isolation*

After fat removal (as above) the skin sample was placed in a 6-well plate with the dermis facing down. In skin samples where the epidermis was removed, the remaining ‘dermal’ component was flipped over such that the sub-

epidermal part was allowed to touch the surface of the T75 culture flask. 1 ml or 10 ml of DMEM containing 4.5 g/L glucose and L-Glutamine (Lonza) and supplemented with 10% FCS or FBS, 0.5 mg/ml streptomycin and 100 U/ml penicillin was added depending on whether a plate or culture flask was used. The plate or flask was placed in 37$^{o}$C, 5% CO2 incubator for fibroblasts to grow out. Culture medium was changed every 2-3 days or after 5 days for dermis cultured in a T75 flask. Fibroblasts began to grow out after 7 days of continuous culture. Cells were then trypsinised and cultured in a T75 culture flask. Cells were usually used between passages 2 and 6.

## 2.4 **Cells passaging**

Cells in culture flasks were usually split when they reached between 80 and 90% confluence. Cells were washed under the safety cabinet with 5-10 ml pre-warmed (PBS, Gibco), depending on the size of flask, by shaking the flask gently for about 4 times. PBS was removed and 3 ml or 5 ml of TE for T75 and T225 respectively was added to the cells. The flask was transferred to the incubator for about 5 minutes in order to detach cells from the flask. For keratinocytes, cells were first treated with 5 ml EDTA and flask was transferred to the incubator for 5 minutes in order to break cell-cell interaction. At this stage, the cells have become single cells and round. EDTA was removed and TE added as above but for about 3 minutes. TNS or serum containing DMEM was added to keratinocytes and fibroblasts respectively in order stop the activity of trypsin. Amount added was equal to the volume of TE. Cells were transferred into 15 ml Falcon tube and centrifuged at 1500

RPM for 3 minutes. Cell pellet was either resuspended in culture medium (and plated for experiments or further culture) or frozen as described below.

## 2.5 **Freezing and thawing of cells**

Cell suspension obtained in the context of cell passaging was transferred to a 15 ml Falcon tube. Cells were centrifuged at 1500 RPM for 3 minutes. Supernatant was discarded and CryoSFM freezing medium (1 ml per 1-2 $x10^6$ cells) was added to the cell pellet. Cells were resuspended and transferred into the freezing vial. The vial was transferred into Mr. Frosty which was taken to the -80$^oC$ freezer overnight before cells were transferred into the liquid nitrogen (196$^oC$) until needed.

To thaw cells, vials containing the cells were left in 37$^oC$ water bath until almost completely thawed but with a little bit of ice still floating in the vial. Cells were transferred into already pre-warmed culture medium in a culture flask and the flask transferred into the incubator at 37$^oC$, 5% CO2 for the cells to attach to the flask and to continue proliferating until ready for the next passage.

## 2.6 **RNA isolation from cells**

Total RNA was isolated using quick RNA mini-prep kit (Epigenetics, Cambridge bioscience, Cambridge, UK). Cells were lysed with 600 µl ZR RNA buffer and the lysate was transferred into the Zymo spin IIIC column in a collection tube. This was centrifuged at 13000 g for 1 minute and the eluted liquid was discarded. This was repeated with 400 µl RNA pre-wash buffer (13000 g for 1 minute), 700 µl RNA wash buffer (13000 g for 30 seconds)

and 400 µl RNA wash buffer 13000 g for 30 seconds). Thereafter, the Zymo spin column was centrifuge at 13000 g for 2 minutes in order to ensure complete removal of the wash buffer. The spin column was placed in a RNase-free tube and 35 µl of DNase/RNase free water was added directly to the column matrix and allowed to stand at room temperature for 1 minute. This was centrifuged at 13000 g for 30 seconds. The eluted RNA was either stored at 70$^{o}$C or used immediately in reverse transcriptase (RT) reaction.

## 2.7 **RNA isolation from animal tissue**

RNA was isolated from tissue biopsies using the Animal tissue RNA purification kit (Norgen Biotek, Geneflow limited, Lichfield, UK). Samples were initially embedded in optimal cutting temperature (OCT) compound. Embedded skin tissue was cut into 100 µm shaves using cryostat. Excess OCT was removed physically using scalpels at -20$^{o}$C. The shaved sample was then homogenised by adding 300 µl of buffer RL and sample was ground or vortexed vigorously until homogenised. Lysate was transferred into RNase-free microcentrifuge tube. 600 µl RNase-free water was added to the lysate which was then vortexed. 20 µl of proteinase K was added to the lysate and was incubated at 55$^{o}$C for 15 minutes with intermittent mixing (vortex). The lysate was then spun for 1 minute to pellet any cell debris. The obtained supernatant was transferred to a new RNase-free microcentrifuge tube. 450 µl of 100% ethanol was added to the lysate which was submitted to vortexing. 650 µl of lysate with ethanol was transferred into a spin column placed on top of a collection tube. The column was centrifuged at 3500 g for 1 minute. Eluted liquid was discarded and the step was repeated using 400

µl of wash solution A (centrifuged for 2 minutes). Thereafter the collection tube was placed in a new collection tube. 100 µl of enzyme incubation buffer A and 15 µl of DNase I were added to the column and centrifuged at 14000 g for 1 minutes. The eluted liquid was put back into the column and was incubated at room temperature for 15 minutes. 400 µl of wash solution A was added to the column containing DNase I mix and was centrifuged for 1 minute. Eluted liquid was discarded and the step was repeated with the addition of 400 µl of wash solution A. The column was then centrifuged for 2 minutes in order to thoroughly dry the resin. The column was placed into a fresh 1.7 ml elution tube and 50 µl of elution solution A was added to the column, which was then centrifuged for 2 minutes at 200 g and subsequently for 1 minute at 14000 g. Eluted RNA was stored at -70$^{o}$C or reverse transcribed immediately to cDNA.

## 2.8 **Reverse transcriptase reaction**

Total RNA reverse transcription was carried out using first strand cDNA synthesis kit (Fermentas/ Thermo Fisher Scientific, Loughborough, UK). 10 µl of total RNA was transferred to 0.2 ml PCR tube and 1 µl oligo $(dT)_{18}$ primer (15 pmol) was added to it. The tube was heated at 65$^{o}$C for 5 minutes and the following were added to make up a total volume of 20 µl: 4 µl 5x reaction buffer, 1 µl ribolock RNase inhibitor (20 U/µl), 2 µl dNTP, 2 µl MuLV reverse transcriptase (20 U/µl). The tube was centrifuged at 13000 g for 10 seconds and the RT reaction was run as follows: 42$^{o}$C for 60 minutes, 70$^{o}$C for 5 minutes to inactivate the enzymes. The reaction was cooled down at 4$^{o}$C.

cDNA from the reaction was either stored at -70$^{o}$C or used immediately in qRT-PCR reaction.

## 2.9 **Quantitative real time- polymerase chain reaction (qRT-PCR)**

Quantitech primers were purchased from Qiagen except for SOCS3 and U6snRNA primers, which were purchased from Sigma (Table 2.1). cDNA amplification was carried out using QuantiFast qRT-PCR kit (Qiagen) and run in BioRad cfx connect machine (BioRad). The total reaction volume of 20 µl was made up of: 10 µl SYBR green mix (1x final concentration), 6 µl DNase/RNase free water, 2 µl primer (1 µM final concentration) and 2 µl cDNA (<100 ng/reaction). The reaction was run using the following parameters: Initial heat activation, 95$^{o}$C for 5 minutes; denaturation, 95$^{o}$C for 10 seconds; combined annealing and elongation, 60$^{o}$C for 30 seconds for a 40 cycle run. mRNA expression of the gene of interest was normalised to U6snRNA mRNA using the ΔΔCT relative quantitation method.

## 2.10 **Cytokine measurement**

Cell culture supernatants were collected after 24 or 48 hours of continuous stimulation of cells and were analysed for secreted IFNλ1, CXCL10 or CCL2 using commercially available sandwich ELISA kits following manufacturers' protocols (IFNλ1, CCL2; ebioscience) and RnD systems (CXCL10; Abingdon, UK). Maxisorp flat-bottom 96-well plates (Nunc, Thermoscientific, Cramlington, UK) were coated with 100 µl capture antibody overnight at 4$^{o}$C or room temperature. Coated plates were then washed with at least 250 µl of wash buffer (0.05% tween-20; Sigma) in PBS per well for a total of 4 or 5

washes. Plates were blocked with 200 µl block buffer (1% FCS in PBS) or reagent diluent (eBioscience). Supernatants were then added and left at room temperature for 2 hours. The plates were washed with wash buffer as previously done. Detection antibody was then added for either 1 or 2 hours depending on the manufacturer's protocol. Plates were washed with wash buffer and then 100 µl of streptavidin (RnD) or avidin (ebioscience) was added to the wells for 20 or 30 minutes respectively. Plates were washed again and 100 µl substrate solution was added to each well for between 10 and 20 minutes before stopping the reaction with 50 µl 2N $H_2SO_4$. Absorbance of each well was measured at 450 nm and corrected at 570 nm using ELISA plate reader and data collected using Gen5 software (Biotek, Potton, UK) or Magellan 6, 200 series (Tecan, Theale, UK).

### 2.11 **Flow cytometric analysis**

Human primary keratinocytes or dermal fibroblasts were cultured in 6-well plates (Greiner bio-one, Stonehouse, UK). To detach cells for flow cytometry, culture supernatants were removed and cells washed with 500 µl warm 1x PBS without $CaCl_2$ and $MgCl_2$ (Gibco/life technologies, Paisley, UK). Cells were then detached from the culture plate using 250-500 µl Hyqtase (Thermo Scientific) or trypsin/EDTA (Lonza) and the reaction was stopped using equal volume of trypsin neutralising solution (Lonza). Cells were washed once with ice-cold PBS. For surface staining, cells were washed twice with FACS buffer (5% BSA/PBS) and centrifuged at 350 g for 5 minutes. Cells were stained with APC-conjugated mouse anti-human IL-22R1 antibody (1:10; R&D systems) for 30 minutes at 4$^{o}$C. Cells were then washed twice with FACS

buffer and resuspended in 400 µl FACS buffer for analysis. For intracellular staining, cells were fixed by resuspending cell pellet in fixation/permeabilization buffer (ebioscience) and centrifuged at 500 g for 5 minutes. Cells were then resuspended in permeabilization buffer (ebioscience) for 30 minutes at 4$^{o}$C. Cells were washed three times with FACS buffer and centrifuging at 500 g for 5 minutes after each wash. Cells were stained with APC-conjugated mouse anti-human IL-22R1 antibody (RnD systems) or PE-conjugated mouse anti-human IFNλR1 antibody (Biolegend) in FACS buffer for 30 minutes at 4$^{o}$C. Appropriate APC-conjugated (RnD systems) or PE-conjugated isotype (Biolegend) controls was used. Cells were washed two times with FACS buffer and centrifuged at 500 g for 5 minutes. Cells were resuspended in FACS buffer ready for analysis. For flow cytometry, BD LSRFortessa (BD Biosciences, Oxford, UK) was used. Data collected were further analysed using FACSdiva software (BD Biosciences).

### 2.12 **Protein concentration determination**

Protein concentrations in cell lysates were determined by Bradford assay (Pierce/Thermo Fisher Scientific, Cramlington, UK) using manufacturer's protocol. Titrated concentrations of bovine serum albumin were used as standard. 5 µl of standards and lysates were aliquoted into a flat bottomed 96-well plate (Nunc). 200 µl Coomassie protein reagent was added to the wells. The intensity of colour change from violet red to blue indicated the amount of protein present. Absorbance was measured at 560 nm using Magellan 6, 200 series (Tecan).

## 2.13 **Western blotting**

Keratinocytes and dermal fibroblasts cultured in 6-well plates were lysed with CelLytic M lysis buffer (Sigma-Aldrich) containing protease inhibitor cocktail (Roche Applied Bioscience, Rotkreuz, Switzerland) to prevent protein degradation and phosphatase inhibitor (Thermo Scientific) to preserve phosphorylated proteins. Protein concentration was determined by Bradford assay (Thermo Scientific). 30 µg of total protein was separated according to their molecular weight using 4-20 % gradient (any kDa) mini protean gel (BioRad). Proteins were blotted onto 0.2 µm PVDF trans-blot pack (BioRad) and membrane blocked in 5% dried semi-skimmed milk in PBS or TBS containing 0.1% Tween-20 (PBST) for 1 hour on a shaker at room temperature. For phosphorylated experiment, membrane was 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$^{o}$C with gentle shaking. Rabbit anti-human MxA (1:1000), rabbit anti-human IL-22R1, rabbit anti-human IFNλR1 (1:1000) and GAPDH were used in 5% milk PBST overnight at 4$^{o}$C with gentle shaking. Donkey anti-rabbit and donkey anti-mouse HRP-conjugated secondary antibodies were used at 1:5000 and 1:3000 respectively in 5% milk PBST or 0.5% TBST for 1 hour at room temperature. Membranes were incubated in enhanced chemiluminiscence (ECL; BioRad) for 2 to 5 minutes at room temperature and protein bands were detected using Chemidoc imaging system (Bio-Rad). For a repeat Western, membranes were stripped at room temperature for 15 minutes

using stripping buffer (Thermo scientific) and blocked for 30 minutes prior to probing with primary antibody.

### 2.14 **Scratch assay**

Keratinocytes or dermal fibroblasts were seeded into 24-well plates (Greiner bio-one) and allowed to grow to 90-95% confluence. Cells were starved in KGM without hydrocortisone and EGF for keratinocytes or DMEM without serum for dermal fibroblasts for 24 hours. Using 200 µl yellow pipette tips (Greiner bio-one), a single line scratch was created in each well of the 24-well plates containing cells. Cells used for migration experiments were treated with 5 µg/ml mitomycin C for 2 hours before scratching in order to stop cell proliferation. Cells were washed twice with warm PBS in order to remove cell debris. Cells were covered with culture media depending on the growth condition to be examined. For keratinocytes, the four culture conditions were: Full growth medium containing hydrocortisone, EGF (+/+) and KGM without hydrocortisone (+/-), KGM with EGF (-/+) and KGM without hydrocortisone and EGF (-/-). Keratinocytes were then either untreated or treated with IL-22 or IFNλ1 while fibroblasts were treated with IL-22, IFNα or IFNλ1 for 48 hours with images of the cells taken at 0, 24 and 48 hours using 4x objective on a Zeiss microscope and Nikon camera. Results were analysed using Wimscratch software (Wimasis, Munich, Germany) or Tscratch software (www.cse-lab.ethz.ch/software.html).

## 2.15 **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 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 (0 hour) analysis. Cells were trypsinised 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). The different colours used represented different generations of daughter cells that have taken up the cell trace dye. A shift to the left indicated proliferation.

## 2.16 **Statistical analysis**

Data were analysed for statistical significance using analysis of variance (ANOVA; and compared columns using Bonferroni), when the graph involves multiple comparison or *t*-test, when data are normally distributed and only two sets of data are compared or Mann Whitney U test, when data are not

normally distributed and only two sets of data are compared on Graphpad prism statistical analysis software (GraphPad Prism 6, GraphPad Software, San Diego, CA). ‘n’ represents number of independent experiments. All data are expressed as means ± SEM. Values of $p< 0.05$ were considered significant. *p< 0.05, **p< 0.01, ***p< 0.001, ****p<0.0001.

# CHAPTER 3: IL-22 AND IFNλ SIGNALLING IN HUMAN PRIMARY KERATINOCYTES: IMPLICATIONS FOR THE PATHOGENESIS OF CUTANEOUS LUPUS ERYTHEMATOSUS

# 3 IL-22 and IFNλ signalling in human primary keratinocytes: Implications for the pathogenesis of cutaneous lupus erythematosus

## 3.1 Introduction

IL-22 and IFNλ are members of the IL-10 family of cytokines. Well described functional effects of IFNλ (STAT1 activation) include: inhibition of proliferation and induction of apoptosis (Maher et al., 2008; Regis et al., 2008). By contrast, IL-22 (STAT3 activation) is involved in the following processes: cell migration, proliferation, inhibition of differentiation, angiogenesis and prevention of fibrosis (Kumar et al., 2013; Muhl et al., 2013). The role of IL-22 in the pathogenesis of hyperproliferative diseases, such as psoriasis is well described (Boniface et al., 2007; Hao, 2014; Sabat et al., 2014) and increased production of IL-22 in psoriatic lesion by infiltrating CD4+ T effector cells has been reported (Boniface et al., 2007; Wilson et al., 2007). ILC3 were identified recently as one of the main producers of IL-22 and these cells have been implicated in the pathogenesis of psoriasis (Kim, 2015; Villanova et al., 2014). Elevated serum levels of IL-22 has been identified as a biomarker for psoriasis and a significant decrease in the amount of this cytokine, as well as IL-6, paralleled clinical improvement (Cordiali-Fei et al., 2014; Michalak-Stoma et al., 2013). The high expression of IL-22 in psoriatic lesion has been linked with downregulation of genes associated with terminal differentiation in keratinocytes, such as involucrin, loricrin and calmodulin. IL-22 induces the expression of 'STAT3 signature genes', including SOCS3 and JUNB (Brand et al., 2006). SOCS3 has been described to be highly

upregulated in the basal and suprabasal layers of psoriatic skin lesion (Federici et al., 2002). SOCS3 in particular has been shown to be involved in the inhibition of apoptotic pathway in psoriasis (Bruun et al., 2009; Madonna et al., 2012; Tomita et al., 2011) and it acts as negative regulator of the JAK/STAT1/3 signalling pathway (Dalpke et al., 2008; Zeitvogel et al., 2012). IFNλ induces SOCS1; a negative regulator of the JAK/STAT3 pathway and the JAK/STAT1 pathway (Brand et al., 2005). Both *in vitro* and *in vivo* experiments demonstrated a protective role and damaging effect for STAT3 and STAT1 respectively (Barry et al., 2007; Stephanou, 2004). The relative abundance, activation and cross regulation of STAT1 and STAT3 play crucial roles in determining cellular responses to a variety of cytokines and other proteins (Hong et al., 2002). STAT1 acts as negative regulator of the expression of pro survival genes, such as Bcl-xL and Bcl-2 and has been shown to favour apoptosis (Stephanou et al., 2000; Wang et al., 2000). In addition, activation of STAT1 by IFNs results in the upregulation of proinflammatory genes, including ICAM1, CXCL9 and CXCL10. ICAM1 helps with increased leukocytes adhesion to endothelial cell; while CXCL9 and CXCL10 act as chemoattractants for cells of hematopoietic origin into the tissue (Regis et al., 2008). STAT3 is involved in the induction of anti-apoptotic genes of the Bcl family (Regis et al., 2008). Based on the possibility for competition for the usage of the shared second receptor, IL-10R2, and the induction of negative regulators for the JAK/STAT1 pathway, SOCS3, it was hypothesised that IL-22 could be a negative regulator of IFNλ signalling and activities in human primary keratinocytes; hence, of potential therapeutic role in CLE.

## 3.2 **Results**

### *3.2.1 Nucleic acids induce the production of IFNλ protein in human primary keratinocytes*

IFNλ plays an important role in the pathogenesis of CLE and it is the main IFN produced by keratinocytes. In order to understand how IFNλ is produced in keratinocytes, cultured human primary keratinocytes from healthy individuals or CLE patients were stimulated with cytokines or nucleic acids for 48 hours and supernatants were collected for IFNλ1 ELISA. The results showed that in both healthy and CLE keratinocytes, only nucleic acids, such as poly I:C and poly dA:dT were able to induce the production of detectable IFNλ1 levels (Fig. 3.1A-B). Moreover, the available CLE keratinocytes seemed to produce up to 4-fold more IFNλ1 protein than healthy keratinocytes (Fig. 3.1B).

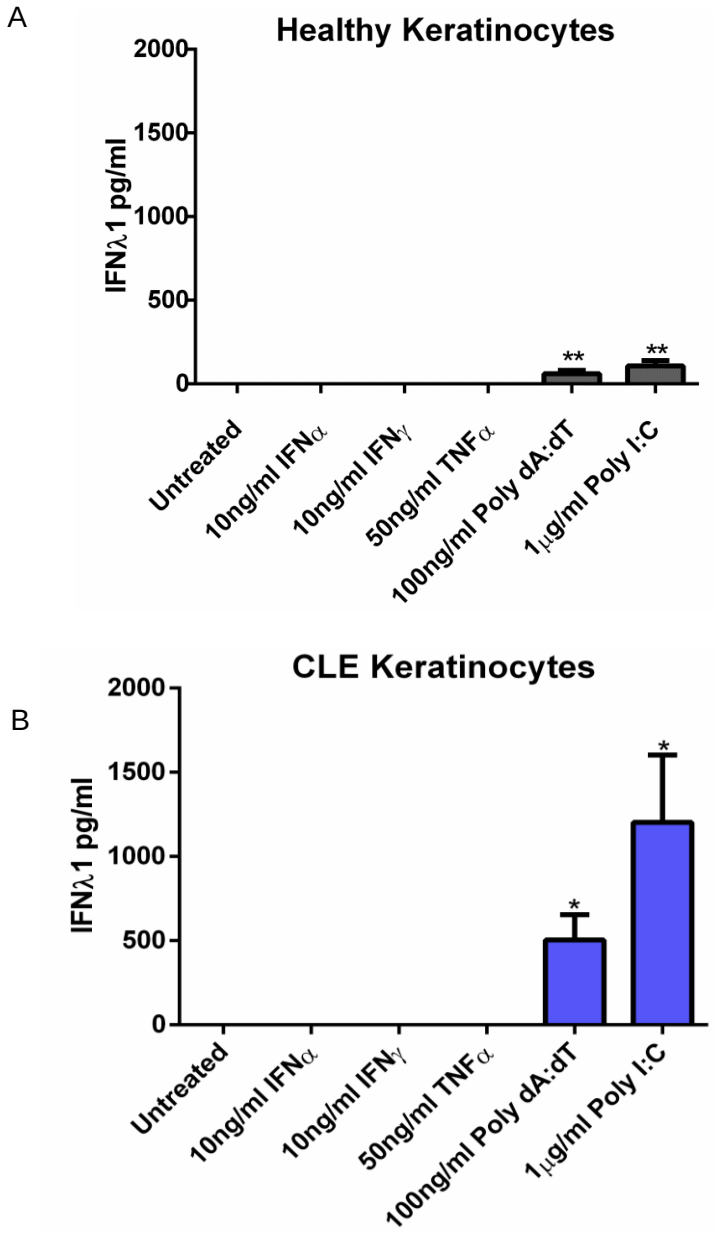


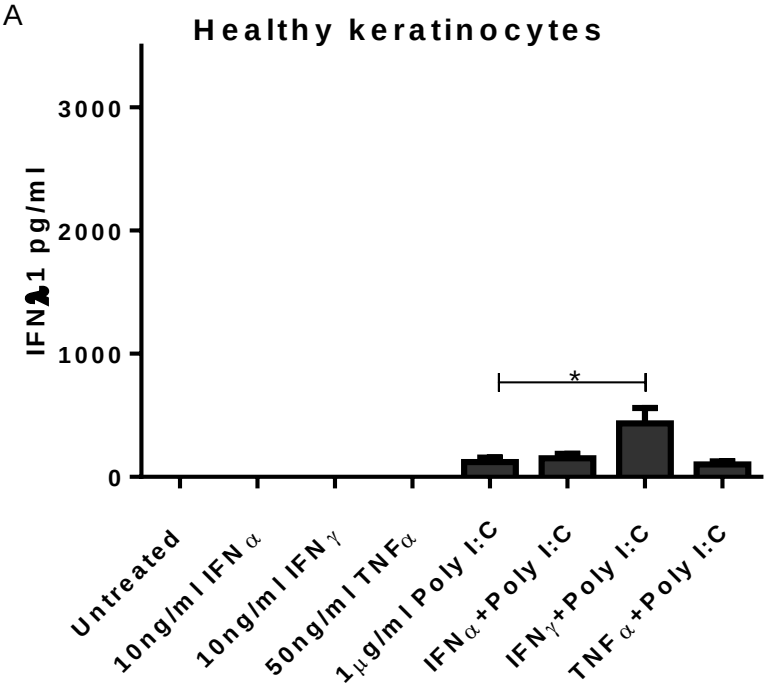

**Figure 3.1: IFNλ1 induction in human primary keratinocytes by nucleic acids.**

Cultured primary keratinocytes from healthy individuals or CLE patients were either untreated or treated with IFNα, IFNγ, TNFα, poly dA:dT, poly I:C for 48 hours. Supernatants were collected and concentration of IFNλ1 protein was determined by ELISA. (A) Healthy keratinocytes, n=5 (B) CLE keratinocytes, n=3. Statistical analysis was done using unpaired *t*-test. Mean±SEM is given; *p< 0.05, **p < 0.01.

### *3.2.2 IFNγ enhances the production of IFNλ in human primary keratinocytes*

IFNs and TNFα are important molecules in the pathogenesis of CLE. The ability of these molecules to enhance IFNλ1 protein induced by poly I:C in cultured human primary keratinocytes was investigated. It was known that these cytokines do not induce the production of detectable levels of IFNλ1 on their own. Healthy or CLE keratinocytes were primed by treating them with IFNα, IFNγ or TNFα for 1 hour before adding poly I:C for 48 hours. The result obtained showed that IFNγ significantly upregulated the production of IFNλ1 in both healthy and CLE keratinocytes (Fig. 3.2A-B). No enhancing effect was observed with IFNα or TNFα in healthy and CLE keratinocytes (Fig. 3.2A-B). The enhancing effect of IFNγ was over 2-fold in both healthy and CLE keratinocytes as compared to poly I:C alone. However, CLE keratinocytes produced about 4-fold more IFNλ1 than healthy keratinocytes (Fig. 3.2B).

A


Healthy keratinocytes
IFNλ1 pg/ml
3000
2000
1000
0
*
Untreated
10ng/ml IFNα
10ng/ml IFNγ
50ng/ml TNFα
1μg/ml Poly I:C
IFNα+Poly I:C
IFNγ+Poly I:C
TNFα+Poly I:C

B

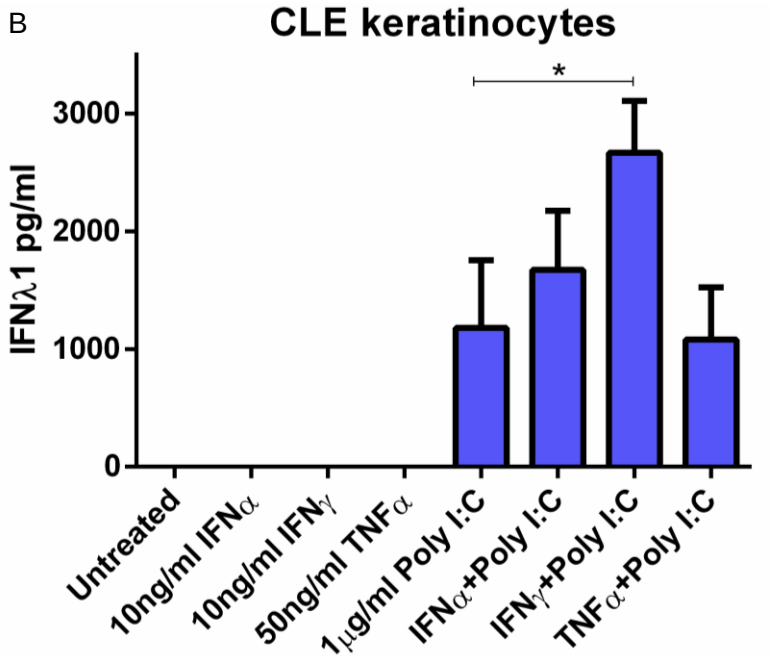

CLE keratinocytes
IFNλ1 pg/ml
3000
2000
1000
0
*
Untreated
10ng/ml IFNα
10ng/ml IFNγ
50ng/ml TNFα
1μg/ml Poly I:C
IFNα+Poly I:C
IFNγ+Poly I:C
TNFα+Poly I:C

**Figure 3.2: IFNγ enhances the production of IFNλ1 in human primary keratinocytes.**

Primary keratinocytes from healthy individuals or CLE patients were either untreated or treated with IFNα, IFNγ, TNFα, poly I:C alone or a combination of cytokine and nucleic acid for 48 hours. Supernatants were collected and concentration of IFNλ1 protein was determined by ELISA. For the experiments, a minimum of three different donors were used in independent experiments. (A) Healthy keratinocytes, n=5 (B) CLE keratinocytes, n=3. Statistical analysis was done using unpaired *t*-test. Values are mean±SEM; *p< 0.05.

### *3.2.3 IFNλ has autocrine activity in healthy and CLE keratinocytes*

In order to see if IFNλ1 could induce its own expression, keratinocytes were stimulated with different concentrations of rhIFNλ1 for 24 hours and gene expression was quantified using qRT-PCR. A dose-dependent upregulation of IFNλ1 mRNA expression by rhIFNλ1 was observed in both healthy and CLE keratinocytes (Fig. 3.3A-B); thus pointing to autocrine activity. Treatment of CLE keratinocytes with IFNλ1 did not result in a much higher expression of IFNλ1 mRNA in comparison to keratinocytes from healthy individuals.

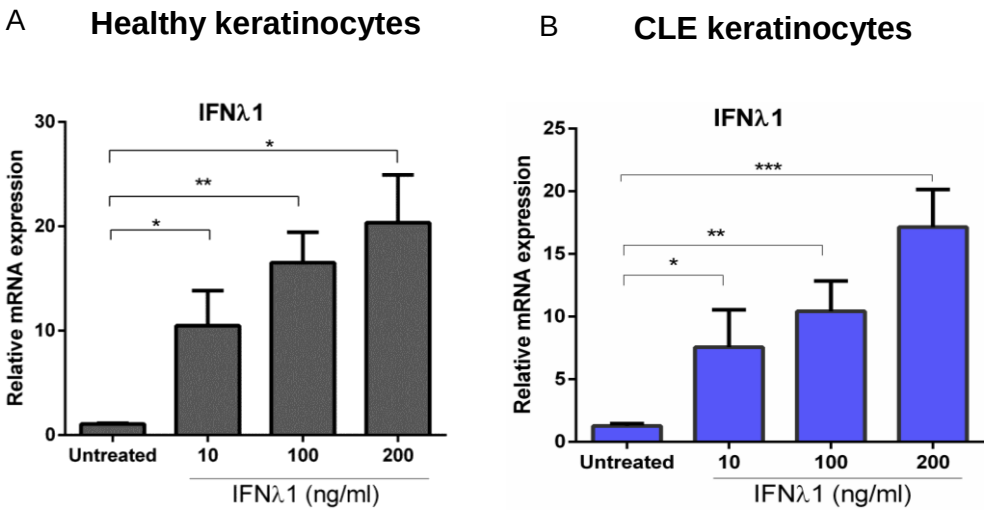


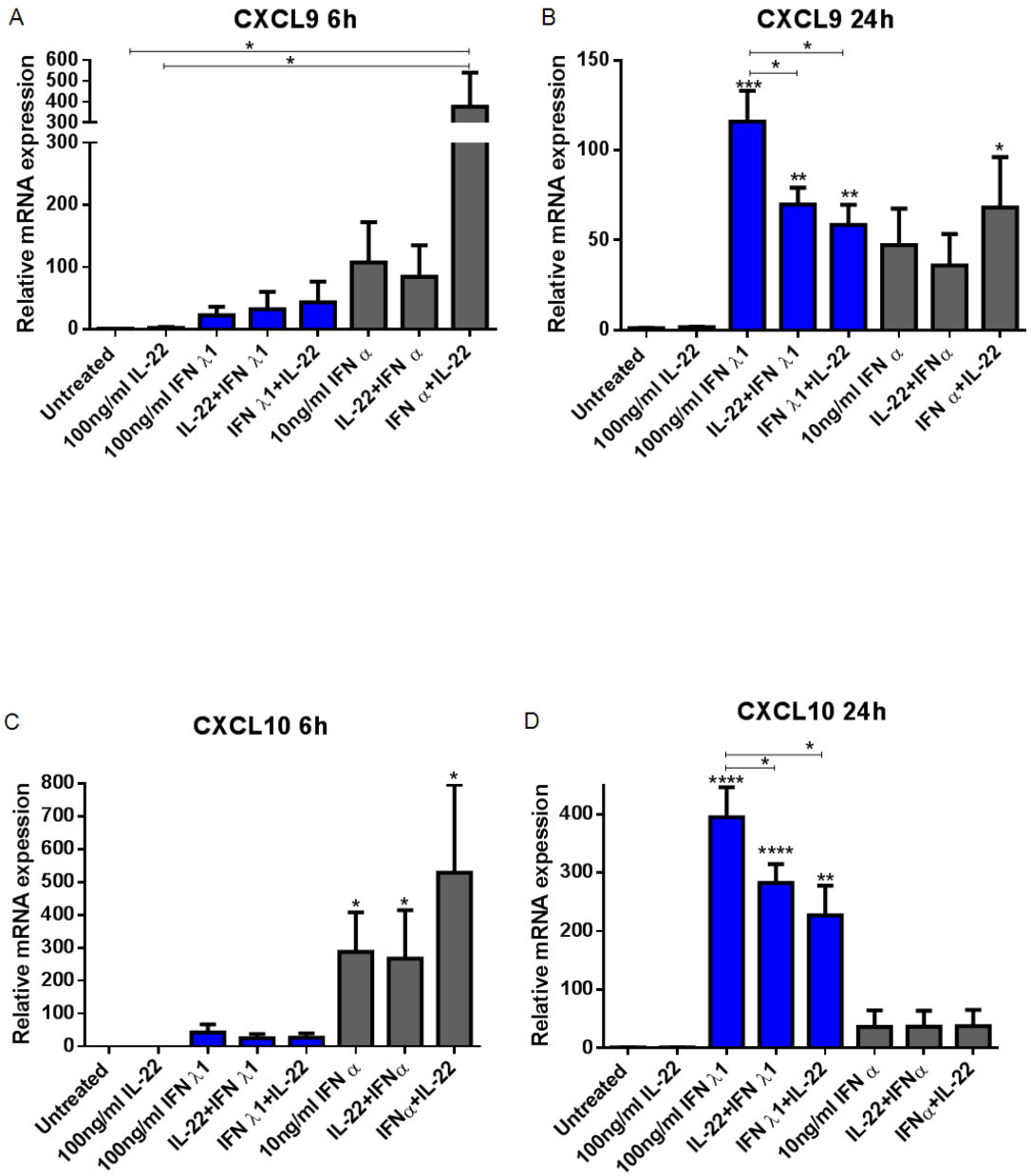

**Figure 3.3: IFNλ1 has autocrine effect on its own expression in human keratinocytes.**

Primary keratinocytes from healthy individuals or CLE patients were either untreated or treated with 10 ng/ml, 100 ng/ml or 200 ng/ml rhIFNλ1 for 24 hours. IFNλ1 mRNA expression was quantified by qRT-PCR. Relative mRNA expression was normalised to U6snRNA and calibrated to untreated control. Minimum of 3 independent experiments were carried out using different donors (A) n=3 (B) n=5. Mann Whitney U test was used to determine statistical significance. Values are mean ± SEM; *p < 0.05, **p < 0.01, ***p<0.001.

### *3.2.4 IL-22 counter-regulates the expression of IFNλ-induced CXCL9 and CXCL10 in healthy primary keratinocytes but not in CLE keratinocytes*

It has been shown that pre-treatment of keratinocytes and some cell lines, such as DLD1 with IFNα prior to treating with IL-22 resulted in enhanced expression of ISGs (Bachmann et al., 2013). In addition, my experiments followed the hypothesis that, IL-22 which shares the second receptor with IFNλ, could have beneficial effects on IFNλ dominated pathologies, such as CLE. In order to investigate if a relationship exists between IL-22 and IFNλ, primary keratinocytes were exposed to IL-22, IFNα, IFNλ1 in different combinations and stimulation sequences. IFNα was used as positive control. Both healthy and CLE keratinocytes failed to show downregulatory effects to IL-22 for any of the ISGs after 6 hours of stimulation (Fig. 3.4A/C/E/G). Interestingly, the result showed that the presence of IL-22 was able to significantly reduce the expression of IFNλ1-induced CXCL9 and CXCL10 but not other ISGs investigated in primary keratinocytes from healthy donors after 24 hours of treatment (Fig. 3.4B/D/F/H). This downregulatory effect was not observed for any ISG in CLE keratinocytes after 24 hours of treatment (Fig. 3.5B/D/F/H). Furthermore, it was observed that pre-treating primary keratinocytes with IFNα prior to IL-22 stimulation for 6 hours resulted in over 5-fold and about 2-fold increase in CXCL9 and CXCL10 expression respectively in comparison to IFNα treatment alone (Fig. 3.4A/C). This effect was lost after 24 hours of treatment. IFNα effect on ISGs expression also decreased after 24 hours in Fig. 3.4A-H. Neither the downregulatory effect of IL-22 on CXCL9 and CXCL10 expression nor the enhancing effect of IL-22

on IFNα-induced expression of CXCL9 and CXCL10 was observable in CLE keratinocytes (Fig. 3.4A/C and Fig. 3.5A-D). CLE keratinocytes had higher basal expression levels of CXCL9 and CXCL10 in comparison to healthy keratinocytes (Fig. 3.4A-D and 3.5A-D).



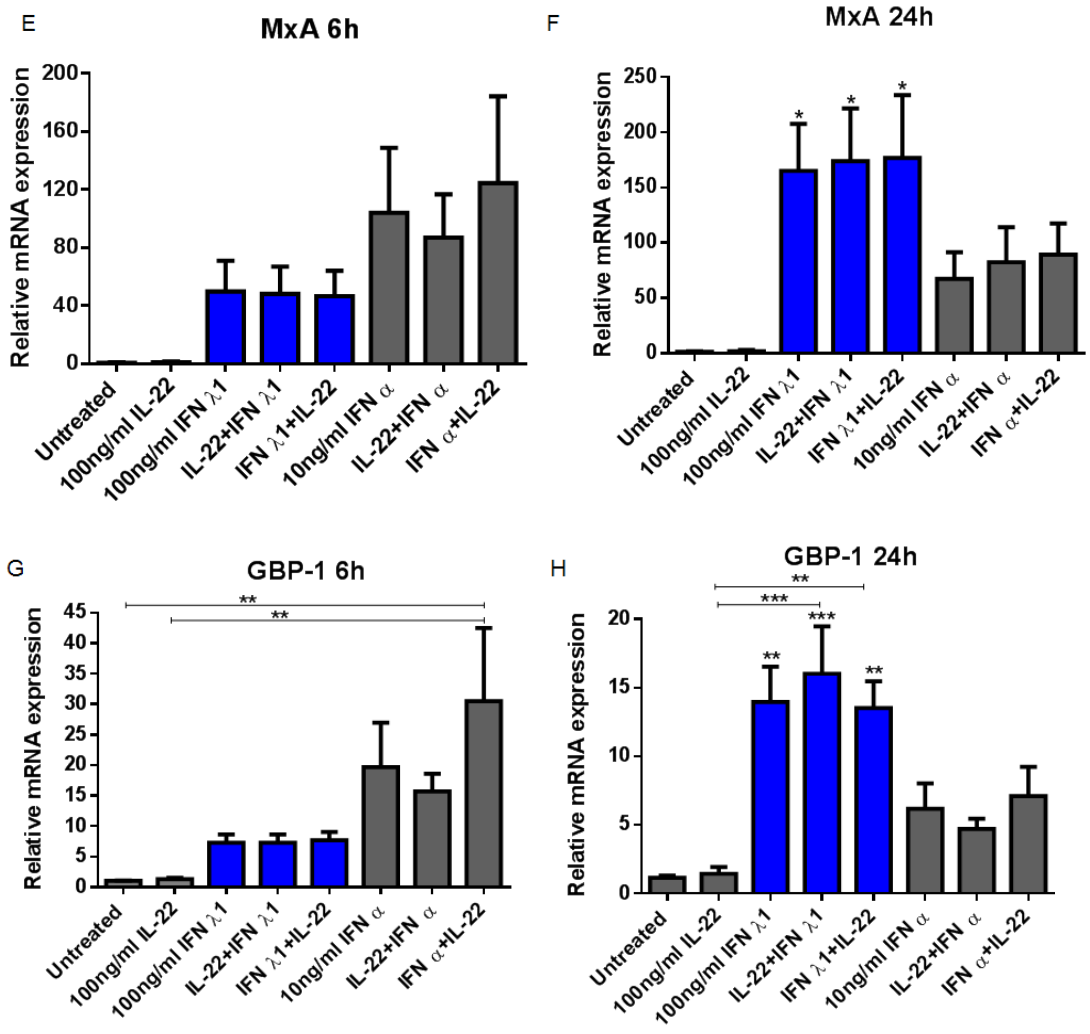
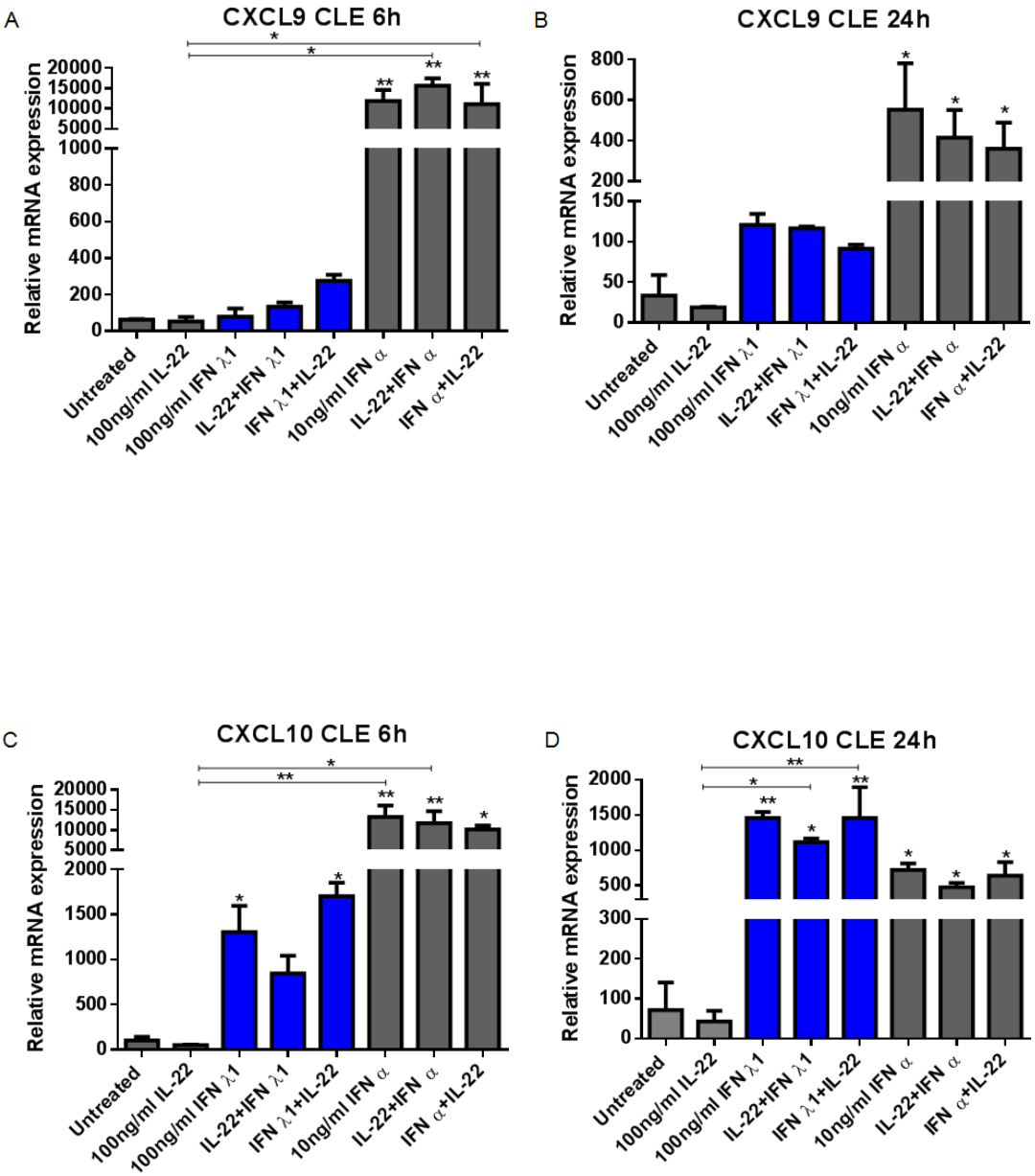



**Figure 3.4: Regulation of ISGs expression by IL-22 in healthy keratinocytes**

Primary keratinocytes from healthy individuals were either untreated or treated with 10 ng/ml IFNα, 100 ng/ml IFNλ1, 100 ng/ml IL-22 alone or with IL-22 for 30 minutes before and after addition of IFNs. Cells were treated for either 6 or 24 hours. Total RNA was isolated and expression of ISGs was quantified by qRT-PCR. (A) CXCL9, 6 hours (B) CXCL9, 24 hours (C) CXCL10, 6 hours (D) CXCL10, 24 hours (E) MxA, 6 hours (F) MxA, 24 hours (G) GBP-1, 6 hours (H) GBP-1, 24 hours. One-way ANOVA was used to determine statistical significance, n=4. Values are mean±SEM; *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.


A
CXCL9 CLE 6h
Relative mRNA expression
20000
15000
10000
5000
1000
800
600
400
200
0
Untreated
100ng/ml IL-22
100ng/ml IFN λ1
IL-22+IFN λ1
IFN λ1+IL-22
10ng/ml IFN α
IL-22+IFN α
IFN α+IL-22
B
CXCL9 CLE 24h
Relative mRNA expression
800
600
400
200
150
100
50
0
Untreated
100ng/ml IL-22
100ng/ml IFN λ1
IL-22+IFN λ1
IFN λ1+IL-22
10ng/ml IFN α
IL-22+IFN α
IFN α+IL-22
C
CXCL10 CLE 6h
Relative mRNA expression
20000
15000
10000
5000
2000
1500
1000
500
0
Untreated
100ng/ml IL-22
100ng/ml IFN λ1
IL-22+IFN λ1
IFN λ1+IL-22
10ng/ml IFN α
IL-22+IFN α
IFN α+IL-22
D
CXCL10 CLE 24h
Relative mRNA expression
2000
1500
1000
500
300
200
100
0
Untreated
100ng/ml IL-22
100ng/ml IFN λ1
IL-22+IFN λ1
IFN λ1+IL-22
10ng/ml IFN α
IL-22+IFN α
IFN α+IL-22

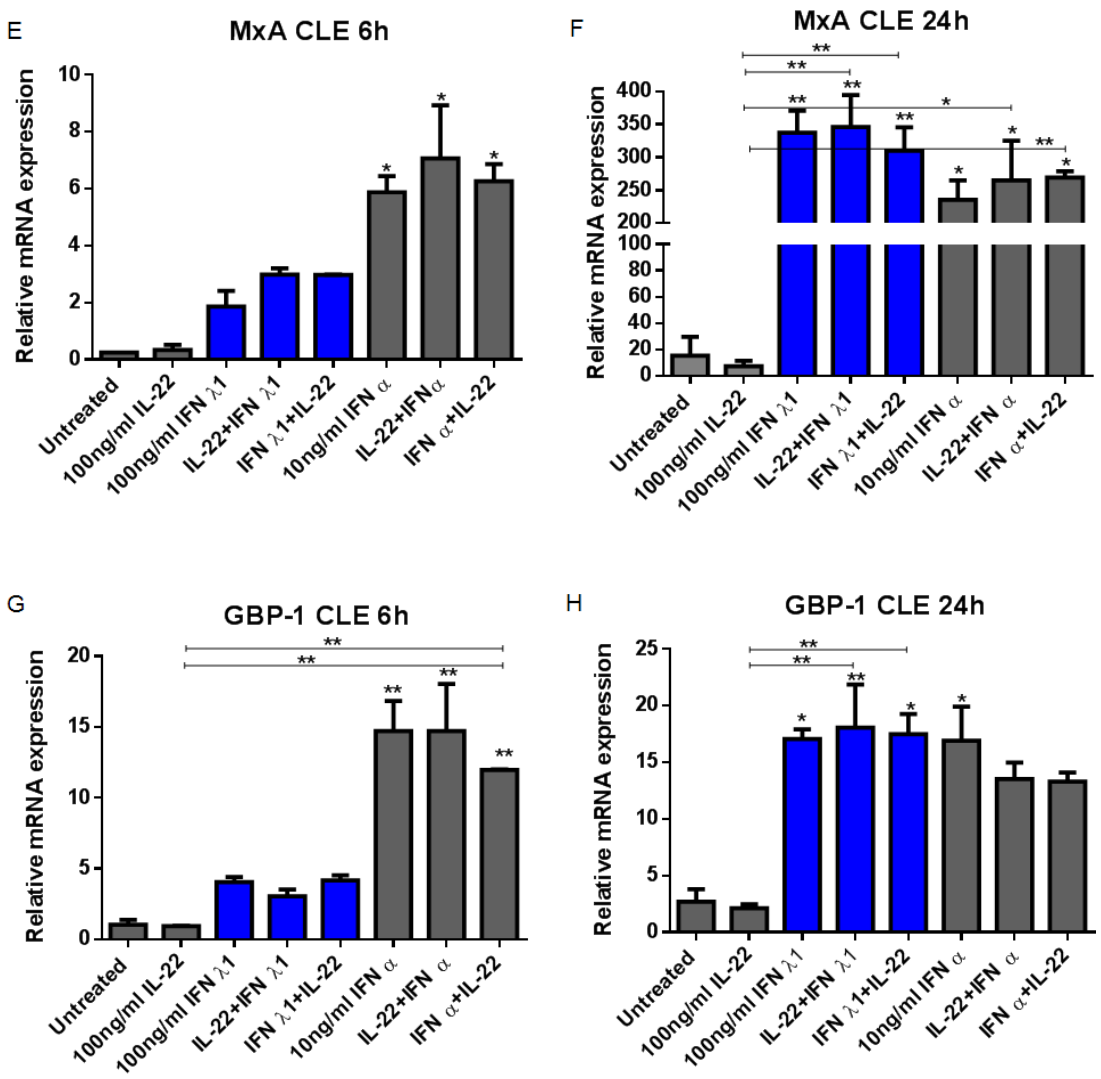



**Figure 3.5: Regulation of ISGs expression by IL-22 in CLE keratinocytes**

Primary keratinocytes from CLE patients were either untreated or treated with 10 ng/ml IFNα, 100 ng/ml IFNλ1, 100 ng/ml IL-22 alone or with IL-22 for 30 minutes before and after treatment with IFNs. Cells were treated for either 6 or 24 hours. Total RNA was isolated and expression of ISGs was quantified by qRT-PCR. (A) CXCL9, 6 hours (B) CXCL9, 24 hours (C) CXCL10, 6 hours (D) CXCL10, 24 hours (E) MxA, 6 hours (F) MxA, 24 hours (G) GBP-1, 6 hours (H) GBP-1, 24 hours. One-way ANOVA was used to determine statistical significance, n=4. Values are mean±SEM; *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.

### *3.2.5 IL-22 downregulated CXCL10 production in human primary keratinocytes*

Figures above show the downregulatory effect of IL-22 on IFNλ1-induced CXCL9 and CXCL10 on the mRNA level in human keratinocytes from healthy individual. Additional experiments were performed for the protein level. Primary keratinocytes were either untreated or treated with 100 ng/ml IFNλ1 or pre-treated with different concentrations of IL-22 for 30 minutes before adding IFNλ1 for 24 hours. Supernatants were harvested and subjected to ELISA assays to determine the amount of CXCL10 secreted. The result showed that only higher concentrations of IL-22 (100 and 250 ng/ml) had significant downregulatory effect on CXCL10 production by keratinocytes (Fig. 3.6).

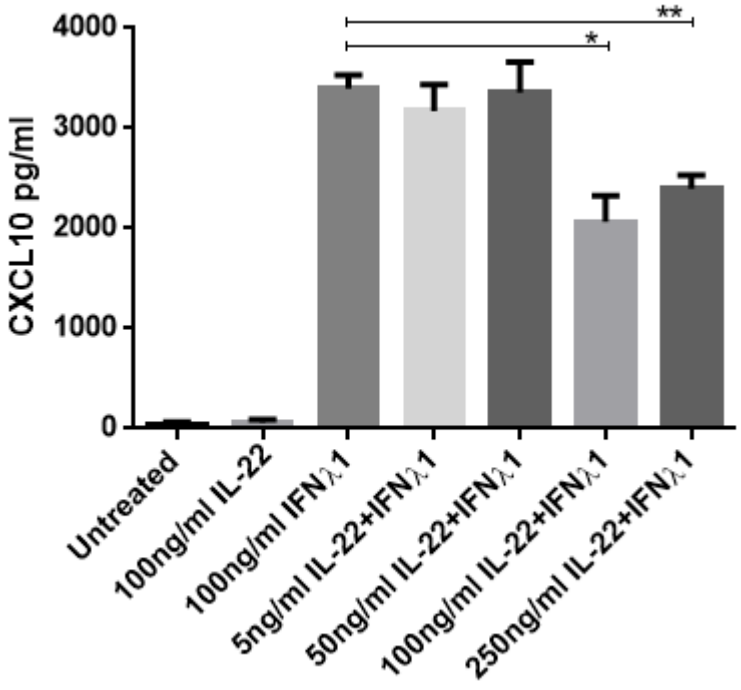


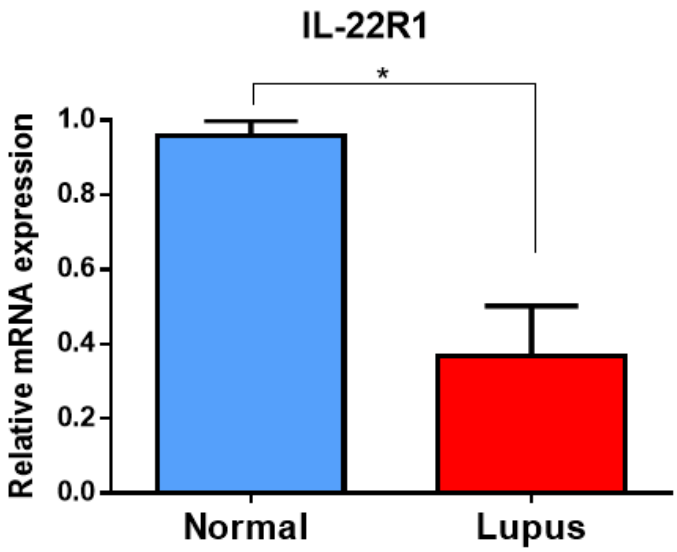

**Figure 3.6: Dose titration showing effect of IL-22 on IFNλ1-induced CXCL10 protein.**

Primary keratinocytes from healthy individuals were either untreated or treated with 100 ng/ml IL-22 or 100 ng/ml IFNλ1 or pre-treated with 5 ng/ml, 50 ng/ml, 100 ng/ml or 250 ng/ml IL-22 for 30 minutes before adding 100 ng/ml IFNλ1. Cells were treated for 24 hours. Supernatants were collected and the amount of secreted CXCL10 was determined by ELISA. Statistical significance was determined using unpaired *t*-test. Values are mean±SEM; *$p < 0.05$.

### *3.2.6 CLE keratinocytes showed reduced expression of IL-22R1 in comparison to healthy keratinocytes*

Based on the findings above on the regulatory effect of IL-22 on chemokines expression and secretion in healthy keratinocytes but not in CLE keratinocytes, it was investigated whether this effect could be due to the differences in expression levels of IL-22R1 on these cells. Experiments performed for the mRNA and protein expression levels showed a significantly lower IL-22R1 expression in CLE keratinocytes compared to healthy keratinocytes. Healthy keratinocytes on the other hand showed high expression of IL-22R1 protein (Fig. 3.7A-C).

A



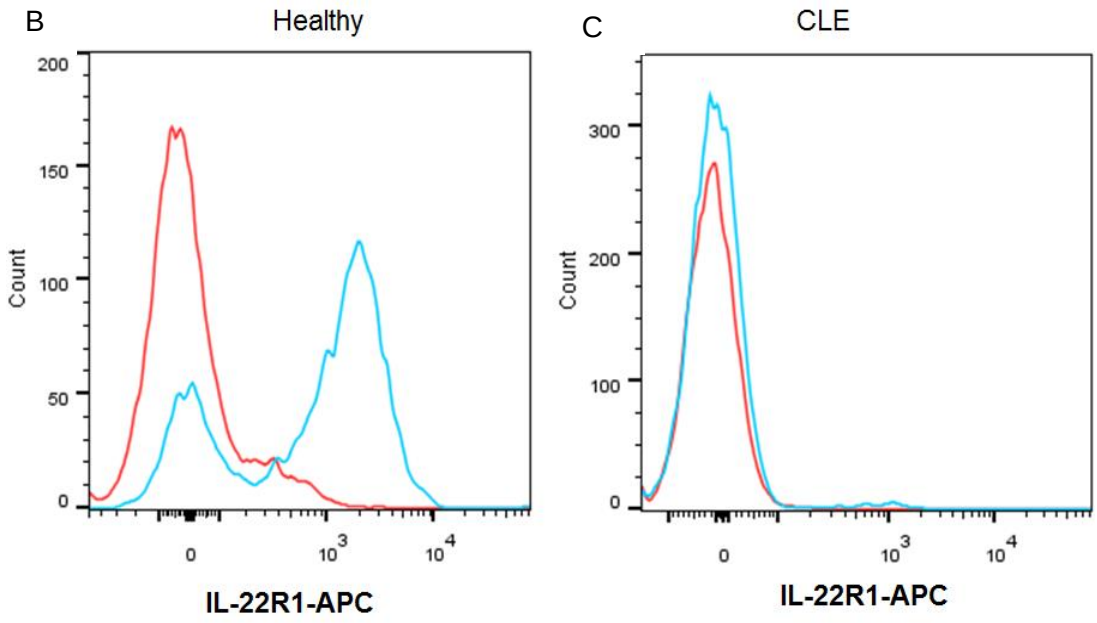



**Figure 3.7: Reduced expression of IL-22R1 in CLE keratinocytes.**

IL-22R1 expression levels were measured in primary keratinocytes from healthy or LE patients by qRT-PCR and flow cytometry. (A) Total RNA was isolated from cells and IL-22R1 mRNA expression was quantified using qRT-PCR. Mann Whitney test was used to determine statistical significance; n=4. Values are mean $\pm$ SEM; *$p < 0.05$ (B) Cells were stained with APC-conjugated anti-human IL-22R1 antibody (blue) and appropriate APC-conjugated isotype control (red) was used. A representative figure from 3 independent experiments from 3 donors is shown.

### *3.2.7 IL-22 induces significant expression of SOCS3 in healthy primary keratinocytes*

SOCS1 and SOCS3 are important negative regulators of IL-22 and IFNλ1 signalling. Like SOCS3, JUNB is also activated downstream of STAT3 and has been shown to be an important molecule in psoriatic inflammation. The ability of IL-22 and IFNλ1 to induce the expression of these molecules over a 6-hour period was monitored. The result showed that IL-22 significantly induced the expression of SOCS3 but not SOCS1 and JUNB in primary keratinocytes (Fig. 3.8A-D). SOCS3 was induced as early as 30 minutes after treatment with peak expression level at 1 hour after treatment with IL-22 and returning to about pre-treatment level after 6 hours of treatment (Fig. 3.8A-E). IFNλ1 did not induce significant expression of any of the genes investigated but showed a tendency for SOCS1 expression (Fig. 3.8A-E).

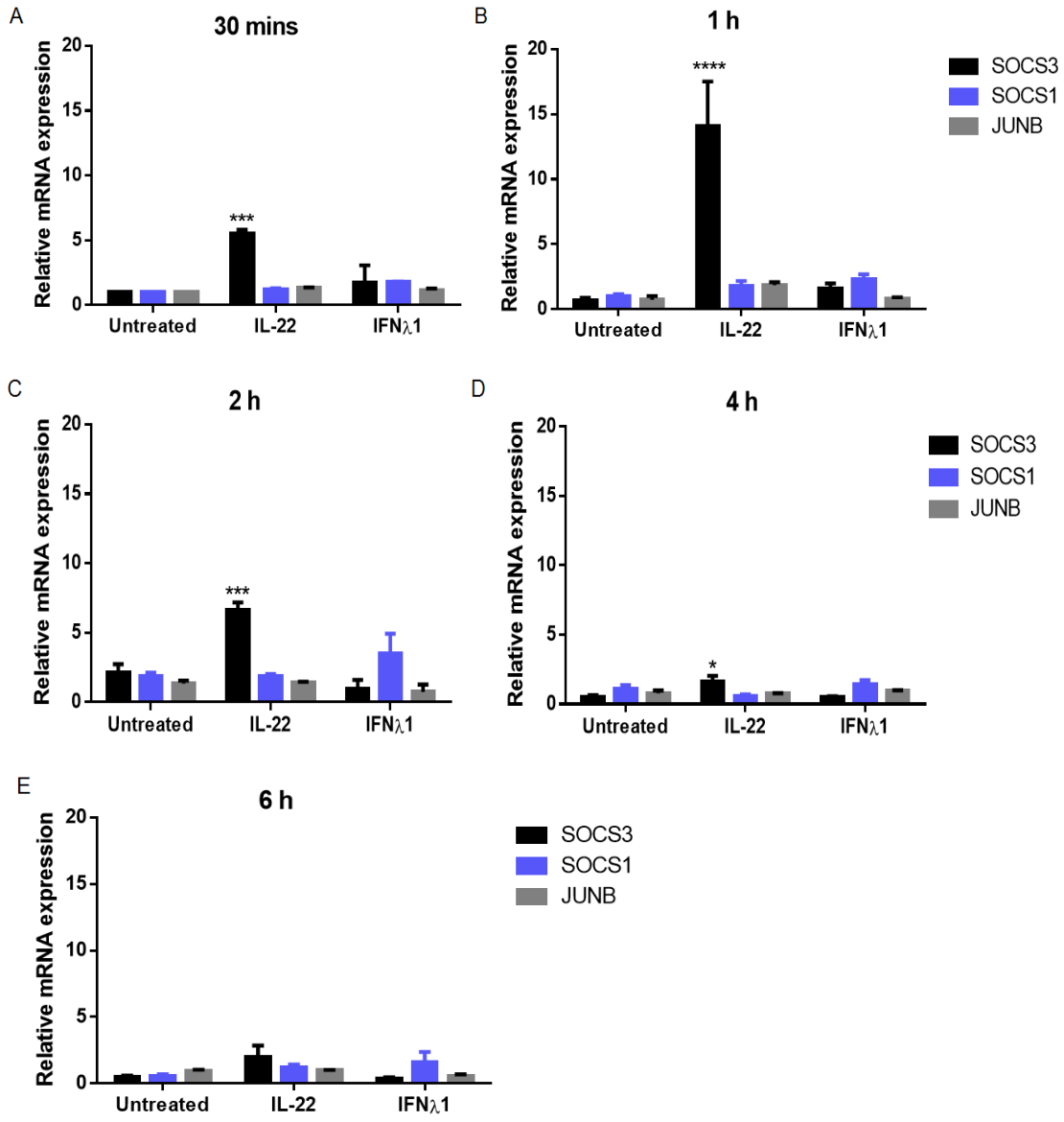



**Figure 3.8: Time course induction of SOCS genes by IL-22 and IFNλ1 in healthy primary keratinocytes.**

Keratinocytes were either not treated or treated with 100 ng/ml IL-22 or 100 ng/ml IFNλ1 for time-points ranging between 30 minutes and 6 hours. Cells were lysed, RNA extracted and mRNA expression of SOCS3, SOCS1 and JUNB was quantified using qRT-PCR. (A) 30 minutes (B) 1 hour (C) 2 hours (D) 4 hours (E) 6 hours. Two-way ANOVA was used to determine statistical significance; n=3. Values are mean±SEM; *p < 0.05, **p < 0.01, ***p<0.001, ****p<0.0001.

### *3.2.8 IFNλ1 upregulated and downregulated the expression of SOCS1 and SOCS3 respectively in CLE keratinocytes*

Based on the finding with healthy keratinocytes, expression levels of SOCS genes in response to IL-22 and IFNλ1 were investigated in CLE keratinocytes. The result showed significant downregulation of SOCS3 by IFNλ1 just after 30 minutes of treatment (Fig. 3.9A). However, this effect was not observed in all the other time points checked (Fig. 3.9B-E). Interestingly, IFNλ1 significantly upregulated the expression of SOCS1 in CLE keratinocytes after 2 hours of treatment (Fig. 3.9C). IL-22 failed to show significant effects on the expression of the genes investigated (Fig. 3.9A-E).

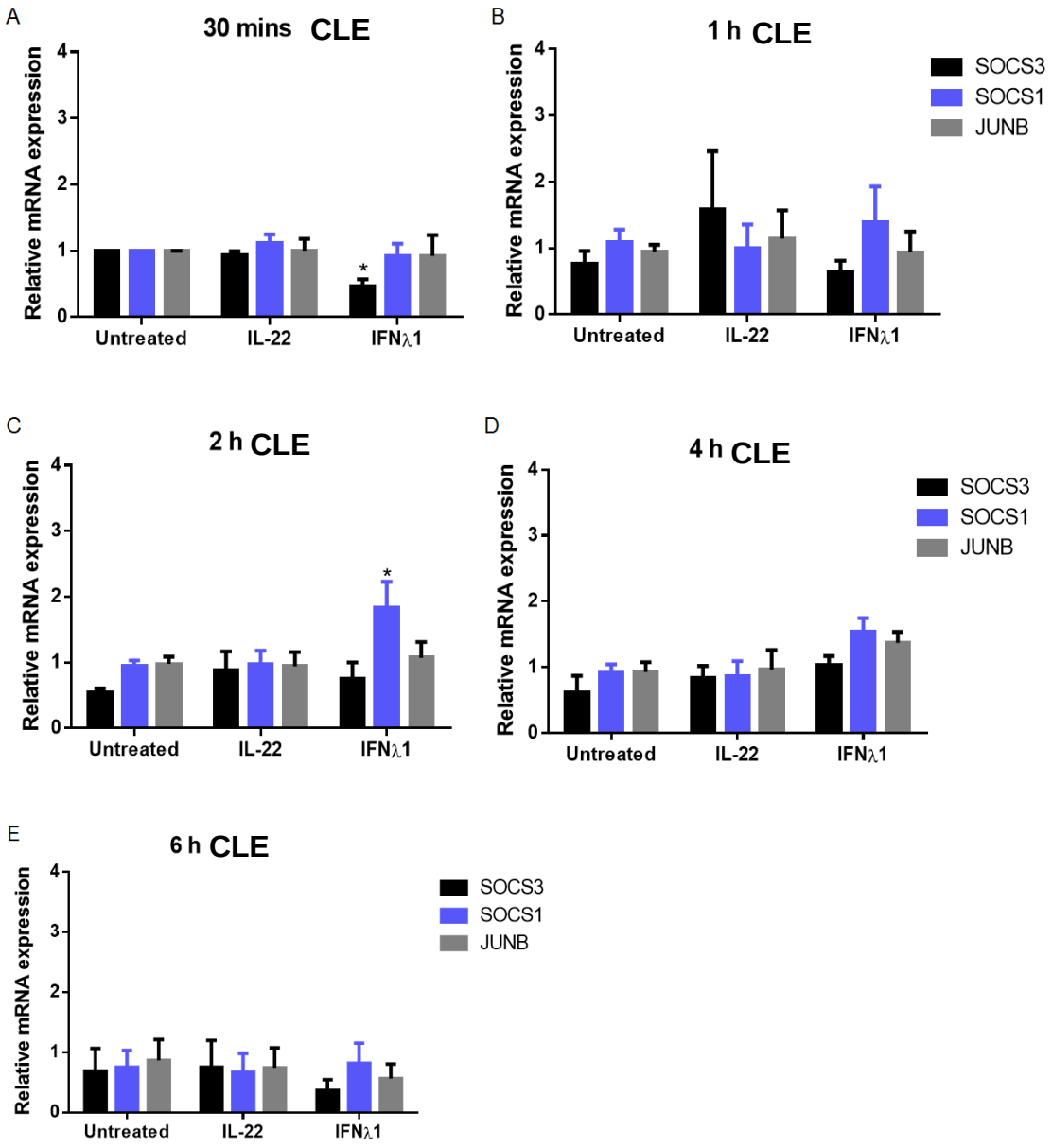



**Figure 3.9: Time course induction of SOCS genes by IL-22 and IFNλ1 in CLE keratinocytes.**

Cultured CLE keratinocytes were either not treated or treated with 100 ng/ml IL-22 or 100 ng/ml IFNλ1 for time-points ranging between 30 minutes and 6 hours. Cells were lysed, RNA extracted and mRNA expression of SOCS3, SOCS1 and JUNB was quantified using qRT-PCR. (A) 30 minutes (B) 1 hour (C) 2 hours (D) 4 hours (E) 6 hours. Two-way ANOVA was used to determine statistical significance; n=3. Values are mean±SEM; *p < 0.05.

### *3.2.9 CLE keratinocytes showed reduced activation of STAT3 upon IL-22 treatment in comparison to healthy cells*

It is well known that IL-22 induces the phosphorylation of STAT3 in many cells, including primary keratinocytes. In order to confirm this and compare the levels of phosphorylation in healthy and CLE keratinocytes, cultured cells were treated with IL-22 for various time points and cell lysates were analysed by western blotting. The result confirmed the ability of IL-22 to significantly induce STAT3 phosphorylation in human primary keratinocytes (Fig. 3.10A-C). Interestingly, reduced STAT3 phosphorylation was observed in CLE keratinocytes in comparison to healthy keratinocytes. This reduced phosphorylation in CLE keratinocytes was observed at all the time points checked (Fig. 3.10A-D). Most pronounced phosphorylation for both healthy and CLE cells was observed 10 minutes after IL-22 stimulation (Fig. 10A-B).

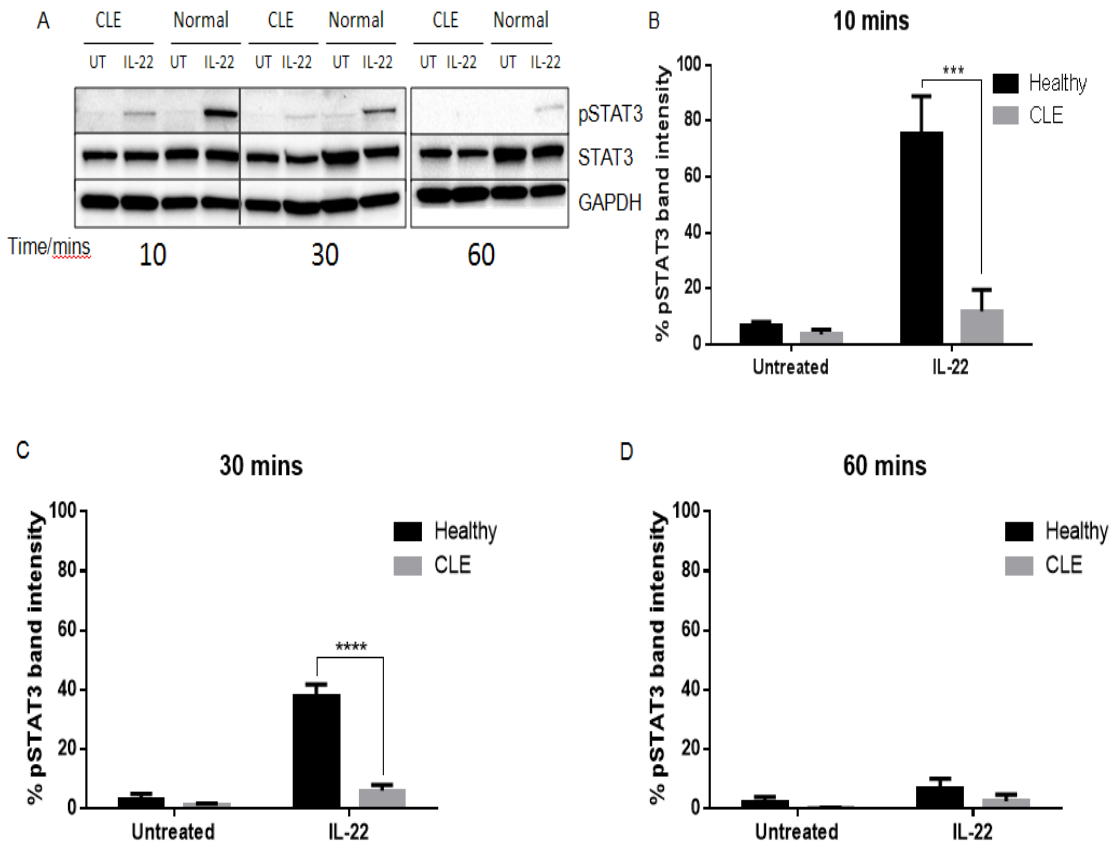


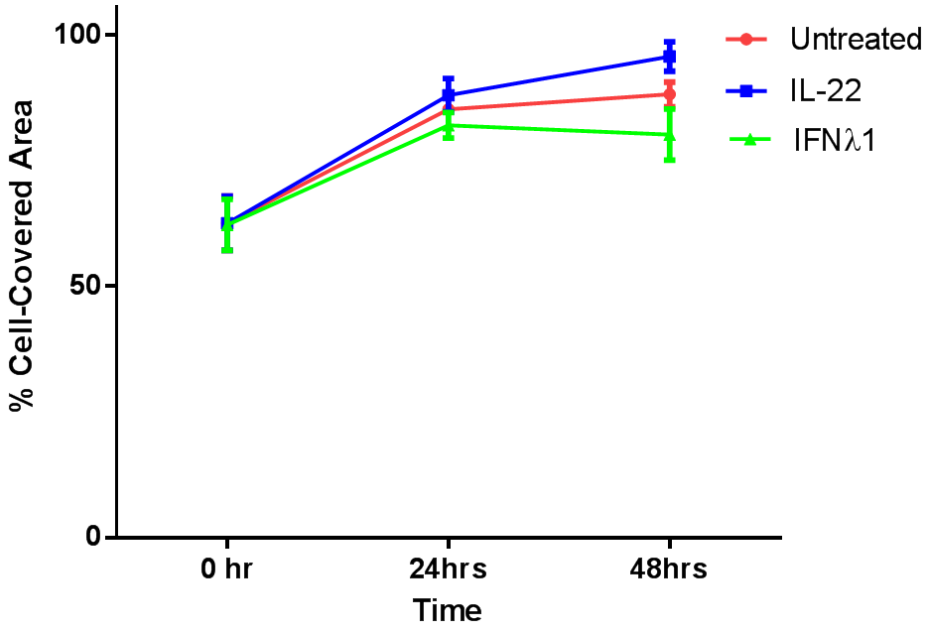

**Figure 3.10: STAT3 phosphorylation in Healthy and CLE keratinocytes by IL-22.**

Keratinocytes from healthy individuals and CLE patients were either untreated or treated with 100 ng/ml IL-22 for 10, 30 and 60 minutes. Supernatants were removed and cells were lysed, protein quantified by Bradford assay and 30 µg of protein was used for Western blotting. (A) Representative Western blot figure from 3 independent experiments; (B) Percentage band intensity of phospho-STAT3 in healthy versus CLE keratinocytes after 10 minutes, 30 min (C), and 60 min (D) of IL-22 treatment;. Two-way ANOVA was used to determine statistical significance; n=3. Values are mean ± SEM; *p < 0.05, **p < 0.01, ***p<0.001, ****p<0.0001. UT: Untreated.

### *3.2.10 IL-22 and IFNλ1 have contrasting effects on keratinocytes 'gap' closure in scratch assay*

In order to understand the functional roles of IL-22 and IFNλ1 in repair processes, confluent keratinocytes monolayer cultures in 24-well plates were scratched to create a clear cell-free area at the centre of the wells. Cells were then stimulated with IL-22 or IFNλ1 for 48 hours. IL-22 stimulated cells were able to close the scratch area quicker than non-stimulated and IFNλ1 stimulated cells i.e. 7% and 18% quicker respectively (Fig. 3.11A-B). On the contrary, IFNλ1 caused a reduction in the rate of gap closure by up to 10% in comparison to the non-stimulated cells (Fig. 3.11A-B). The differential effects of IL-22 and IFNλ1 on scratch/gap closure could be seen after 24 and 48 hours of treatment (Fig. 3.11B).

A


100
50
0
% Cell-Covered Area
0 hr
24hrs
48hrs
Time
Untreated
IL-22
IFNλ1

B

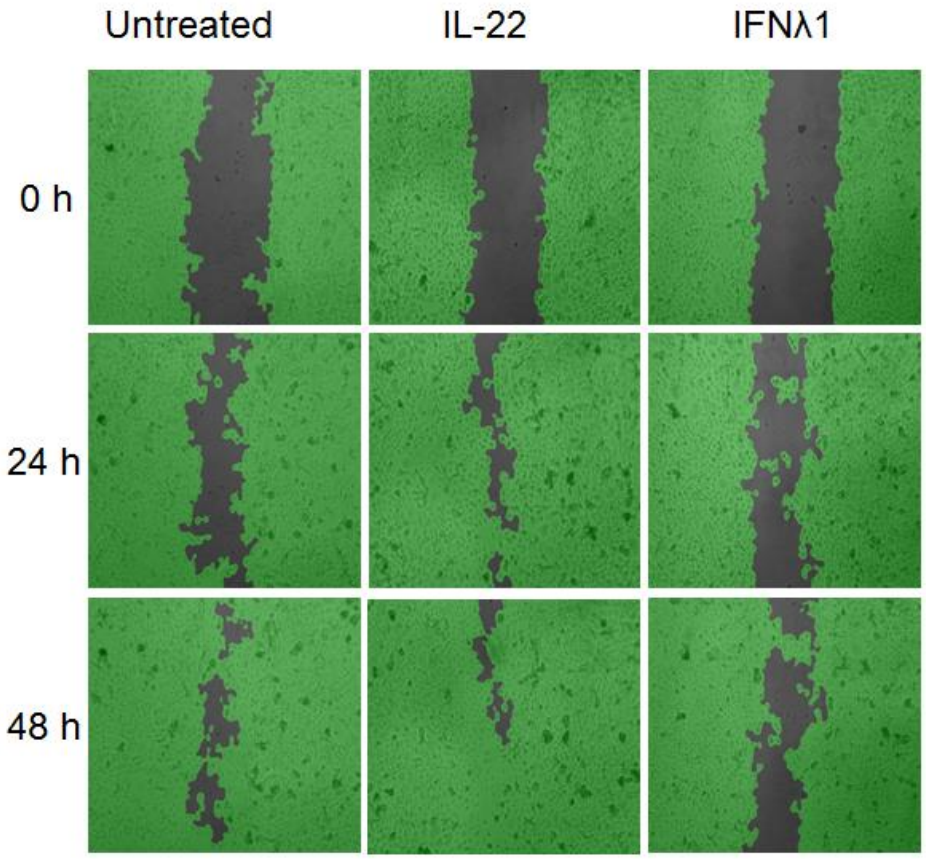
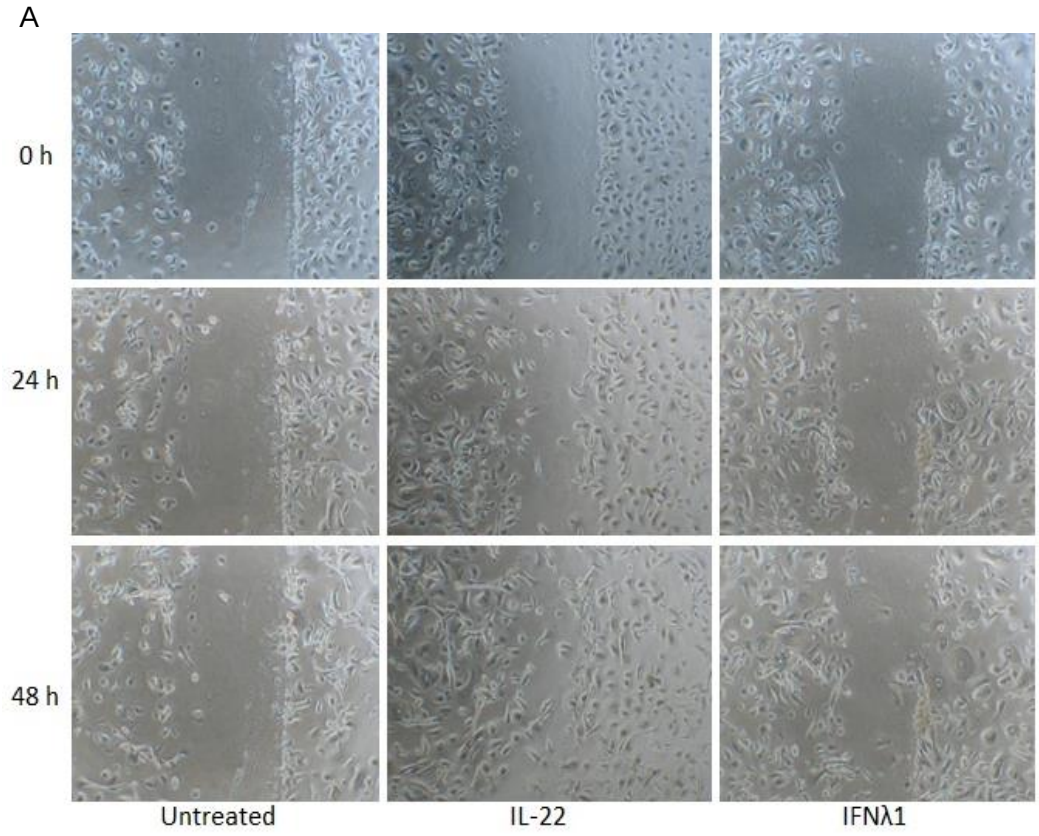

Untreated
IL-22
IFNλ1
0 h
24 h
48 h

**Figure 3.11: IL-22 but not IFNλ1 improves "gap" closure in keratinocytes monolayer culture.**

Keratinocytes from healthy donors were allowed to reach about 95% confluence in a 24-well plate. The cells were then scratched with p200 (yellow) pipette tips to create a clear gap across the wells containing the monolayer culture. Cells were washed with warm PBS twice in order to remove cell debris. Growth medium was replaced and cells were either untreated or treated with IL-22 or IFNλ1. Images were taken at 0, 24 and 48 hours. (A) Analysis of repeat experiments using wimscratch software to show percentage of cell covered area (B) Images comparing IL-22 versus IFNλ1 effects using wimscratch software (Wimasis). Unpaired *t*-test was used to determine statistical significance; n=5. Values represent mean ± SEM, *p < 0.05.

### *3.2.11 IL-22 improves keratinocytes migration*

Having established the role of IL-22 in improvement of gap closure in human primary keratinocytes, further experiments were carried out investigating whether this effect was as a result of cell proliferation or migration or both and mitomycin C which prevents cell proliferation was used for the experimental setup. The results showed that IL-22 improves keratinocytes migration in comparison to untreated cells either in the presence or absence of EGF. By contrast, IFNλ1 does not have any clear effect on keratinocytes migration in comparison to untreated cells (Fig. 3.12A-B).



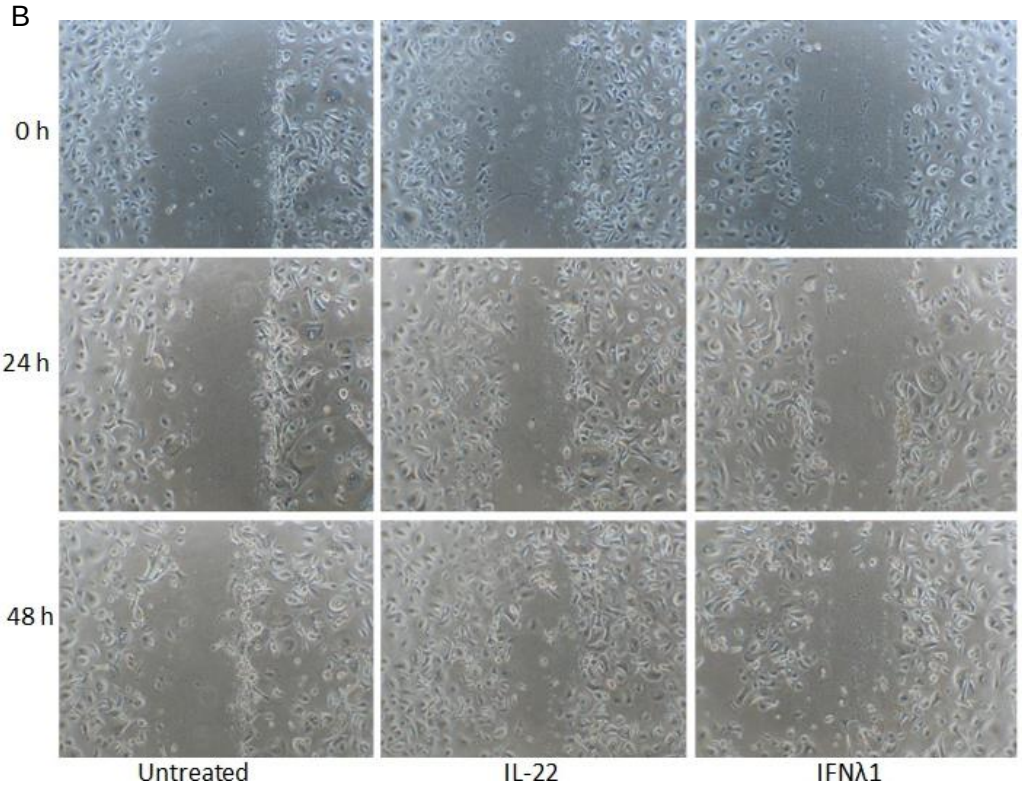


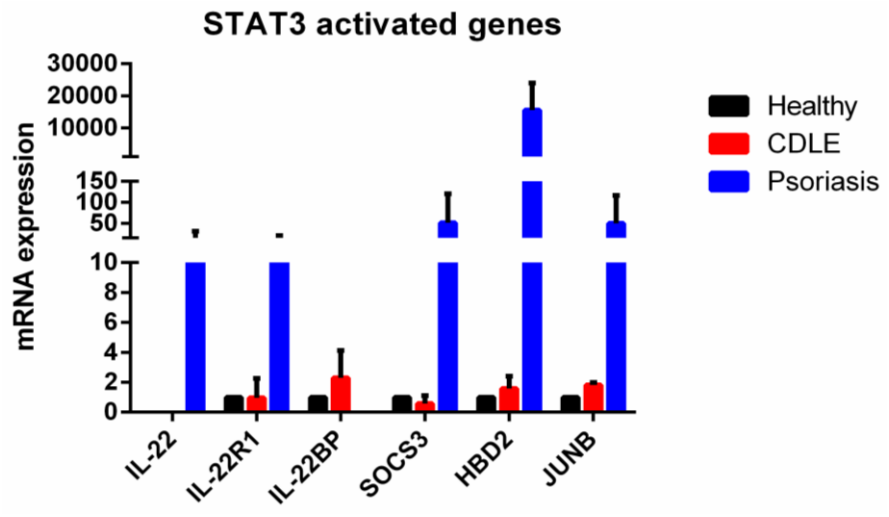

**Figure 3.12: Effect of IL-22 and IFNλ1 on keratinocytes migration.**

Healthy keratinocytes were allowed to reach about 95% confluence in a 24-well plate. Cells were treated with 5 µg/ml mitomycin C for 2 hours at 37$^{o}$C. The cells were then scratched with p200 (yellow) pipette tips to create a clear gap across the wells containing the monolayer culture. Cells were washed with warm PBS twice in order to remove cell debris. Growth medium was replaced with or without EGF and hydrocortisone and cells were either untreated or treated with IL-22 or IFNλ1. Images were taken at 0, 24 and 48 hours. Images comparing IL-22 vs IFNλ1 effects are shown. (A) Cells cultured in full medium (+/+); (B) Cells cultured in medium without hydrocortisone and EGF (-/-).

### *3.2.12 STAT3 and STAT1 'signature' genes are upregulated in psoriasis and CDLE respectively*

Results from previous experiments showed that CLE keratinocytes are not very responsive to IL-22 and that this cytokine downregulated the expression of IFNλ1-induced CXCL9 in healthy keratinocytes. Further mRNA expression analysis of whole thickness biopsies from lesional skin was carried out using qRT-PCR to obtain further information on the potential clinical relevance of these findings. The results showed that most of the genes downstream of STAT3, such as *SOCS3, IL-22, JUNB, hBD2 and IL-22R1* were highly upregulated in psoriatic lesion while many genes downstream of STAT1, such as *MxA, CXCL9, CXCL10, GBP1, IFI16, IFNλ1 and SOCS1* were upregulated in both diseases but more pronounced in CDLE lesion (Fig. 3.13). Interestingly, IL-22BP was upregulated in CDLE lesion but no IL-22 expression was found in these biopsies. On the contrary, no IL-22BP expression was found in psoriatic lesion; paralleled by high IL-22 expression (Fig. 3.13).

A




B

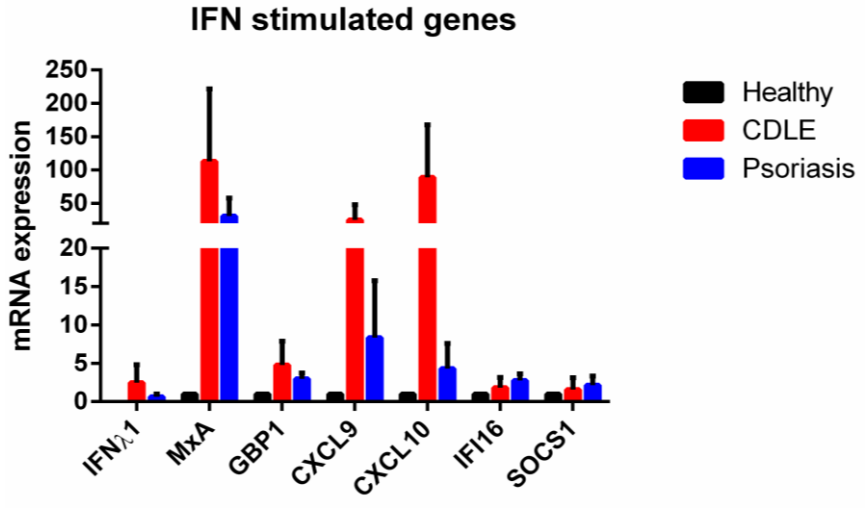



**Figure 3.13: Expression of IFN stimulated and STAT3 activated genes in human skin biopsies**

4 mm skin biopsies were taken from upper back, active lesions of patients with CDLE and psoriasis or healthy control. These biopsies were stored in OCT and RNA was isolated in order to measure gene expression of selected genes. mRNA expression was measured using qRT-PCR. Relative expression in lesional skin was normalised to U6snRNA (housekeeping gene) and calibrated to the healthy control (A) STAT3 activated genes (B) IFN stimulated genes. n=5 (2 biopsies for each disease condition and 1 healthy donor). Values represent mean ± SEM.

## 3.3 **Discussion**

CLE is a complex autoimmune disease which manifests under the influence of environmental, immunological and genetic factors. It is well known that UV irradiation, different cell types, such as pDCs, cytotoxic T cells and keratinocytes, and soluble mediators, such as IFNs play active roles in the pathogenesis of CLE. This work is mainly focussed on cytokine regulation and/or dysregulation in the skin compartment in the context of inflammatory skin diseases.

A hallmark of LE is immunoreactivity to nucleic acids. Autoantibodies to nucleic acids (ANA – antinuclear Ab) are believed to be important in the pathogenesis of CLE even in the absence of detectable serum ANA in routine laboratory tests. As outlined above, processes (nucleic acid immunoreactivity) leading to ANA formation, such as defective clearance of apoptotic cells can also lead to production of proinflammatory cytokines, such as IFNs. Results of experiments using keratinocytes from both healthy and LE patients support the importance of nucleic acids in the initial trigger of skin expressed IFNs.TLR3, the main receptor for poly I:C and RIG-I, receptor for poly dA:dT have been implicated in the pathogenesis of LE (Hari et al., 2010; Wong et al., 2009). It is possible, though not investigated in this project, that TLR3 dependent pathway may be involved in the observation that CLE keratinocytes show a 4-fold increase in IFNλ1 production in comparison to healthy keratinocytes upon nucleic acid stimulation. These receptors may already be highly upregulated in LE keratinocytes prior to nucleic acids stimulation. IFNs have been implicated in CLE and the ability of IFNα to enhance the responsiveness of keratinocytes and other cell types to poly I:C,

viral particles or LPS for subsequent production of inflammatory mediators has been described (Siren et al., 2005; Tissari et al., 2005). It has been suggested that a cross-talk exists between type II and type III IFNs (Li et al., 2008; Liu et al., 2011). IFNγ has also been shown to upregulate TLR3 and MDA5 in smooth muscle cells leading to induction of proinflammatory cytokines (Ahmad et al., 2010). The ability of IFNγ to enhance IFNλ1 production in both healthy and CLE keratinocytes may be due to its upregulatory effect on TLR3 expression in keratinocytes and even more so in CLE keratinocytes leading to about 4-fold increase in protein production (Kajita et al., 2015). Although IFNγ and IFNα are not ISGs, the autocrine effect of IFNλ1 as shown in this study and that of others (Ank et al., 2006) suggests that IFNλ1 may be the only IFN which also is an ISG; this may allow it to act in a positive feedback loop. The autocrine activity may be of relevance in epidermal CLE inflammation. Using immunohistological staining, highly upregulated expression of IFNλR1 has been reported in cutaneous lesions taken from CLE patients (Zahn et al., 2010). Keratinocytes from CLE patients failed to show highly increased IFNλ1 mRNA expression upon IFNλ1 stimulation in comparison to healthy keratinocytes despite reported high expression of IFNλR1 in CLE lesions.

As outlined above, IL-22 and IFNλ1 are suggested to have opposing functions in healing and repair processes. Using reconstituted human epidermis, Boniface et al. observed that IL-22 inhibited epidermal differentiation but did not induce the expression of Ki-67, a marker for cell proliferation (Boniface et al., 2005). This suggests that the mode of action of

IL-22 in the skin may be inhibition of differentiation and not active induction of hyperproliferation. Contrasting effects of IL-22 and IFNλ1 on scratch closure in keratinocytes monolayer culture *in vitro* were observed. These effects were however, modest. Migration and proliferation are two active processes in wound healing. The ability of IL-22 to close the scratch on cultured keratinocytes monolayer quicker than the non-stimulated control supports the role of IL-22 in promoting migration and proliferation in keratinocytes and suggests that this cytokine may play an active role in normal wound healing. A similar effect of IL-22 on gap closure in keratinocytes monolayer culture *in vitro* has been reported (Eyerich et al., 2009). IFNλ1 on the other hand caused delay in gap closure in comparison to untreated control. This observation supports the role of IFNλ1 as anti-proliferative agent (Maher et al., 2008); suggesting that it may be involved in delayed epidermal healing responses. IL-22 and IFNλ1 share the IL-10R2 and in line with some predicted competition for ligand binding, the presence of IL-22 in the cell culture resulted in significant downregulation of the expression of IFNλ1-induced CXCL9 and CXCL10 after 24 hours of treatment with the cytokine in comparison to IFNλ1 alone. Although this finding is significant, the downregulatory effect was not consistently found for other ISGs investigated in this work. This may be attributed to slight differences in the transcription factors activated upstream of these genes. IFNα has been shown to activate ISGF3 upstream of MxA expression while it activates STAT1 homodimer upstream of *CXCL9* and *CXCL10* genes (Ivashkiv and Donlin, 2014; Tomasello et al., 2014). The implication of this is that the regulatory role of IL-22 on IFN signalling may be restricted to the inflammatory/pro-apoptotic/anti-

proliferative pathway and may not interfere with their antiviral function. This assumption is also supported by the fact that the enhancing effect of IL-22 on IFNα signalling in keratinocytes was observed for CXCL9 and CXCL10 expression but not on other ISGs checked (Fig. 3.3A-D; (Bachmann et al., 2013). A recent report has however shown a synergistic relationship between IFNλ2 and IL-22 on the induction of ISGs in rotavirus-infected mouse and human intestinal epithelial cell lines through enhanced activation of STAT1 (Bird, 2015; Hernandez et al., 2015). It is possible that the downregulatory effect of IL-22 on IFNλ-induced CXCL9 and CXCL10 expression may not be solely due to competition for IL-10R2 but also based on interaction at JAK/STAT signalling level. It has been reported that both IL-22 and IFNλ bind to their signalling receptors (IL-22R1 and IFNλR1 respectively) with high affinity, forming IL-22/IL-22R1 and IFNλ/IL-28R1 binary complex respectively. IL-10R2 then recognises and binds to the binary complex to form a ternary complex, resulting in full activation of the pathways (Bleicher et al., 2008; Li et al., 2004a).

IL-22 induces SOCS3 in keratinocytes and overexpression of both SOCS3 and SOCS1 has been shown to downregulate IFNγ-induced expression of ICAM-1, MHC class II molecules, CXCL9/10 and CCL2 through the inhibition of STAT1 and STAT3 activities (Albanesi et al., 2007; Federici et al., 2002). The level of regulation between IFNλ and IL-22 with regards to ISGs induction clearly needs further investigation and this work highlights that cross-regulation may not simply be due to ligand competition on receptor level. A downregulatory effect of IL-22 on IFNλ1-induced CXCL9 and

CXCL10 was not observed after 6 hours of treatment in keratinocytes and this may be due to the fact that it takes longer for IFNλ1 to fully activate the STAT1 pathway in comparison to IFNα (Maher et al., 2008). It was initially unexpected that the presence of IL-22 failed to downregulate IFNλ1-induced CXCL9 and CXCL10 expression in CLE keratinocytes. However, as observed in this work, this may be well explained by the reduced IL-22R1 expression on these cells compared to primary keratinocytes from healthy donors (Fig. 3.5A-C). The results from this work and that of other have shown that IFNα is a quick and strong activator of STAT1 (Bachmann et al., 2013). While pre-treatment of healthy keratinocytes with IL-22 prior to IFNα treatment for 6 hours showed no significant downregulatory effect on all ISGs investigated in comparison to IFNα alone, pre-treatment of keratinocytes with IFNα prior to IL-22 treatment for the same period of time surprisingly, resulted in upregulation of CXCL9 and CXCL10 expression in comparison to IFNα alone. A similar phenomenon has been reported in macrophages. Pre-treatment of macrophages with either IFNα or IFNγ before treatment with IL-10 switches the IL-10 activation pattern from predominantly STAT3, which is anti-inflammatory, to mainly STAT1, which is pro-inflammatory (Regis et al., 2008). The report by Bachmann et al. also showed that priming of keratinocytes with IFNα switches IL-22 downstream activity from STAT3 response to STAT1 (Bachmann et al., 2013). The authors argued that this is only possible because IFNα effect on STAT1 is strong and quick. A seemingly contrary report suggests that IFNα priming of keratinocytes upregulates IL-22R1 thereby enhancing IL-22 activity through increased activation of STAT3 (Tohyama et al., 2012). The enhancing effect of IL-22 on

IFNα-induced CXCL9 expression was however lost after 24 hours of treatment and the priming effect was not observed in CLE keratinocytes. The inability to observe IL-22 enhancing effect (STAT3 to STAT1 switch) on ISGs expression in IFNλ1 primed cells may be due to delayed and not very strong, but long lasting STAT1 activation by IFNλ1 (Maher et al., 2008). The ability of IL-22 to switch between which STAT pathway to activate is very interesting. IL-22 has been shown to protect the liver from immune damage during hepatitis infection (Zenewicz et al., 2007) and also protected mice from lung epithelial damage (fibrosis) following influenza A virus infection (Paget et al., 2012). This protective function has been linked to its ability to counteract the activities of IFNs and other Th1 associated molecules. IL-22 is important in the pathogenesis of psoriasis and increased expression of IL-22 and IL-22R1 has been reported in active lesions of this disease (Sabat et al., 2014; Wolk et al., 2009b). Reduced expression of IL-22R1 in CLE keratinocytes relative to that of keratinocytes from healthy individuals is an interesting finding, which has not been reported. IL-22 induces the expression of SOCS3 through the activation of STAT3 (Brand et al., 2005). The reduced IL-22R1 expression in CLE keratinocytes may be responsible for reduced activation of STAT3 and subsequently reduced expression of SOCS3 upon IL-22 stimulation. The ability of keratinocytes to induce SOCS3 seems important in regulating STAT1 activated chemokines, such as CXCL9 and CXCL10, which are important in pathogenesis of CLE. The regulatory role of SOCS3 on ISGs expression was reported by Zeitvogel et al. for SOCS3 dependent downregulated IL-27-induced CXCL10 in human primary keratinocytes (Zeitvogel et al., 2012).

Gene expression analysis comparing matched biopsies from active lesions from CDLE and psoriasis patients showed a distinct pattern of expression. The high expression of STAT3 signature genes in psoriatic lesion points to the activity of STAT3 activating molecules, such as IL-22 in lesional skin. On the other hand, CDLE samples showed high expression of mainly STAT1 signature genes; suggesting the importance of IFNs in lesion formation. No expression of IL-22 was detected in CDLE sample as also recently reported (Jabbari et al., 2014) while some IL-22BP was detected. By contrast, high expression of IL-22 was detected in psoriatic sample with almost no expression of IL-22BP. It has been reported that IL-22BP is often downregulated in diseases with elevated levels of IL-22 (Sabat et al., 2014).

As found in these lesional biopsies, high expression of SOCS3 has also been reported before and this has been linked with the sustenance of the AKT pathway, which prevents keratinocytes apoptosis (Madonna et al., 2012; Mitra et al., 2012b; Takekoshi et al., 2013). Although growth-inhibiting functions of SOCS3 have been reported, this molecule undoubtedly plays an important role in downregulating inflammatory molecules and reducing apoptosis in various mouse models of hepatic inflammation therapy (Jo et al., 2005). There seems to be contrasting reports as to the role and expression levels of SOCS3 in psoriasis. Contrary to findings in this work and that of some others (Madonna et al., 2012; Takekoshi et al., 2013), deficient expression of SOCS3 by keratinocytes and T cells from psoriasis patients relative to healthy cells has been reported (Eriksen et al., 2010; Sonkoly et al., 2007). These differences could be as a result of donor-to-donor variation or

on the inflammatory “stage” of the disease. Of note, the dynamic of induction of SOCS, an immediate early gene, needs to be considered (Fig. 3.8). The downregulation of IL22BP, which is the natural antagonist for IL-22 could be of importance in the pathogenic mechanisms in psoriasis. Taken together, these results suggest that the IL-22/IL-22R pathway could have potential therapeutic importance not only in psoriasis but also in CLE. CXCL9 and CXCL10 are key chemokines involved in the pathogenesis of CLE. They are involved in the recruitment of CXCR3+ IFNγ-producing T cells, and enhance CXCL12-induced pDCs migration to the dermo-epidermal junction (Vanbervliet et al., 2003; Wenzel et al., 2005b). As seen in healthy keratinocytes, enhancing the ability of IL-22 to downregulate these important chemokines in CLE keratinocytes could be beneficial in the treatment of CDLE. IFNλ, which is inducible by nucleic acids is the main IFN produced by keratinocytes (Zahn et al., 2010). IFNλ in turn induces the expression of ISGs, one of which is CXCL9. In order to block the recruitment of pathogenic immune cells to the skin and to avoid the priming effect of IFNα and the upregulation of IFNλ production by IFNγ thus exacerbating CLE condition, early cutaneous administration or upregulation of IL-22 could present an approach to prevent lesion progression. However, once this stage is missed, administration of IL-22 to CLE lesion could exacerbate the condition rather than ameliorate it. IL-22 could also be useful, via SOCS3 induction, to reduce excessive apoptosis of keratinocytes as seen in CDLE (Kuhn et al., 2006). To properly tackle the problem of scarring in CDLE, it may be essential that an appropriate balance is maintained between STAT1 (Th1) and STAT3 (Th17) signalling pathways (Jabbari et al., 2014). It is important to point out that

using scratch assay to understanding the roles of IL-22 and IFNλ1 in cell monolayer wound *in vitro*, has its own limitations. Scratching the monolayer culture could result in the cells releasing danger signals, such as HMGB1; therefore, it is possible that the effects on gap closure seen may not just be that of the cytokines of interesting but a combination of many cellular factors. The differences in effects of cytokines on gap closure are also usually very subtle; hence, the need to repeat experiments several times in order to see any appreciable difference between different treatments.

# CHAPTER 4: IL-22 INDUCES SOCS3 EXPRESSION IN HUMAN DERMAL FIBROBLASTS THROUGH THE PHOSPHORYLATION OF STAT3

# 4 IL-22 induces SOCS3 expression in human dermal fibroblasts through the phosphorylation of STAT3

## 4.1 Introduction

IL-22 is mainly produced by hematopoietic cells but efficiently stimulates tissue cells (Kumar et al., 2013). In the skin, responsiveness to IL-22 was thought to be mainly mediated by its action on keratinocytes. The presence of IL-*22R1* gene and mRNA expression has been reported in human dermal fibroblasts but it was suggested that these cells do not respond to IL-22, mainly because of the low expression of the receptor in the dermal compartment of the skin (Wolk et al., 2009a). Other groups have also reported the presence of this receptor in stromal cells (Nagalakshmi et al., 2004). However, the responsiveness of stromal/ mesenchymal cells to IL-22 is a subject of intense research at the moment. It has been reported that fibroblasts-like synoviocytes from psoriatic arthritis (PsA) and rheumatoid arthritis (RA) patients express IL-22R1 (Mitra et al., 2012a). In addition, human synovial fibroblasts from RA patients functionally respond to IL-22 by inducing RANKL and MMP9 (Kim et al., 2012). In colonic subepithelial myofibroblasts, IL-22 induces the expression of IL-6, IL8, IL-11 and MMPs through the activation of the MAPK/NFkB pathway (Andoh et al., 2005). The ability of IL-22 to induce the expression of MMPs in different cell types has been linked with cancer progression and invasion of surrounding tissue (Wen et al., 2014). IL-22 acts in synergy with TGFβ1 to mediate EMT in asthmatic airway epithelium (Johnson et al., 2013). So far, the responsiveness of dermal fibroblasts to IL-22 has only been shown in mouse dermal fibroblasts,

where IL-22 induced the expression of ECM and improved wound healing in mice (McGee et al., 2013).

The aim of this study was to investigate if human dermal fibroblasts are sensitive to IL-22 treatment and to see if these cells can contribute to IL-22 responsiveness of the skin compartment, in particular with regard to biological effects involved in tissue repair and healing.

## 4.2 **Results**

### *4.2.1 IL-22R1 is present in human dermal fibroblasts*

The expression of very low levels of IL-22R1 mRNA in human dermal fibroblasts has been reported but its functionality has not been fully investigated (Wolk et al., 2009a). In order to confirm the expression of IL-22R1 in fibroblasts and to see if it is regulated, qPCR, Western blotting and flow cytometry techniques were used. For qPCR, total RNA was isolated and reverse transcribed to cDNA prior to amplification. For Western blotting, cells were lysed and the lysate was used to assess IL-22R1 protein expression. Primary keratinocytes lysate was used as positive control. For flow cytometry analysis, cells were fixed, permeabilised and stained with APC-conjugated anti-human IL-22R1 antibody and appropriate isotype control was used. The results confirmed both mRNA and protein expression of IL-22R1 in human dermal fibroblasts (Fig. 4.1A-D). In Western blotting, major protein bands migrating at about 72 kDa were observed; the same size as the band seen in keratinocytes lysate used as positive control (Fig. 4.1C). The size of the bands was about 9 kDa higher than the predicted molecular weight of IL-22R1, 63 kDa.

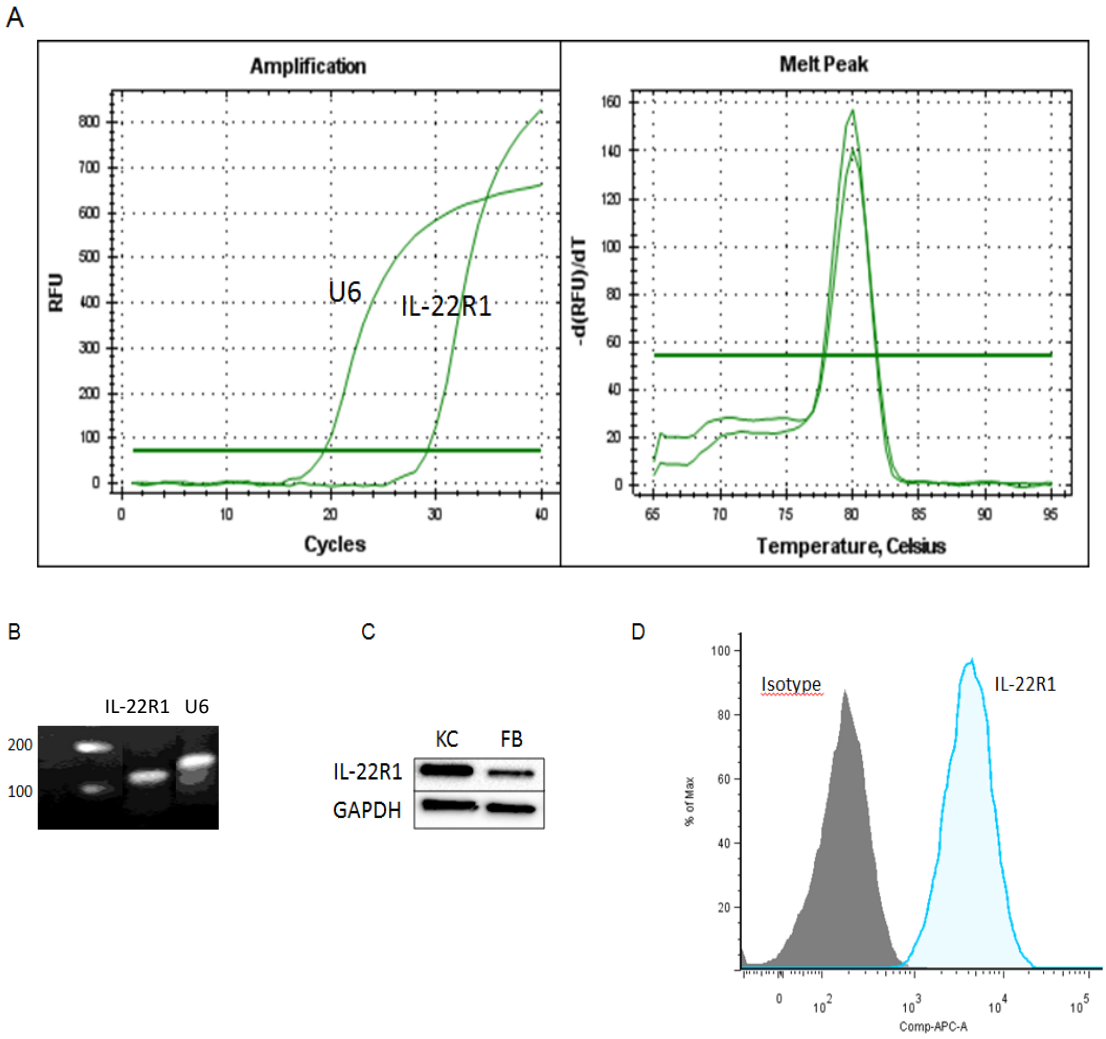



**Figure 4.1: IL-22R1 is expressed in human dermal fibroblasts.**

Fibroblasts (non-stimulated) were passaged and the presence of IL-22R1 was verified using qRT-PCR, Western blotting and Flow cytometry. (A) qRT-PCR quantification and melting peak for U6snRNA and IL-22R1 in non-stimulated cells; (B) qPCR products for IL-22R1 and the housekeeping gene U6snRNA on 2% agarose gel are shown as a representative experiment out of three independent experiments; (C) Western blot analysis using keratinocytes as positive control; (D) IL-22R1 expression in dermal fibroblasts using flow cytometry. Figures are representatives of 3 independent experiments from 3 donors. KC: Keratinocytes; FB: Fibroblasts.

### *4.2.2 IL-22 activated STAT3 pathway in human dermal fibroblasts*

Since IL-22 is capable of activating the STAT3 pathway in many cells types, this effect was investigated in human dermal fibroblasts in order to establish the responsiveness of these cells to the cytokine. The result showed that IL-22 is capable of activating the STAT3 pathway in dermal fibroblasts. The best effect of IL-22 on STAT3 phosphorylation was observed after 10 minutes of treatment and this effect decreased over the time points investigated. The activation of the STAT3 pathway was lost completely after 120 minutes of fibroblasts treatment with IL-22 (Fig. 4.2). The phosphorylation kinetics is similar to what was observed in primary keratinocytes (Fig. 3.10).

In order to investigate if the activation of STAT3 would result in expression of downstream genes, fibroblasts were treated with IL-22 for various duration of time. The result showed SOCS3 upregulation in IL-22 treated fibroblasts (Fig. 4.3A-E). The peak time point for this SOCS3 expression was 1 hour after treatment with IL-22 (Fig. 4.3B). The expression level of SOCS3 began to drop after 2 hours of treatment with IL-22. IL-22 did not induce significant expression of JUNB, which is another gene downstream of STAT3 (Fig. 4.3A-E). However, there was about 1.5 fold increase in expression of JUNB after 1 hour of treatment in comparison to untreated cells (Fig. 4.3B).

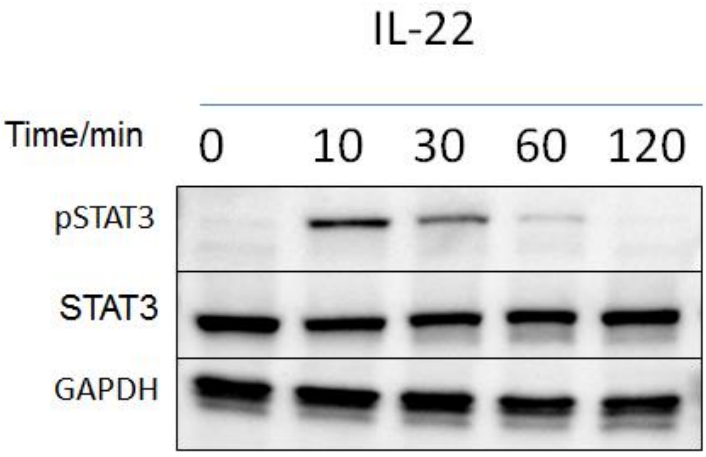



**Figure 4.2: STAT3 is activated in IL-22 treated dermal fibroblasts.**

Fibroblasts were either not treated or treated with 100 ng/ml IL-22 in serum free DMEM for 10, 30, 60 or 120 minutes. STAT3 phosphorylation was assessed using Western blotting. Results shown are representative of 3 independent experiments using 3 different donors.

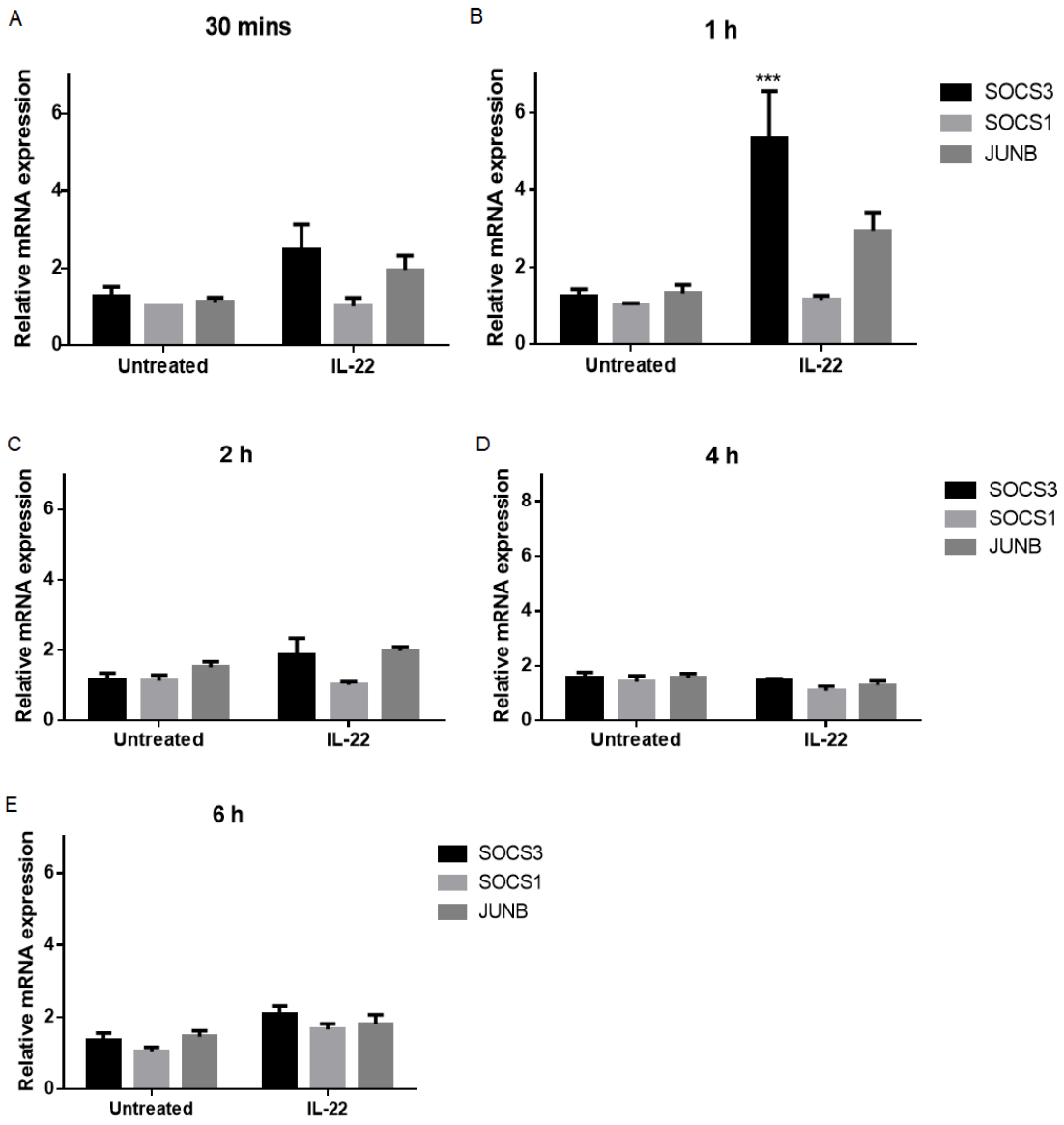



**Figure 4.3: IL-22 treated dermal fibroblasts express SOCS3.**

Fibroblasts were either not treated or treated with 100 ng/ml IL-22 for time-points ranging between 30 minutes and 6 hours. mRNA expression of SOCS3, SOCS1 and JUNB was quantified using qRT-PCR and results for cells exposed to the following stimulation periods are shown: (A) 30 minutes; (B) 1 hour; (C) 2 hours; (D) 4 hours; (E) 6 hours. 3 independent experiments were carried out using 3 different donors. Two-way ANOVA was used to determine statistical significance; n=3. Values are mean±SEM; *p < 0.05, **p < 0.01, ***p<0.001, ****p<0.0001.

### *4.2.3 IL-22 induced the expression of SOCS3 in a dose-dependent manner*

Having observed that IL-22 was able to induce the expression of SOCS3 in dermal fibroblasts with the best time point being 1 hour, dose titrations were then carried out. The result showed that both 50 and 100 ng/ml IL-22 can induce significant expression of SOCS3; however, 100 ng/ml had a more significant effect. There was a similar dose-dependent tendency on JUNB expression (Fig. 4.4).

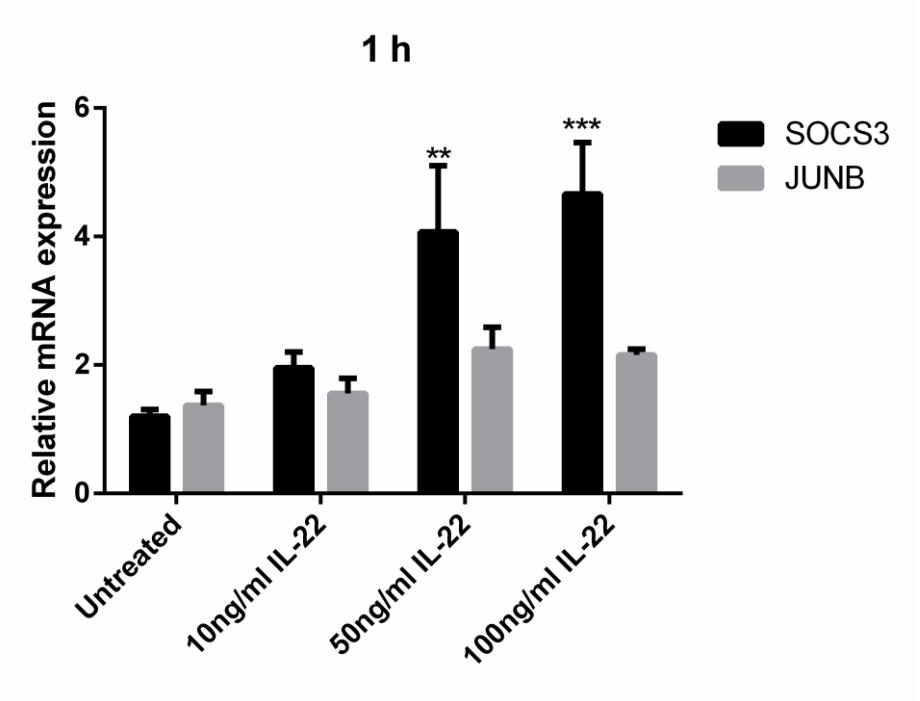



**Figure 4.4: Dermal fibroblasts respond to IL-22 dose-dependently.**

Fibroblasts were either untreated or treated with different concentration of rhIL-22 ranging from 1 ng/ml to 100 ng/ml for 1 hour. SOCS3 and JUNB mRNA expression was quantified using qRT-PCR. For statistical analysis, two-way ANOVA was used to compare columns. n=3, values represent mean± SEM. *p<0.05, **p<0.01, ***p<0.001.

### *4.2.4 IL-22R1 upregulation resulted in increased SOCS3 expression*

In order to investigate whether receptor upregulated resulted in an enhanced functional response to IL-22, dermal fibroblasts were pre-treated with TNFα or TGFβ1 prior to IL-22 stimulation. IL-22R1 protein expression was measured using flow cytometry while SOCS3 and JUNB mRNA expression was measured using qRT-PCR. Treatment of dermal fibroblasts with either TNFα or TGFβ1 for 16 hours resulted in increased expression of IL-22R1 (Fig. 4.5A-B). Interestingly, the result also showed that pre-treatment of dermal fibroblasts with either TGFβ1 or TNFα enhanced IL-22-induced expression of SOCS3 and JUNB. This increased expression of SOCS3 and JUNB was at least 3-fold and 1.5 fold respectively more than in cells that were not pre-treated but treated with IL-22 alone (Fig. 4.5C).

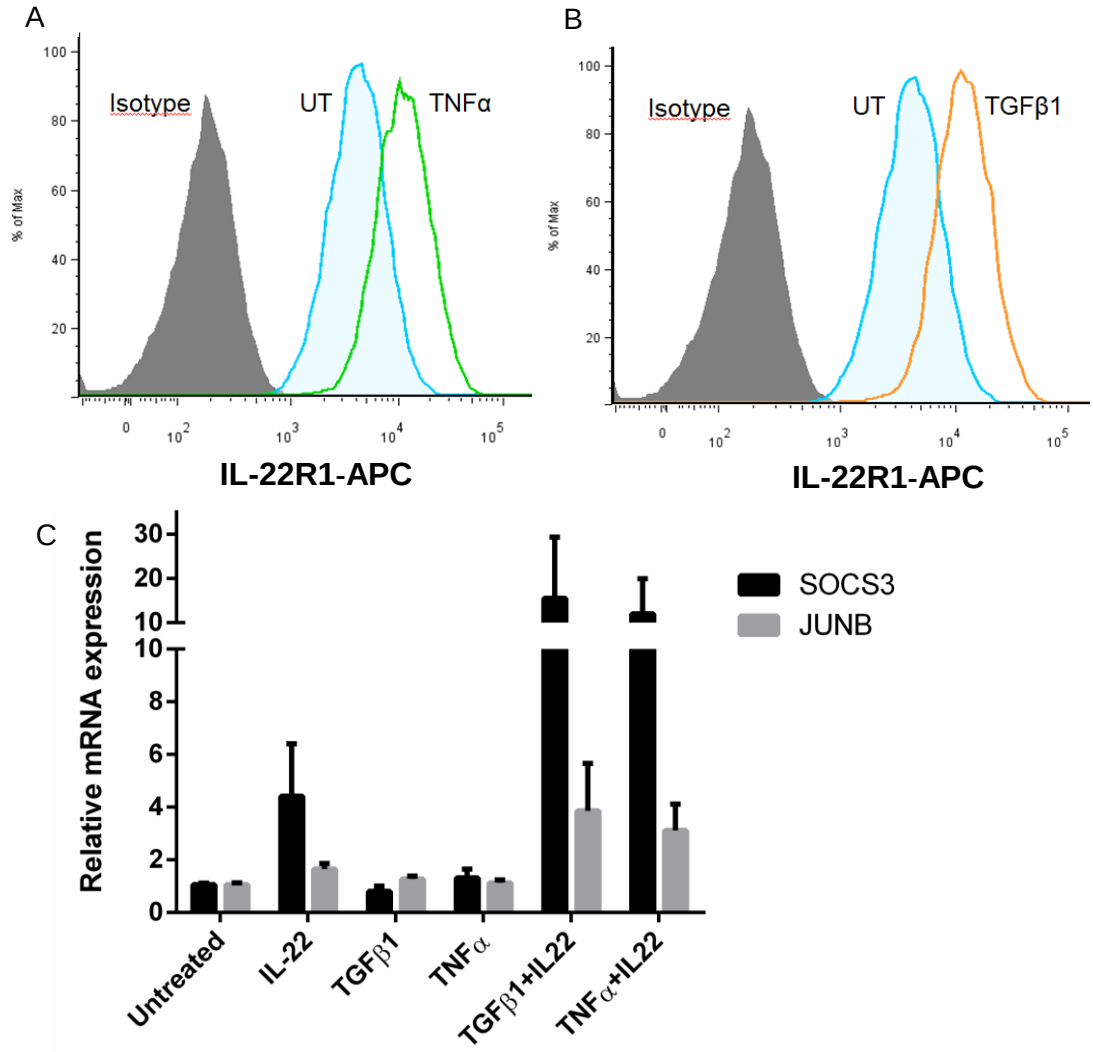
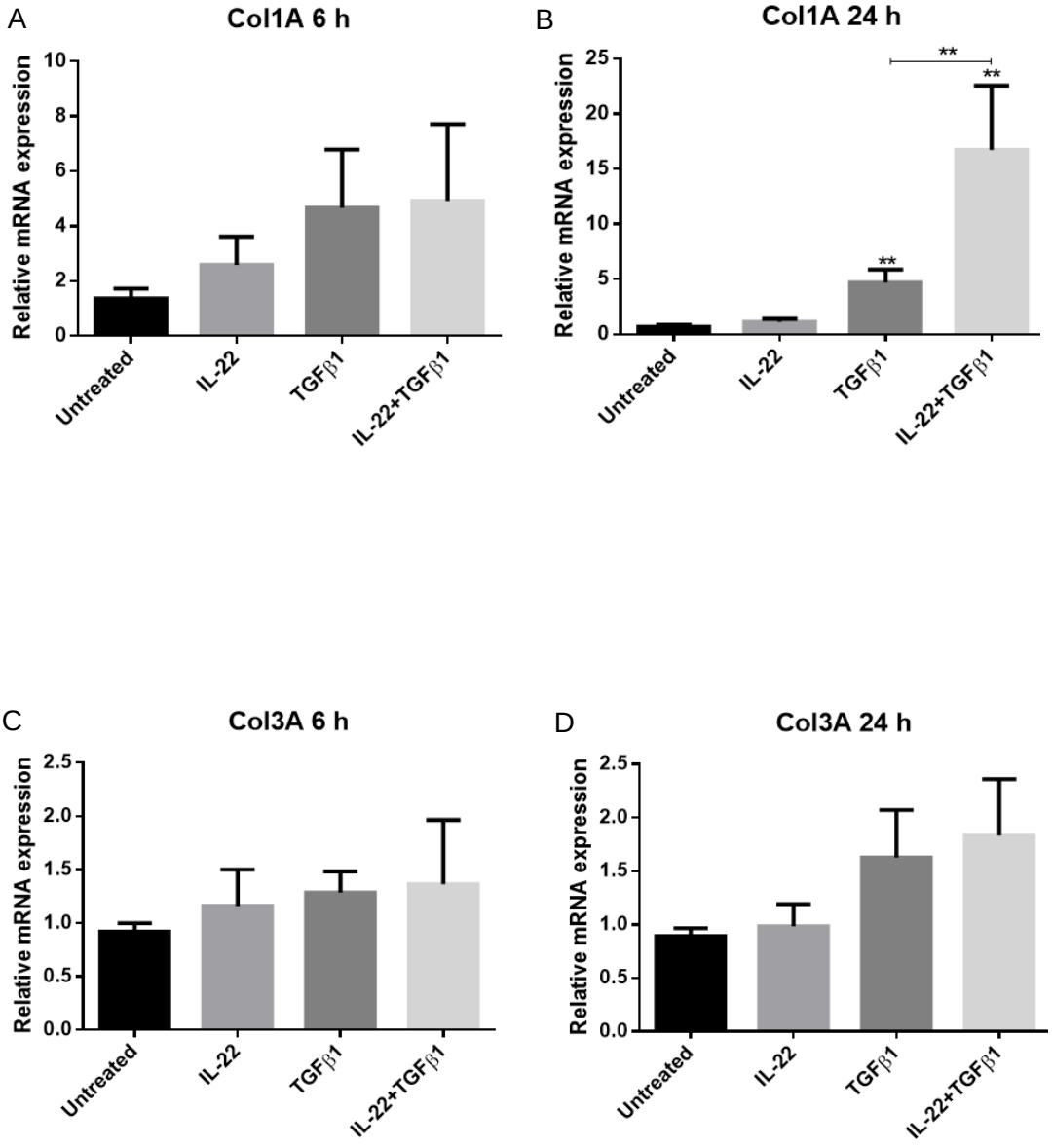



**Figure 4.5: Upregulation of IL22R1 expression leads to increased STAT3 signature gene expression.**

Fibroblasts were pre-treated with 5 ng/ml TGFβ1 or 20 ng/ml TNFα for 16 hours with untreated control. Cells were stained with APC-conjugated IL-22R1 and appropriate isotype prior to flow cytometric analysis. (A) Untreated (blue) and TNFα (green) (B) Untreated (blue) and TGFβ1 (orange) (C) Cells pretreated with TGFβ1 or TNFα were washed twice with warm PBS prior to stimulation with 100 ng/ml IL-22 for 1 hour. SOCS3 and JUNB mRNA expression was quantified using qRT-PCR. Values are mean±SEM. n=2. UT: Untreated.

### *4.2.5 IL-22 enhanced TGFβ1-induced collagen expression but had no effect on CCL2 production*

The ability of IL-22 to induce collagen expression in murine dermal fibroblasts has been described (McGee et al., 2013). In order to investigate if IL-22 is able to induce the expression of collagens in human dermal fibroblasts, cells were treated with IL-22, TGFβ1 or a combination of both. The results showed that IL-22 is not able to induce the expression of *Col1A1*, *Col3A1*, *Col4A2, Col7A1* in dermal fibroblasts at the time points investigated (Fig. 4.6A-H). TGFβ1, which is known as an inducer of collagen expression, was able to induce significant expression of *Col1A1* and *Col3A1* after 24 hours of treatment but not after 6 hours (Fig. 4.6A-D). Interestingly, the results showed that IL-22 is able to significantly enhance the expression of TGFβ1-induced *Col1A1*, *Col4A2* and *Col7A1* but not *Col3A1* expression after 24 but not 6 hours of treatment (Fig. 4.6). Supernatants from IL-22 treated cells were used for CCL2 determination. As shown in Figure 4.7, IL-22 did not induce significant amount of CCL2 protein relative to the untreated control. The result also showed that dermal fibroblasts produce high CCL2 levels constitutively (Fig. 4.7).


A
Col1A 6 h
Relative mRNA expression
10
8
6
4
2
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Untreated
IL-22
TGFβ1
IL-22+TGFβ1
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Col1A 24 h
Relative mRNA expression
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Untreated
IL-22
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IL-22+TGFβ1
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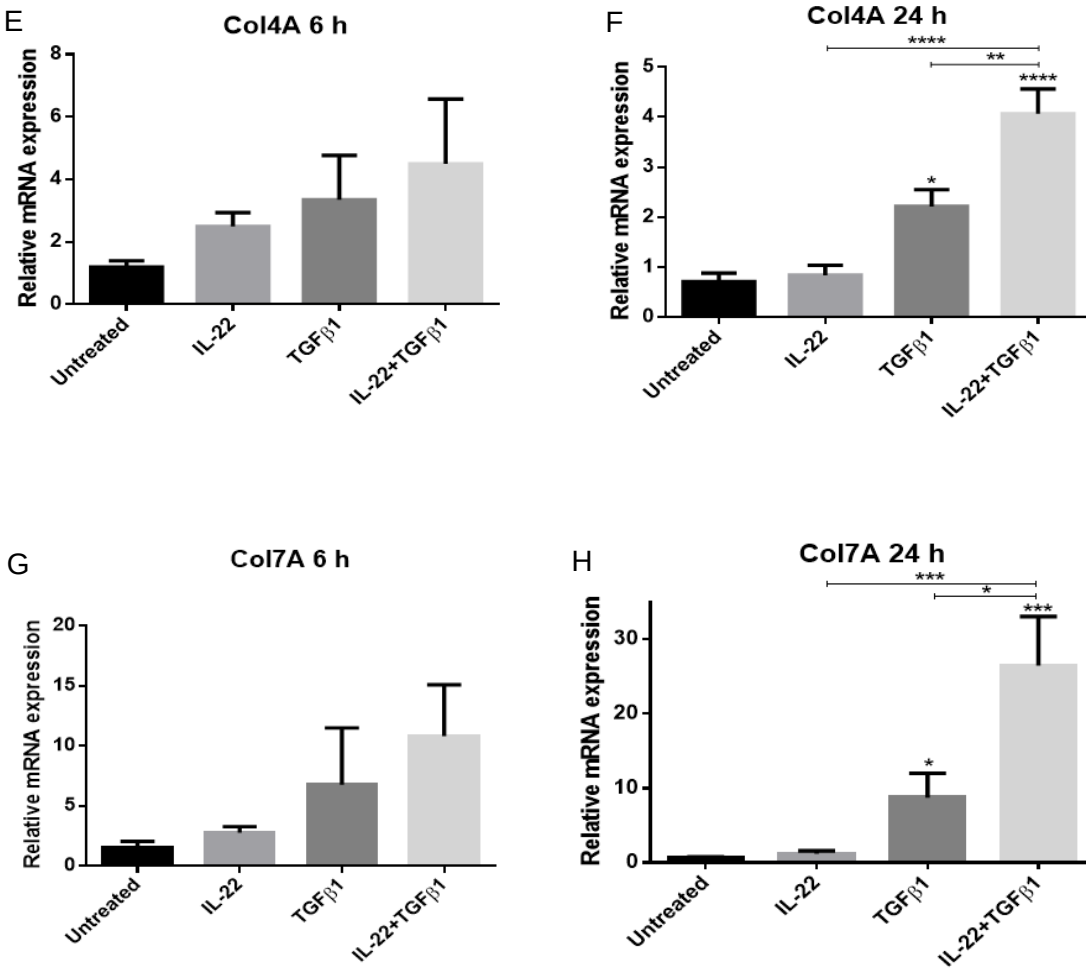



**Figure 4.6: Effect of IL-22 on collagen expression in dermal fibroblasts**

Fibroblasts were treated with 100 ng/ml IL-22 or 5 ng/ml TGFβ1 or combination of IL-22 and TGFβ1 for 6 or 24 hours in serum free medium. mRNA expression of collagens was measured by qRT-PCR. (A) *Col1A1* expression after 6 hours; (B) *Col1A1* expression after 24 hours; (C) *Col3A1* expression after 6 hours; (D) *Col3A1* expression after 24 hours; (E) *Col4A2* expression after 6 hours; (F) *Col4A2* expression after 24 hours; (G) *Col7A1* expression after 6 hours (H) *Col7A1* expression after 24 hours. For statistical analysis, one-way ANOVA was used to compare columns. n=4, values represent mean± SEM; *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

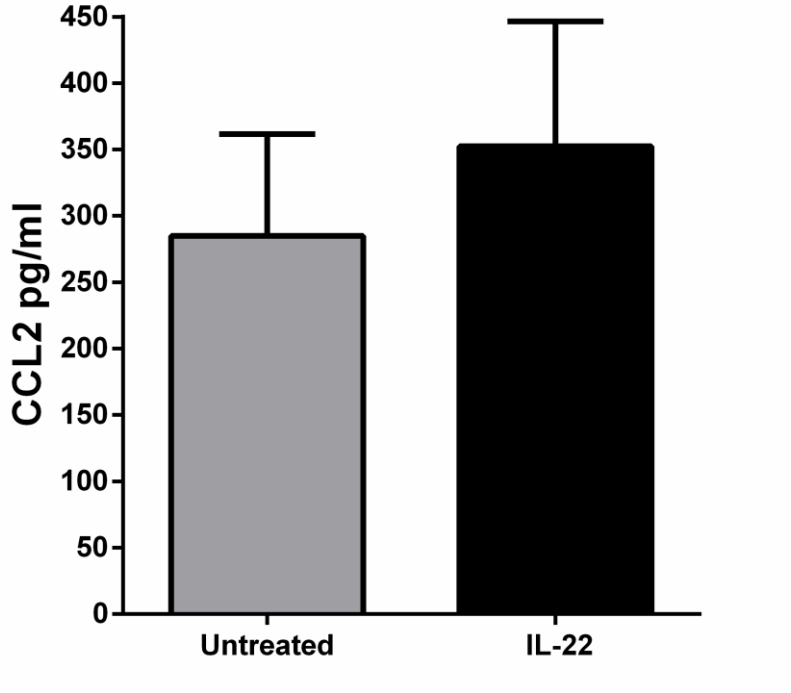



**Figure 4.7: Effect of IL-22 on CCL2 production in dermal fibroblasts.**

Cultured dermal fibroblasts were either untreated or treated with 100 ng/ml IL-22 for 24 hours. Supernatants were collected and the amount of secreted CCL2 in culture supernatant was determined using ELISA. n=4, values represent mean± SEM.

### *4.2.6 IL-22 does not induce fibroblasts proliferation and does not affect 'scratch' gap closure*

In order to investigate whether IL-22 is able to induce fibroblasts proliferation, human dermal fibroblasts were stained with cell tracer dye and were treated with IL-22 for 72 hours. The results showed that IL-22 does not induce any detectable level of proliferation in dermal fibroblasts (Fig. 4.8A-B). The effect of IL-22 on cell monolayer gap closure was also investigated by treating dermal fibroblasts with IL-22 for 48 hours. The results showed that IL-22 does not affect 'scratch' closure in these monolayer cultures (Fig. 4.9).

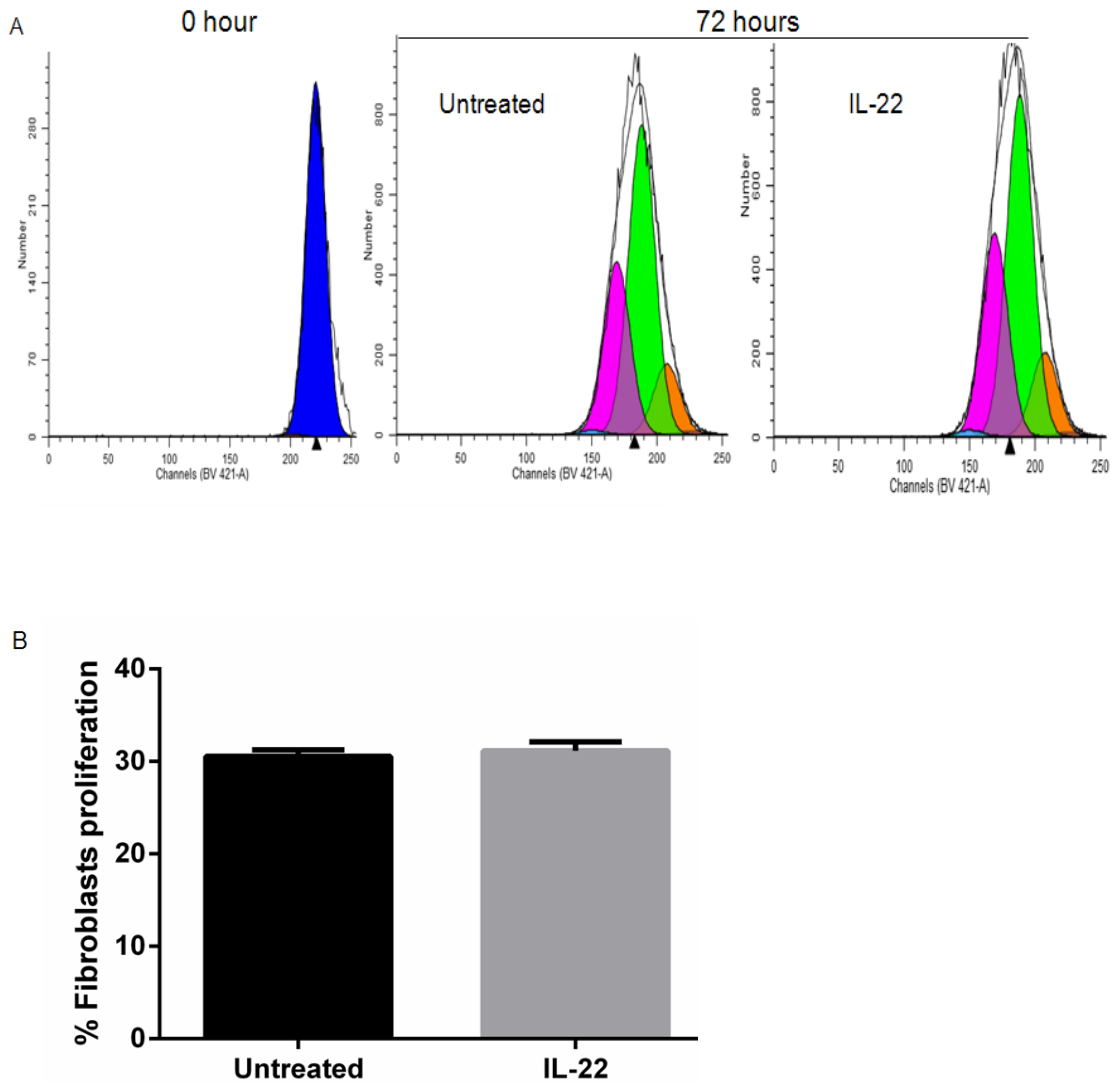



**Figure 4.8: Effects of IL-22 on proliferation of dermal fibroblasts**

Human dermal fibroblasts were labelled with cell trace violet. Cells were treated with IL-22 (100 ng/ml) for 72 h. Proliferation as a measure of fluorescence signal was determined by flow cytometric analysis. (A) As the cells divide, the cell trace violet is diluted between daughter cells causing a shift in fluorescence peak towards the left side. The histogram of daughter cells populations is depicted; (B) Graphical representation of percentage cell proliferation; n=3, values represent mean± SEM.

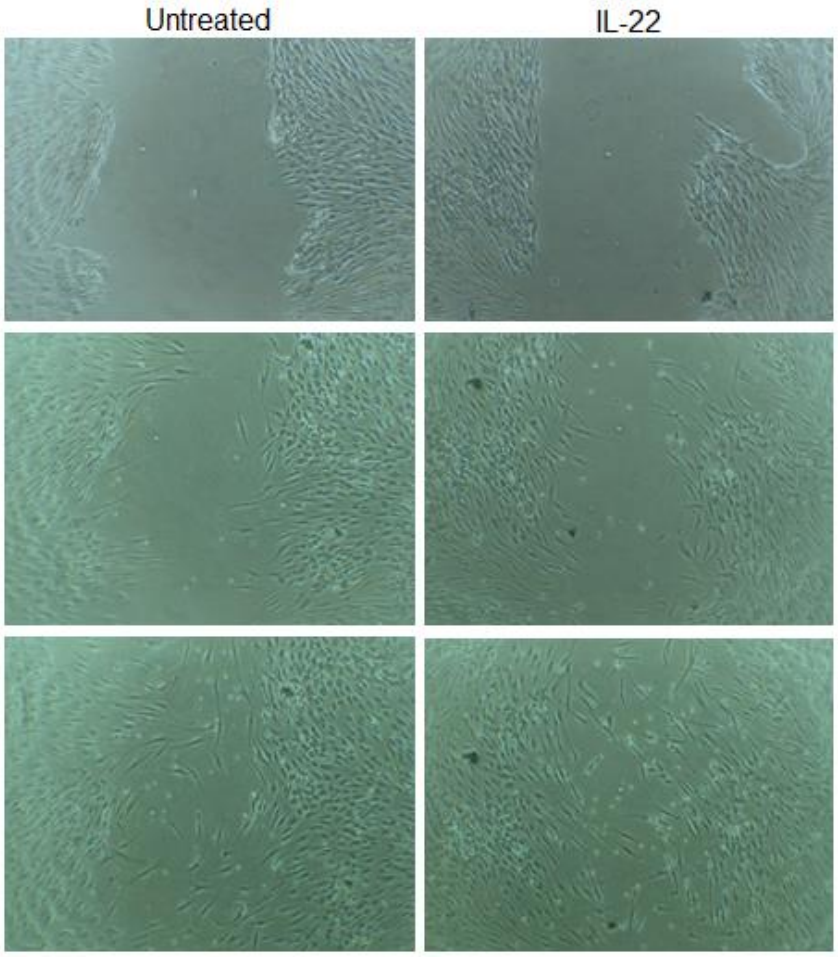



**Figure 4.9: IL-22 does not enhance a 'scratch' gap closure in fibroblast monolayers.**

Monolayer culture of human dermal fibroblasts was allowed to grow to 95% confluency and scratched with p200 (yellow) pipette tip. Cells were either untreated or treated with IL-22 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 2 independent experiments using 2 different donors. No obvious acceleration in gap closure in IL-22 compared to non-treated cells was observed.

## 4.3 **Discussion**

As outlined above, IL-22 has been shown to play important role in cell migration, cell proliferation, prevention of tissues damage and angiogenesis (Lim and Savan, 2014; Ren et al., 2010; Zenewicz et al., 2007) particularly in epithelial compartments. IL-22R1 determines cellular responsiveness and its expression is restricted to tissue cells and not cells of hematopoietic origin (Lim and Savan, 2014). Very low levels of IL-22R1 mRNA expression has been reported in human dermal fibroblasts; however, the responsiveness of these cells to IL-22 treatment has not been established (Wolk et al., 2009a). Protein data showing the presence of IL-22R1 in human dermal fibroblasts has also not been reported. IL-22R1 has been described in mouse dermal fibroblasts and these cells have shown responsiveness to IL-22 stimulation resulting in STAT3 activation, myofibroblasts differentiation and extracellular matrix production (McGee et al., 2013). It is thus a major finding of this project that IL-22R1 mRNA and protein are expressed in untreated human dermal fibroblasts. The predicted molecular weight of IL-22R1 is 63kDa; however, the IL-22R1 protein band was observed at 72kDa on the PVDF membrane, the same size as observed in human primary keratinocytes. The 9kDa difference in predicted molecular weight and the actual size seen in Western blotting may be attributed to glycosylation of IL-22R1 at three putative N-linked glycosylation (Asn-X-Thr) sites in the extracellular domain of the receptor (Sabat et al., 2014). It is interesting that the expression of IL-22R1 in dermal fibroblasts is regulated by TGFβ1 and TNFα, which are generally regarded as anti-inflammatory and proinflammatory cytokines, respectively. Increased expression of IL-22R1 in the presence of TNFα has

been reported in fibroblasts-like synoviocytes (FLSs) from patients suffering from RA and osteoarthritis (OA) (Carrion et al., 2013). It is well known that IL-22 exerts its biological effect on cells through the activation of mainly STAT3 (Sabat et al., 2014; Wolk et al., 2004). The above results confirm that IL-22 activates this signalling pathway also in human dermal fibroblasts, which has not been shown before. The induction of SOCS3 expression by dermal fibroblasts following IL-22 treatment suggests that these cells may contribute to high expression of SOCS3 seen in skin lesion of patients with active psoriasis (Fig. 3.14; (Takekoshi et al., 2013)) and it is interesting that TNFα – known to be involved for psoriasis plaque formation - could enhance this effect. The role of TNFα in lupus is controversial. Our group has previously shown that this cytokine may be important in counteracting the damaging effect of IFNs on skin cells (Alase, 2013). By positively regulating SOCS3 expression in dermal fibroblasts, TNFα may enhance the beneficial effect of IL-22 in reducing apoptosis in CLE skin. Although IL-22 treatment alone did not have any effect on collagen expression in dermal fibroblasts, this cytokine enhanced TGFβ1-induced collagen types I, IV and VII expression. This finding suggests a positive role for IL-22 in basement membrane formation and maintenance of skin integrity. The enhancing effect of IL-22 on TGFβ1-induced EMT in asthmatic bronchial epithelial cells has previously been reported (Johnson et al., 2013). The importance of IL-22 in cell proliferation has been identified by many groups (Brand et al., 2006; Carrion et al., 2013). However, some have suggested that the IL-22 effect is limited to inhibition of expression of markers for terminal differentiation in cells and that it does not actively drive cell proliferation (Boniface et al., 2005). As also seen in primary

keratinocytes, IL-22 did not significantly induce proliferation in dermal fibroblast. The differences between the findings in this work and that of Brand et al. and Carrion et al. regarding the role of IL-22 on cells proliferation may be explained by the fact that these two groups measured cells proliferation using MTS assay, a technique that determines metabolic activities of the cells rather than true proliferation (i.e higher metabolic activity not always equals increased proliferation). IL-22 also failed to induce proliferation in colonic subepithelial myofibroblasts (Andoh et al., 2005). While IL-22 improved the rate of 'scratch gap' closure in primary keratinocytes ((Scarponi et al., 2014); Fig. 3.11A-B), no significant effect on gap closure was observed in dermal fibroblasts. This finding is contrary to what was observed in mouse dermal fibroblasts, where IL-22 improved wound healing (McGee et al., 2013). The ability of IL-22 to induce the expression of CCL2 in epithelial cells and hepatocytes has been reported (Donnelly et al., 2004; Scarponi et al., 2014). IL-22 failed to induce significant amount of CCL2 protein in dermal fibroblasts. This finding suggests that the dermal compartment may not be involved in IL-22 induced recruitment of leukocytes and monocytic infiltrates into the skin during skin inflammation in diseases, such as psoriasis.

Taken together, these findings suggest that human dermal fibroblasts may play an important role in inflammatory conditions with high expression of IL-22. IL-22 may support healing responses in synergy with TGFβ. The lack of chemokine production may well be in line with the rather inflammation "limiting" role of the dermal as compared to the epidermal compartment as hypothesised by our group (Wittmann et al., 2012). Thus, IL-22 could play a beneficial role in CLE by inducing anti-apoptotic pathways and sustaining cell

survival through active induction of SOCS3 expression. These functional responses have not been addressed in this work and it would be of high interest to gain further insight in the disease specific impact of IL-22 on the dermal compartment (e.g. in CLE and psoriasis). However, results presented here contribute to the growing evidence of involvement of IL-22 in wound healing and maintenance of skin integrity through enhanced collagen deposition. In addition, this study further highlights that dermal fibroblasts are not just connective tissue cells but are active players in the overall skin immunology.

# CHAPTER 5: IFNλ STIMULATES MXA PRODUCTION IN HUMAN DERMAL FIBROBLASTS VIA A MAPK-DEPENDENT STAT1-INDEPENDENT MECHANISM

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

## 5.1 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 promoting repair and healing responses. Although IFNλ is structurally related to IL-10, it resembles type I IFNs in its antiviral and anti-proliferative 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), coding for proteins, 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; Steen and Gamero, 2010); which has also been reported for HaCaT keratinocyte cell lines (Maher et al., 2008). 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 CLE and here increased expression is linked with disease severity (Naschberger et al., 2010; Wenzel et al., 2005a).

The aim of this study was to understand if human dermal fibroblasts are responsive to IFNλ1 through the activation of STAT1 and subsequent downstream induction of ISGs. There was also need to understand the role

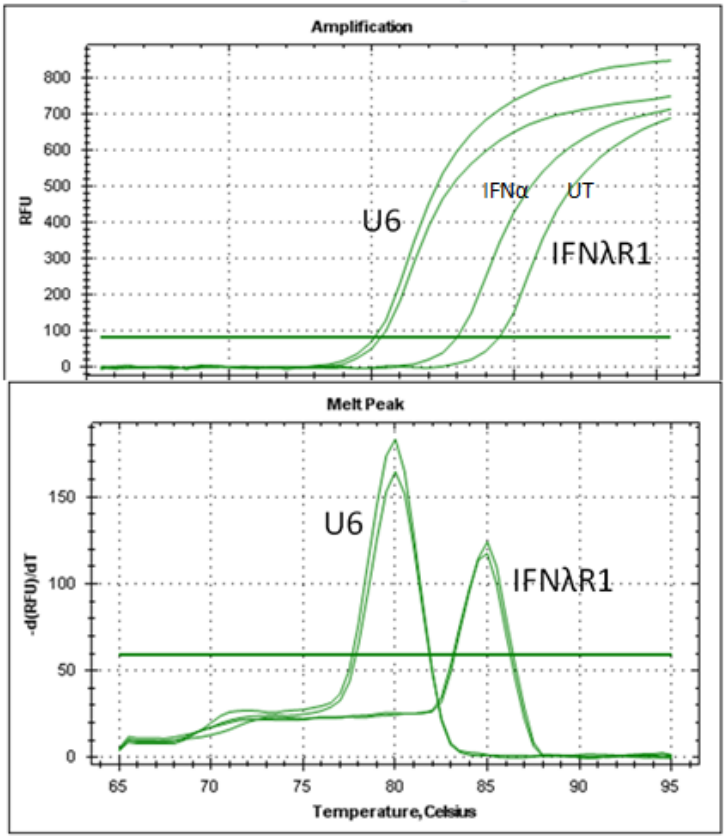
of this cytokine in maintenance of skin integrity with regard to collagen expression and proliferation of skin resident cells.

## 5.2 **Results**

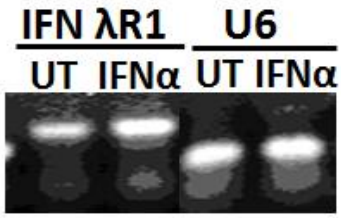
### *5.2.1 IFNλR1 is expressed by human dermal fibroblasts*

The presence of low levels of IFNλR1 mRNA in human dermal fibroblasts has been reported (Witte et al., 2009). In order to confirm the expression of IFNλR1 in fibroblasts mRNA expression was measured by qRT-PCR and protein expression was measured by Western blotting and flow cytometry. The result showed that IFNλR1 is expressed in dermal fibroblasts and it is upregulated in the presence of IFNα (Fig. 5.1A-F).

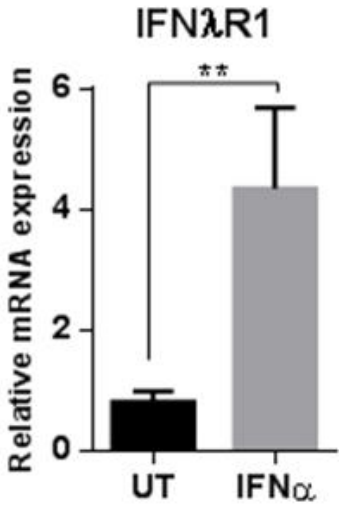
A



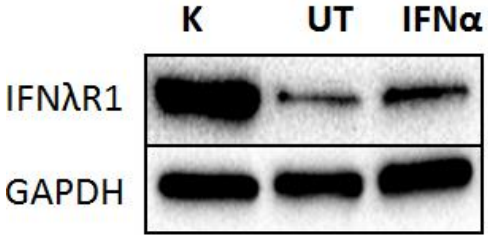
B


IFN λR1
U6
UT IFNα UT IFNα

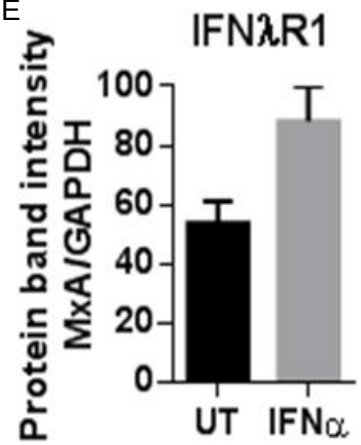
C


IFNλR1
**
Relative mRNA expression
6
4
2
0
UT
IFNα

D


K
UT
IFNα
IFNλR1
GAPDH

E


IFNλR1
Protein band intensity
MxA/GAPDH
100
80
60
40
20
0
UT
IFNα

F

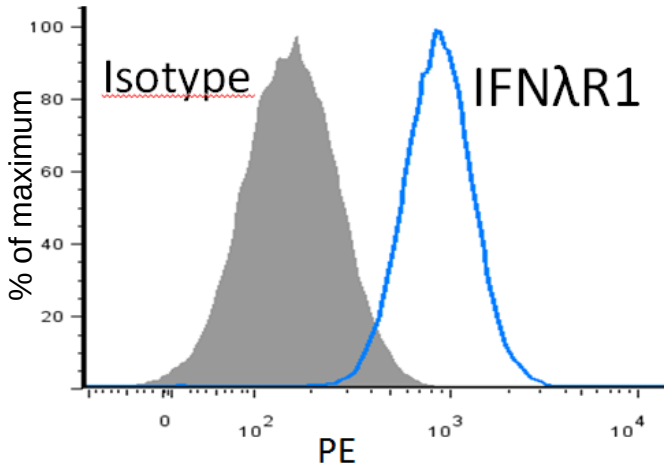

Isotype
IFNλR1
% of maximum
100
80
60
40
20
0
10^2
10^3
10^4
PE

**Figure 5.1: IFNλR1 expression and production in human dermal fibroblasts**

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. (A) Amplification and melting peak showing IFNλR1 and U6snRNA; (B) qPCR products for IFNλR1 and the housekeeping gene U6snRNA on 2% agarose gel are shown as a representative experiment out of five independent experiments. (C) Relative mRNA expression is shown for five independent experiments. (D) A representative Western blot result for IFNλR1 protein and (E) protein band intensity analysis are depicted; n=3. (F) A representative (n=3) histogram of flow cytometry analysis depicting the staining intensity for the IFNλR1 and appropriate isotype control. For statistical analysis, Mann Whitney U test was used. Values represent mean ± SEM. *$p<0.005$, **$p<0.01$. UT: Untreated; K: keratinocytes.

### *5.2.2 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). mRNA expression of ISGs was quantified using qRT-PCR. The result showed significant expression of MxA but not GBP-1 and OAS2 after 4 and 24 hours of IFNλ1 treatment (Fig. 5.2A-F). As expected, when compared to IFNλ1, IFNα induced significantly higher expression of MxA and GBP-1 after 4 hours of treatment. However, while the expression of MxA and OAS2 remained high after 24 hours of treatment with IFNα, GBP-1 expression dropped significantly over this time period (Fig. 5.2B/D/F). In fibroblasts, IFNα appeared to induce higher upregulation of MxA as compared to OAS2 and GBP-1 (Fig. 5.2A-F).

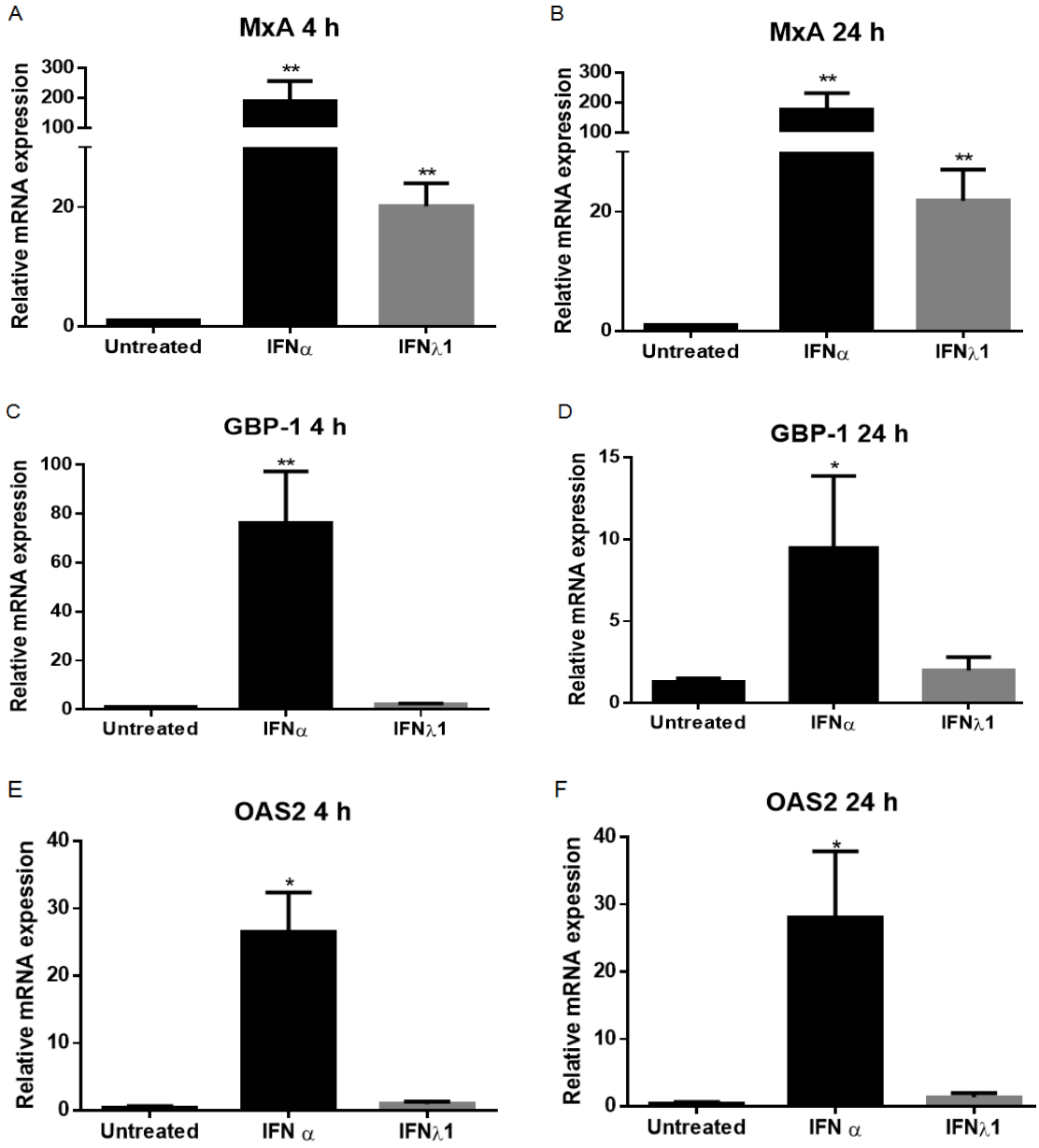



**Figure 5.2: Interferon stimulated genes (ISGs) expression in human dermal fibroblasts**

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. mRNA expression of ISGs was measured using qRT-PCR. (A) MxA, 4 hours; (B) MxA, 24 hours; (C) GBP-1, 4 hours; (D) GBP-1, 24 hours; (E) OAS2, 4 hours; (F) OAS2, 24 hours. For statistical analysis, Mann Whitney U test was used. n=5, values represent mean ± SEM. *p<0.05, **p<0.01.

### *5.2.3 IFNα and IFNλ induce ISG expression in human primary keratinocytes*

The findings on ISGs expression in fibroblasts was compared 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). The result showed 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 (Fig. 5.3A-F). Interestingly, a different time kinetic was observed for the ISG response to IFNα as compared to IFNλ1. IFNα showed a stronger upregulation of MxA, OAS2 and GBP-1 at 6 hours in comparison to IFNλ1, while IFNλ1 stimulation resulted in a strong increase in MxA, OAS2 and GBP-1 expression between 6 and 24 hours. This suggests that IFNλ1 stimulation may result in more delayed and/or longer lasting effects (Fig. 5.3A-F) as previously observed in hepatocytes (Bolen et al., 2014).

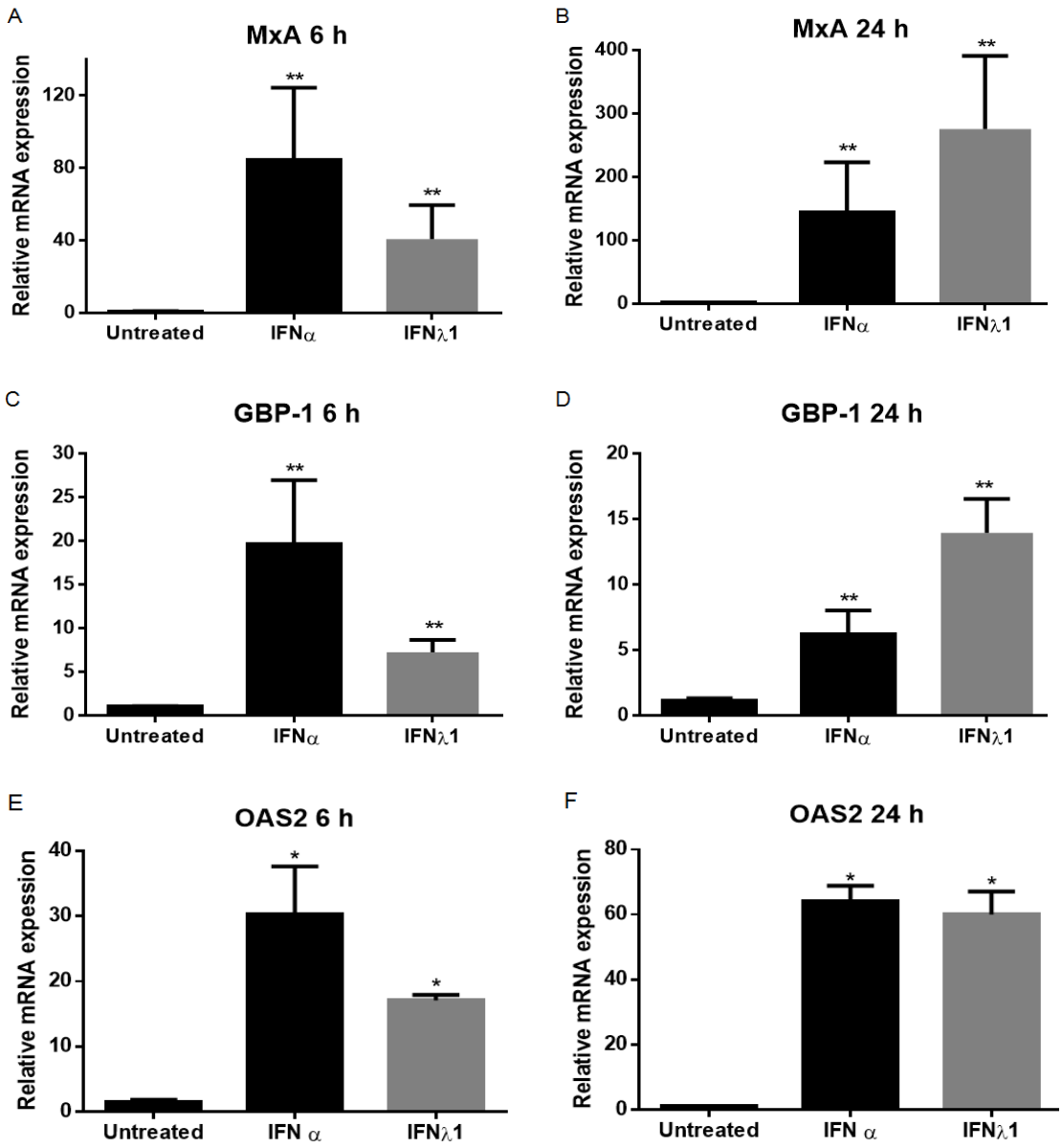



**Figure 5.3: Interferon stimulated genes (ISGs) expression in human primary keratinocytes.**

Human primary keratinocytes were either untreated or treated with 10 ng/ml IFNα or 100 ng/ml IFNλ1 for 6 or 24 hours in KGM (-/-). mRNA expression of ISGs was measured using qRT-PCR. (A) MxA, 4 hours; (B) MxA, 24 hours; (C) GBP-1, 4 hours; (D) GBP-1, 24 hours; (E) OAS2, 4 hours; (F) OAS2, 24 hours. For statistical analysis, Mann Whitney U test was used. n=5, values represent mean ± SEM. *p<0.05, **p<0.01.

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

Type I and III IFNs are known to activate the canonical JAK/STAT pathway to induce downstream gene expression. Having established that dermal fibroblasts respond to IFNλ1, the mechanism of action of this cytokine was then investigated. To achieve this, dermal fibroblasts were treated with either IFNλ1 or IFNα for different time periods and protein phosphorylation was determined using western blotting. As expected, IFNα-induced STAT1 phosphorylation with maximal phosphorylation detected after 30 minutes of stimulation (Fig. 5.4A). However, no STAT1 phosphorylation in IFNλ1 treated cells was observed 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). Therefore, the ability of IFNλ1 to induce the phosphorylation of p38 in dermal fibroblasts was investigated and we clearly found activation of this MAPK (Fig. 5.4A). IFNα treatment resulted in p38 phosphorylation in addition to STAT1 phosphorylation (Fig. 5.4A). Furthermore, both IFNα and IFNλ1 induced phosphorylation of ERK pathway in dermal fibroblasts. As expected, both IFNα and IFNλ1 induced STAT1 phosphorylation in human primary keratinocytes (Fig. 5.4B). However, no clear IFNλ dependent activation of either the p38 or ERK pathway upon treatment with IFNs was observed (Fig. 5.4B).

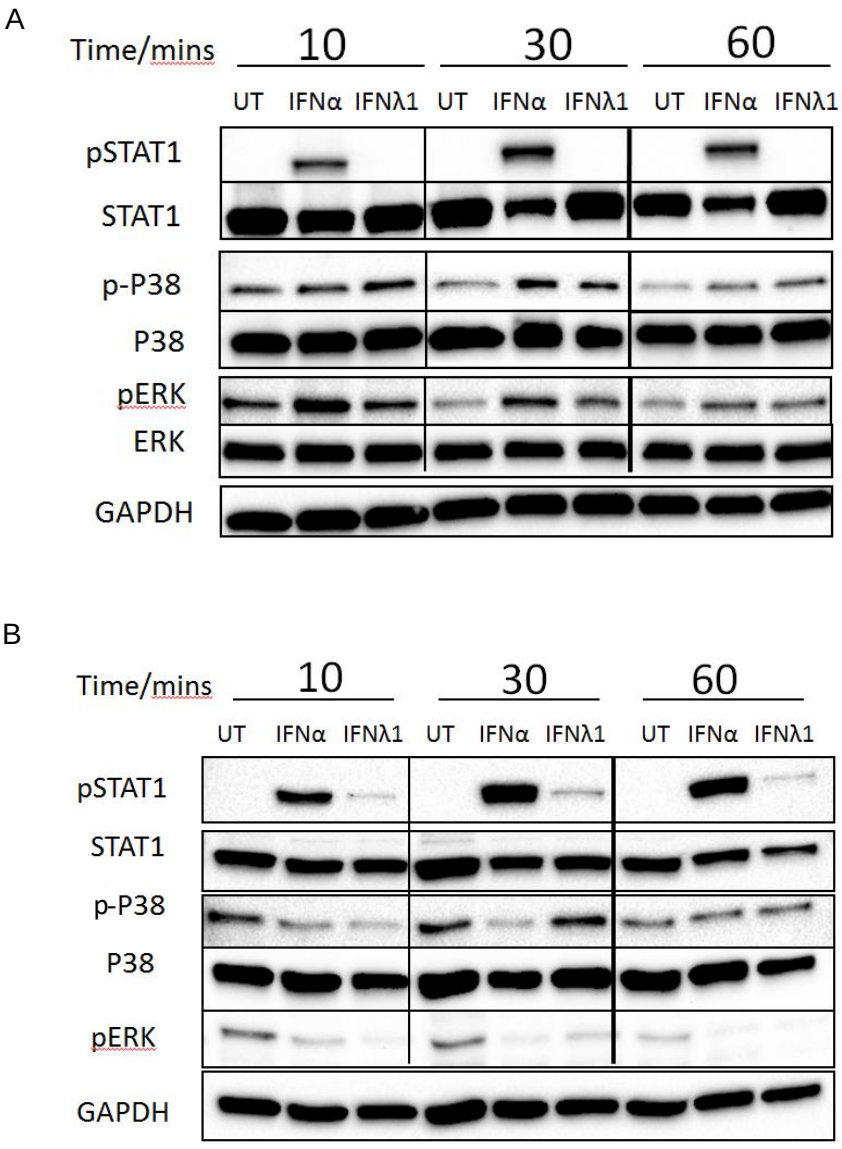
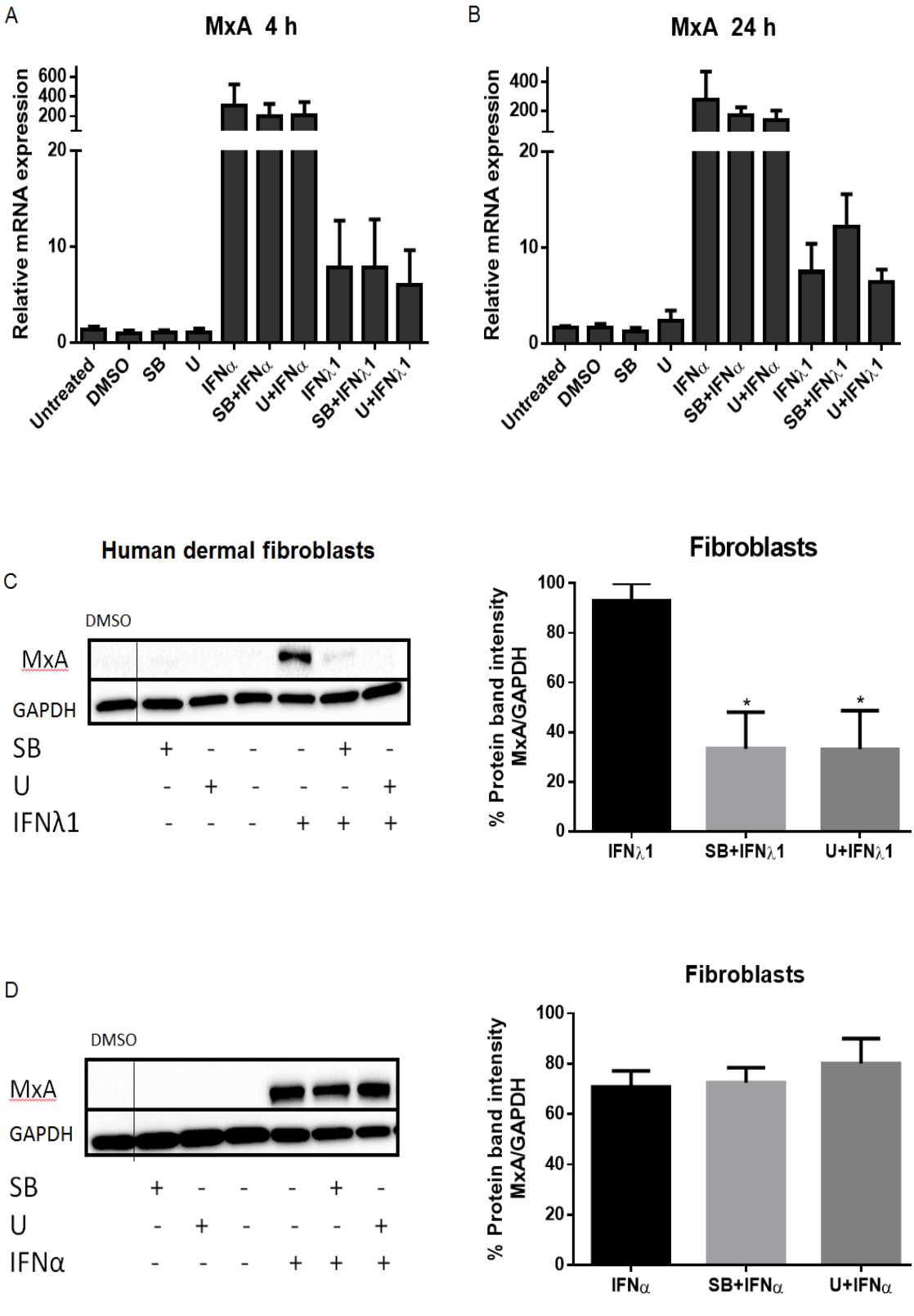



**Figure 5.4: IFNλ1 induced STAT1 and MAPKs phosphorylation fibroblasts and keratinocytes**

Human dermal fibroblasts (5.4A) or primary keratinocytes (5.4B) 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 is depicted. UT: untreated.

### *5.2.5 P38 and ERK inhibitors attenuate IFNλ1-induced MxA protein expression*

Based on the finding that IFNλ1 induces the activation of the p38 and ERK MAPKs, there was need to understand if these were 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 and analysis of mRNA or protein expression. Interestingly, the results showed that the expression of MxA was not regulated at the mRNA level by the MAPK inhibitors, as no significant difference in MxA mRNA expression in their presence or absence upon stimulation with cytokines was observed (Fig. 5.5A-B). However, at the protein level, the presence of either the p38 or ERK inhibitor abrogated IFNλ1 induced MxA protein production (Fig. 5.5C). No decrease in IFNα induced MxA protein production in fibroblasts in the presence of SB203580, PD98059 or U0126 was observed (Fig. 5.5D). It was also investigated whether MAPKs played a role in IFNλ1-induced MxA protein expression in human primary keratinocytes. When these cells were treated in the same manner as the fibroblasts, no significant effect on IFNλ1 and IFNα induced MxA protein production in the context of p38 or ERK inhibition was observed (Fig. 5.5E).


A
MxA 4 h
Relative mRNA expression
600
400
200
20
10
0
Untreated
DMSO
SB
U
IFNα
SB+IFNα
U+IFNα
IFNλ1
SB+IFNλ1
U+IFNλ1
B
MxA 24 h
Relative mRNA expression
400
200
20
10
0
Untreated
DMSO
SB
U
IFNα
SB+IFNα
U+IFNα
IFNλ1
SB+IFNλ1
U+IFNλ1
Human dermal fibroblasts
C
DMSO
MxA
GAPDH
SB + - - - + -
U - + - - - +
IFNλ1 - - - + + +
Fibroblasts
% Protein band intensity MxA/GAPDH
100
80
60
40
20
0
*
*
IFNλ1
SB+IFNλ1
U+IFNλ1
D
DMSO
MxA
GAPDH
SB + - - - + -
U - + - - - +
IFNα - - - + + +
Fibroblasts
% Protein band intensity MxA/GAPDH
100
80
60
40
20
0
IFNα
SB+IFNα
U+IFNα

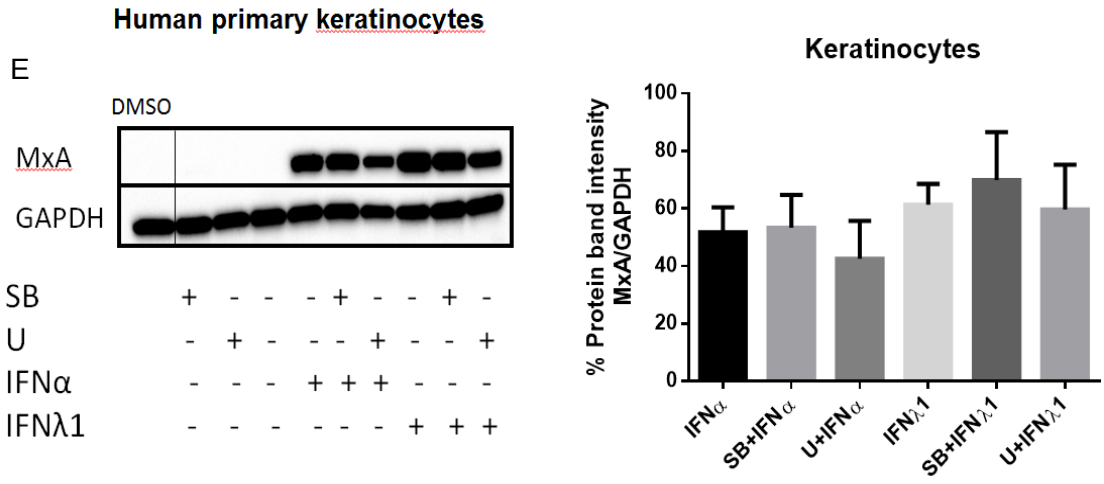



**Figure 5.5: Inhibition of MxA production by p38 and ERK inhibitors**

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). (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).

### *5.2.6 IFNλ enhances collagen expression in TGFβ1-treated human dermal fibroblasts*

Dermal fibroblasts are essential for extracellular dermal matrix (ECM) homeostasis and also contribute to basement membrane formation and thus play an important role in repair processes. As conditions with high type I IFN expression can be linked to impaired healing responses, it was investigated whether IFNλ1 had any influence on collagen expression. Fibroblasts were treated with IFNλ1, IFNα, TGFβ1 or a combination of IFN and TGFβ1 for 24 hours. TGFβ1 was used as a control, given its well described stimulation of fibroblast proliferation and collagen synthesis. *Col1A1*, *Col3A1*, *Col4A2* and *Col7A1* expression was measured using qRT-PCR. 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 (Fig. 5.6A-D).

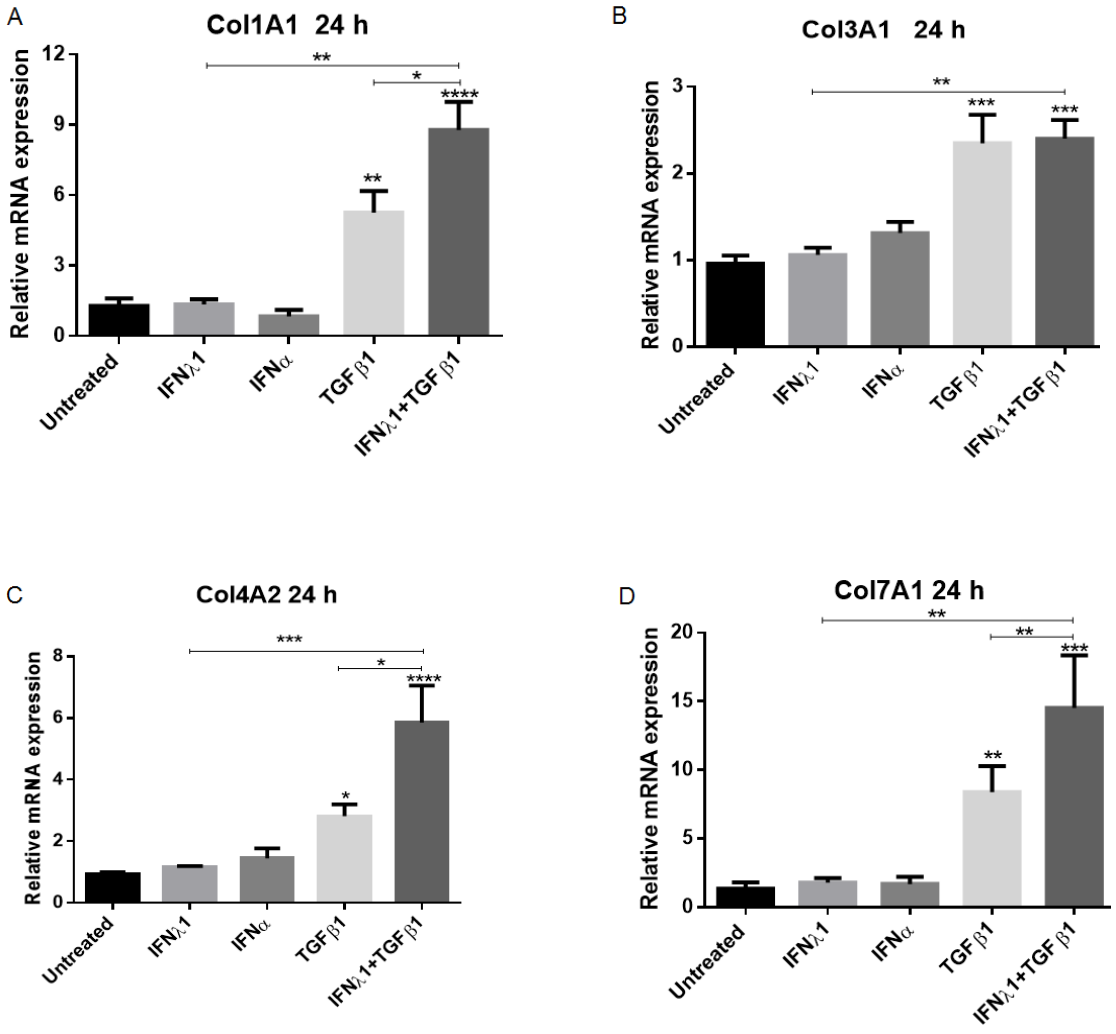


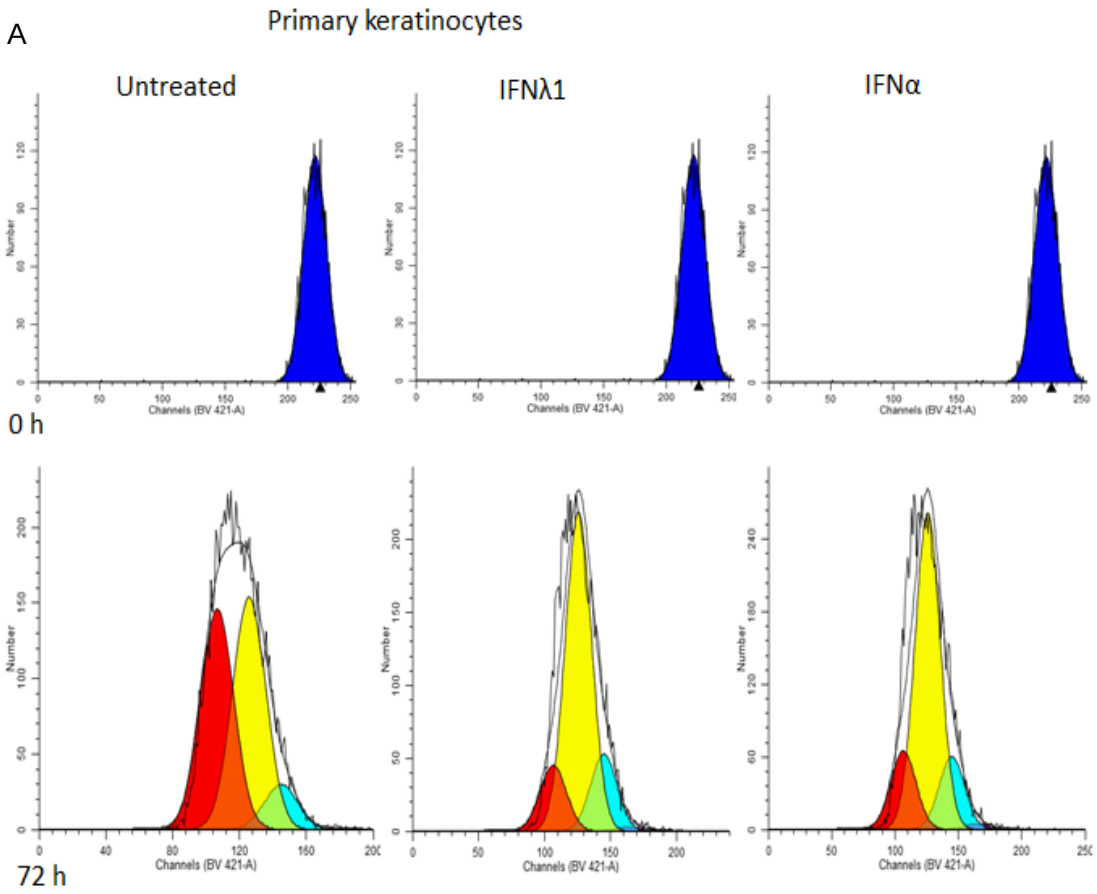

**Figure 5.6: Effect of IFNλ of collagen expression in human dermal fibroblasts.**

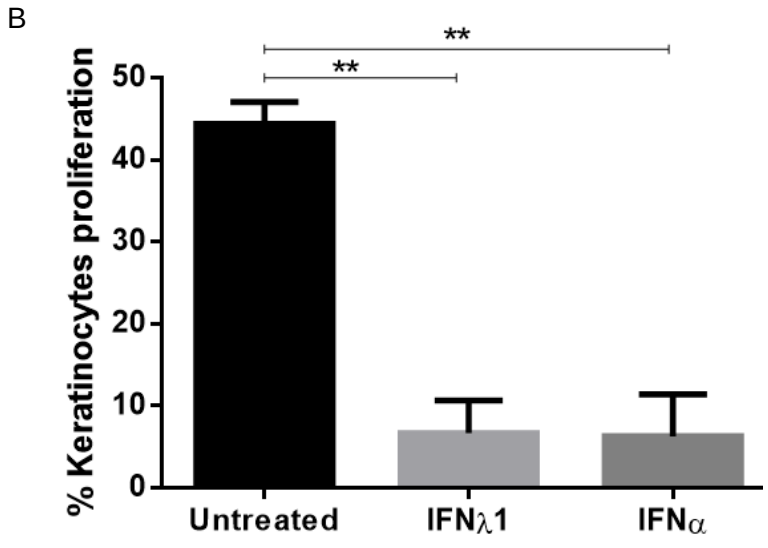
Cultured dermal fibroblasts were either untreated or treated with 10 ng/ml IFNα, 100 ng/ml IFNλ1, 5 ng/ml TGFβ1 or combination of IFNλ1 and TGFβ1 for 24 hours. mRNA expression of ISGs was measured using qRT-PCR. Depicted is *Col1A1* (a) *Col3A1* (b) *Col4A2* (c) and *Col7A1* (d) expression. For statistical analysis, one-way ANOVA was used. n=5, values represent mean± SEM; *p<0.005, **p<0.01, ***p<0.001, ****p<0.0001.

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

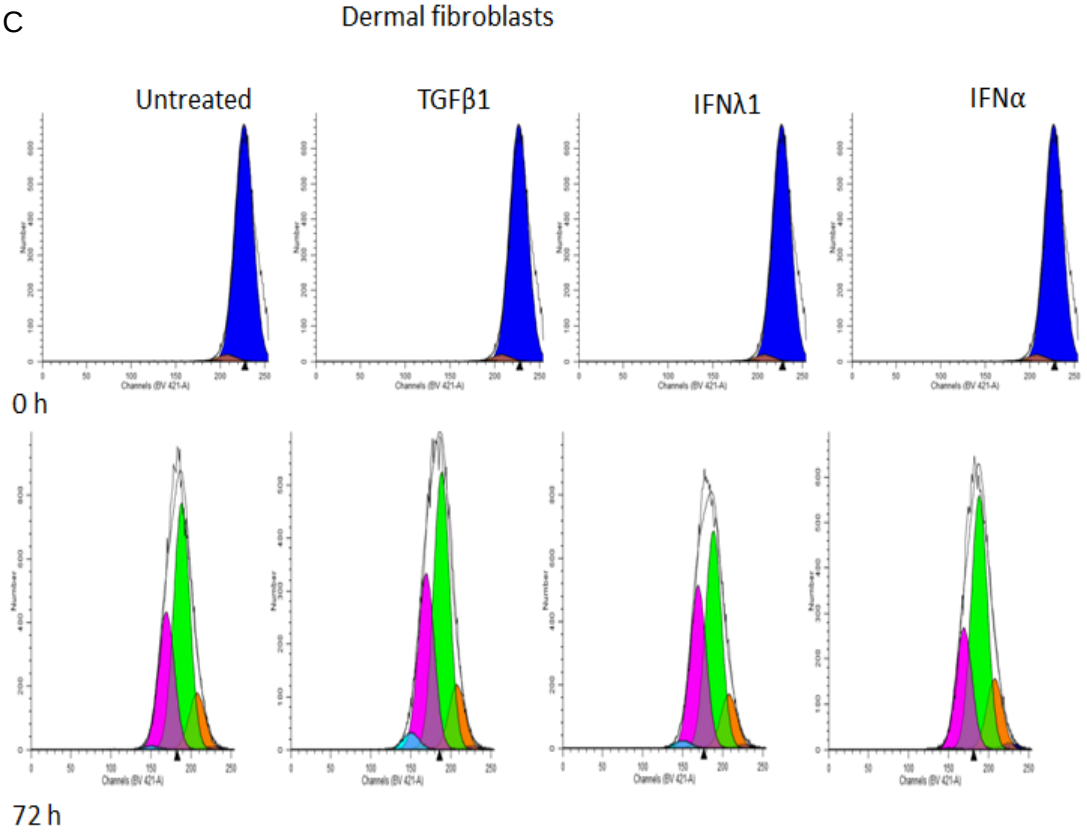
The anti-proliferative effect of IFNλ1 on HaCaT keratinocyte cell lines has been described (Maher et al., 2008). It was therefore important to compare the effect of both IFNλ1 and IFNα on primary skin cells. Cultured human primary keratinocytes and dermal fibroblasts were stained with cell tracer dye and subsequently stimulated with either IFNλ or IFNα for 72 hours. As expected, both IFNs clearly reduced keratinocyte proliferation (Fig. 5.7A-B). By contrast, we observed that IFNα but not IFNλ1 inhibits dermal fibroblast proliferation (Fig. 5.7C-D). TGFβ1, known to induce fibroblast proliferation, was used as positive control (Fig. 5.7C-D).

The effects of IFNλ1 and IFNα on "gap" closure in monolayer cultures of dermal fibroblasts to which both migration and proliferation of cells contribute was investigated. Confluent fibroblast cultures were "scratched" and stimulated with IFNs. Gap closure was monitored at time 0, 24 and 48 hours. The result showed that IFNλ1 even improved gap closure in fibroblasts while IFNα delayed gap closure after 48 hours of stimulation in comparison to the untreated cells (Fig. 5.8).


A
Primary keratinocytes
Untreated
IFNλ1
IFNα
0 h
72 h
Number
Channels (BV 421-A)


B
% Keratinocytes proliferation
**
**
Untreated
IFNλ1
IFNα

C


Dermal fibroblasts
Untreated
TGFβ1
IFNλ1
IFNα
0 h
72 h

D

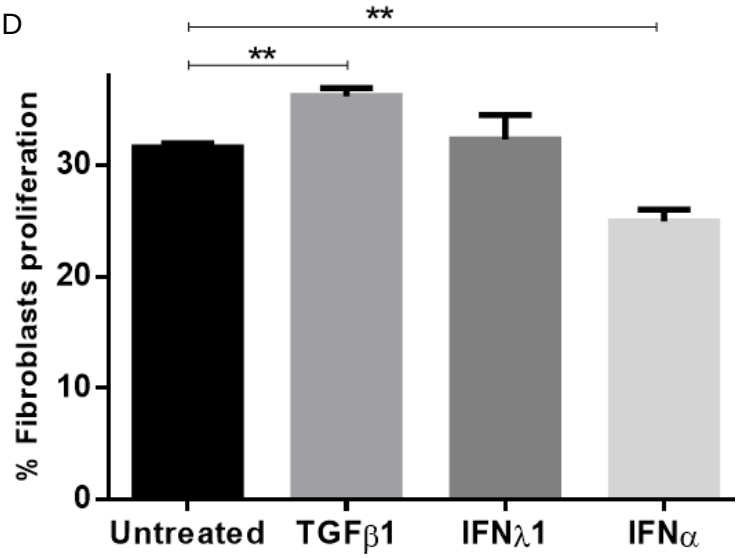

**
**
% Fibroblasts proliferation
30
20
10
0
Untreated
TGFβ1
IFNλ1
IFNα

**Figure 5.7: Effects of IFNs on proliferation of skin resident cells**

Human dermal fibroblasts or primary keratinocytes were 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 (highlighted by different colours) of dividing cells were visualised by flow cytometric analysis after 72 hours of stimulation. Division leads to decrease in fluorescence and thus increased proliferation results in an increase of daughter cell populations on the left end of the histogram. (A) Cell tracer dependent fluorescent signal in keratinocytes at 0 hour and after exposure to IFNλ1/IFNα/medium alone for 72 hours is depicted in histograms. (B) Percentage proliferation of keratinocytes after 72 hours is shown (n=3). (C) Histograms for cell tracer dependent fluorescent signal in fibroblasts at day 0 and 72 hours after stimulation with TGFβ/IFNλ1/IFNα/medium. (D) Percentage proliferation of fibroblasts after 72 hours is shown (n=3). For statistical analysis, unpaired *t* test was used; n=3; values represent mean± SEM. *p<0.05, **p<0.01.

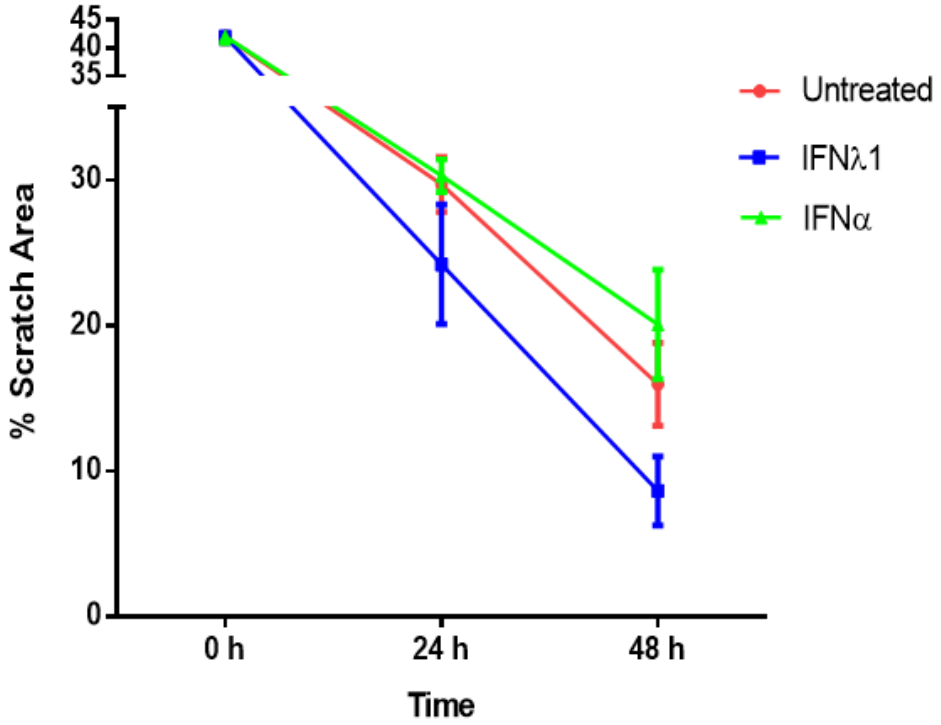



**Figure 5.8: Differential effects of IFNλ1 and IFNα on dermal fibroblasts monolayer culture gap closure.**

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. Graphical representation of three independent experiments using three different donors. n=3; values represent mean ± SEM.

## 5.3 **Discussion**

Types I and III innate IFNs were initially identified for their potent antiviral activities (MacMicking, 2012; Sadler and Williams, 2008). IFNs can regulate around 2000 different genes (Hertzog et al., 2011) and among the ISGs, MX1, which encodes for MxA, is highly inducible and one of the most potent anti-viral factors which has been shown to block the early replication events of different RNA and DNA viruses (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). They are also important mediators in the pathogenesis of autoimmune diseases, such as CLE (Meyer, 2009; Ronnblom and Eloranta, 2013).

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, such as pDCs, monocyte-derived macrophages, and T cells (Dai et al., 2009; Liu et al., 2011; Mennechet and Uze, 2006; Yin et al., 2012). Apart from the canonical JAK/STAT pathway for IFN signalling, 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). The contribution and significance 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). However, so far, only few studies show data of IFNλ induced signalling in primary skin cells. This work is novel

because it shows 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 have been considered to be unresponsive to this IFNλ (Abushahba et al., 2010). The results presented here challenge this previously held view by showing that human fibroblasts respond to IFNλ1 treatment with significant expression of MxA mRNA and protein. In line with findings in this work, 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, significant expression of GBP-1 or OAS2 upon IFNλ1 treatment was not observed (Fig. 5.2D/F). 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, the findings in this work 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., 2004b). It is interesting that despite these findings, inhibition of p38 and ERK in IFNα treated dermal fibroblasts did not result in significant downregulation of MxA protein expression; although a slight

reduction in MxA production by primary keratinocytes was observed following inhibition of ERK but not p38. A more recent report has shown that both IFNλ1 and IFNα induce the activation of PI3K-AKT and Raf-MEK-ERK pathways in HepG2.2.15 cell lines (Chai et al., 2011). With regard to skin tissue cells i.e. keratinocytes and fibroblasts, no activation of the AKT pathway was observed. A proposed mechanism of IFNα and IFNλ1 signalling in dermal fibroblasts is shown in Figure 5.9

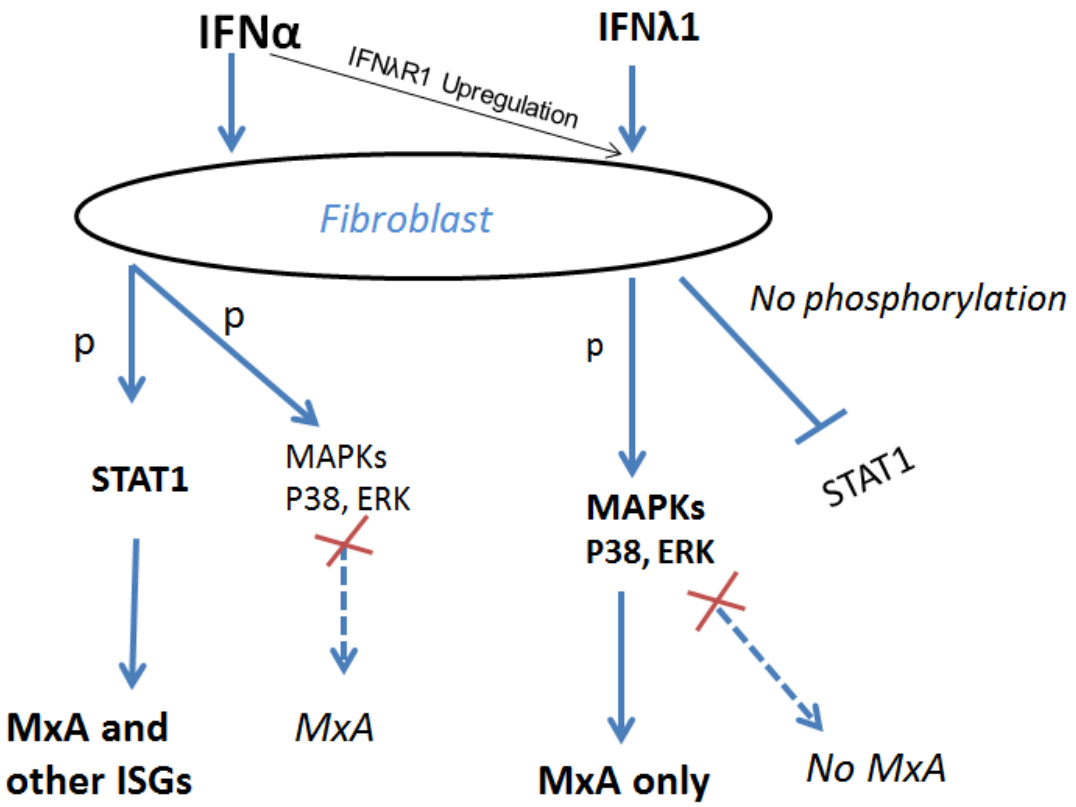



**Figure 5.9: Proposed schematics of IFNα and IFNλ1 signalling in human dermal fibroblasts**

Upon stimulation of fibroblasts with IFNα, there is mainly the 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 for 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 the other ISGs. Blocking these MAPKs pathways results in attenuation of MxA production.

It was indeed surprising that no significant regulatory effects of the MAPK inhibitors was observed at the transcriptional level, suggesting that p38 and ERK inhibition result in posttranscriptional 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). Mnk1 has been shown to prevent proteosomal degradation in response to growth factor stimulation in CHO-K1/COS1 cells (DaSilva et al., 2006). Posttranscriptional regulation of TNFα gene expression has been described in the presence of p38 inhibitor (Clark et al., 2003). Future experiments will show the underlying mechanism of the posttranscriptional regulation observed for MxA in human dermal fibroblasts. This study 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. A similar effect of IFNα on IFNλR1 has been reported in primary human hepatocytes (Duong et al., 2014).

TGFβ1 is well known to induce collagen expression in fibroblasts. Types I and III collagens are the main ECM components, type IV collagen is the main component of the lamina densa in the basement membrane zone and Type VII collagen is a component of the anchoring fibrils lying beneath the lamina densa at the dermal-epidermal junction (Tiedtke, 2007). Results from this work shows that IFNλ1 potentiated the TGFβ-associated induction of collagen I, but not collagen type III, expression. IFNγ, a type II IFN has been

shown to have an inhibitory effect on TGFβ1-induced ECM or collagen deposition in primary human lung (Eickelberg et al., 2001) and in human foreskin fibroblasts through the blocking of Smad signalling (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 has been linked with disorganised fibre structure and 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 it may support basement membrane integrity (Nystrom et al., 2013).

The results of the proliferation experiments in human primary keratinocytes are consistent with the roles of IFNα and IFNλ1 as anti-proliferative agents. The results show that IFNλ1 does not have an inhibitory effect on dermal fibroblast 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 in fibroblasts, no GBP-1 induction in the context of type III IFN stimulation was observed.

This study has shown that IFNλR1 is active in human dermal fibroblasts through the activation of p38 and ERK pathways. It further shows 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. The data in this work 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). Data are lacking so far describing the response of tissue cells to type I and III IFN in CLE disease subtypes.

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 susceptibility to IFNλ. It is thus possible that IFNλ, while important for the epidermal antiviral competence (Wolk et al., 2013), 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. Despite the inhibitory effect of MAPK inhibitors seen in this work, it is important to note that off-target effects have also been reported, particularly for MEK1/2 inhibitors. Both U0126 and PD98059 have been shown to activate AMP-activated protein kinase; thus leading to increase in cellular AMP (Dokladda et al., 2005). Inhibition of calcium entry into the cell and reduction in mitochondrial respiration have been reported following the use of MEK1/2 inhibitors (Ripple et al., 2013; Wauson et al., 2013); these effects were not linked with inhibition of the MAPK pathway. Hence, interpretation of results following the use of these drugs should be done with some measure of caution and their use limited to short term studies. As an alternative to inhibition of the MAPK pathway using small molecule inhibitors, knocking down specific genes on the MAPK pathway using siRNA technology could be a better option. In addition, use of a positive control, such as a molecule or

protein that is well known to activate the MAPK pathway can help to monitor the effectiveness and specificity of the inhibitors. This will be examined by checking reduction/inhibition of MAPK phosphorylation and inhibition of expression of genes downstream ERK.

# CHAPTER 6: DISCUSSION AND FUTURE WORK

# 6 Discussion

The skin is a target organ of LE inflammation and CLE impacts negatively on the quality of life of patients (Klein et al., 2011). Scarring in visible areas of the body including irreversible hair loss is of great concern for affected patients. This work so far points to the importance of IFNλ and indeed IFNα in the pathogenesis of CLE. The abundance of these cytokines and associated ISGs may be very important in prognosis of scarring and non-scarring subtypes of CLE. Nucleic acids (Poly I:C and polydA:dT) are important in the pathogenesis of CLE and the importance of TLR3 and RIG-I agonists has been shown in this work. TLR3 itself is very highly expressed in cutaneous lesion of patients with active LE (Zahn et al., 2010); however, the source of dsRNA *in vivo* is still unknown (Wittmann, 2011). Recent attention regarding IL-22 has focused on its active role in normal wound healing (Lim and Savan, 2014). The double-edge nature of IL-22 (proinflammatory and repair promoting) may be very crucial in CLE and the ability to modulate the signalling pathways of this cytokine may determine its contribution to fibrosis; either to enhance inflammation, which is essential for development of scarring or to drive repair mechanism thereby reducing damaging effect of molecules, such as IFNs. As also highlighted in this work, the role of IL-22 in cell proliferation is still controversial. The effect of IL-22 on cell proliferation *in vitro* may be better evaluated by using fast growing cells, such as epithelial carcinoma cells. As observed in this work and that of others, IL-22 is involved in wound healing and cell migration (Ji et al., 2014). Scar formation is the hallmark of the outcome of chronic discoid lupus erythematosus (CDLE) and once the pathway has been initiated, it becomes impossible to stop the

eventual scarring outcome. The treatment options available, such as the use of antimalarial (chloroquine and hydroxylchloroquine) and methotrexate have failed to completely cure the disease and also prevent scar formation. Moreover, it has been reported that smoking negatively affects disease activity and significantly reduces the efficacy of hydroxylchloroquine and other antimalarials (Kuhn et al., 2014; Piette et al., 2012). Therefore, there is need to develop new therapy to combat this disease. With regards to scarring versus non-scarring outcomes, very early intervention may be necessary in order to halt the final scarring outcome. Results from this work could lead to the hypothesis that early administration or upregulation of IL-22 activity prior to full-onset CLE may be therapeutically beneficial. It would be interesting to understand why CLE keratinocytes have reduced expression of IL-22R1.

It is now well known that IFNα plays important role in the pathogenesis of psoriasis (Lowes et al., 2014) and a possible role for IFNλ has been suggested (Wolk et al., 2013). Both IFNα and IFNλ are important molecules in the pathogenesis of CLE (Wenzel et al., 2010; Wittmann, 2011; Zahn et al., 2011; Zahn et al., 2010). While psoriasis heals without permanent scar, CDLE heals with permanent scar. This study and the work of others have shown high expression of IL-22, IL-22R1 and SOCS3 in cutaneous lesion of patients with psoriasis (Sabat et al., 2014). By contrast, this study shows the absence/low expression of IL-22, low IL-22R1 expression in active lesions from CLE patients and reduced responsiveness of keratinocytes from CLE patients to IL-22. The absence of IL-22 expression in CDLE lesion has been reported elsewhere (Jabbari et al., 2014). These findings suggest that a balance in the type 1/type 17 activities in inflammatory skin diseases may be

essential in ensuring a scarless outcome. Although not produced by skin cells, IL-22 plays active roles in epidermal hyperproliferation, acanthosis, reduced apoptosis and epidermal thickening observed in psoriatic skin (Madonna et al., 2012; Sabat et al., 2014). On the other hand, increased apoptosis has been identified as one of the most important pathogenic factors in CLE (Kuhn and Bijl, 2008; Kuhn et al., 2006). IL-22 could be an important determining factor with regards to the eventual disease outcome in CLE and psoriasis. SOCS3 is induced by IFNα in human T cells and it is involved in the negative regulation of IFNα signalling as a form of feedback mechanism to control excessive stimulation of cells by IFNα (Bode et al., 2003; Brender et al., 2001; Zimmerer et al., 2007). Damage to the bulge region (epidermal stem cell niche) of the hair follicle as a result of severe inflammation and presence of high number of CD8+ T lymphocytes has been reported in CDLE lesions (Al-Refu et al., 2009). Severe inflammation at the pilosebaceous gland and the length of inflammation were identified as important factors in acne scar. Inflammatory reaction was slower in patients with scar than in patients without scar (Fabbrocini et al., 2010; Holland et al., 2004). Acne is a common disorder that results in atrophic scaring (ice pick scars) (Ghodsi et al., 2009). These reports show a strong link between severity and duration of inflammation and scar formation. Therefore, prevention or controlling early inflammation may offer a therapeutic “window” to treatment of scarring conditions.

In a wider context of this study, early local administration of IL-22 or local upregulation of IL-22 activity by e.g. inhibiting IL-22BP, may help to reduce

pathogenic chemokines, such as CXCL9 and CXCL10 produced by skin resident cells; hence, reduction in tissues destruction.

The dual role of IL-22 as both pro- and anti-inflammatory cytokine has been reported (Muhl et al., 2013; Sabat et al., 2014). The results presented here suggest a potential beneficial role of IL-22 in CLE; however, results are clearly very preliminary. Therefore, further and more extensive work has to be done in order to see if its use will be of any benefit to patients. This study also identifies the important immunological roles played by mesenchymal cells in the skin. The effects of IL-22 and IFNλ on dermal fibroblasts are only just emerging. The IL-10 family members can help maintain skin integrity and prevent excessive damage of the dermal compartment of the skin through their ability to enhance collagen expression and counteract the damaging effect of type I IFNs during viral infection and in pathogenesis of autoimmune diseases.

## 6.1 **Future work**

Future work on the therapeutic potential or counter-regulatory role of IL-22 in inflammatory skin diseases will focus on the use of *ex vivo* or *in vivo* model of CLE. Despite the interesting finding on the counter-regulatory role of IL-22 on IFNλ-induced chemokines expression, it is important that more samples from different donors are used in order to fully confirm this finding. It would be interesting to be able to strongly upregulate IL-22R1 expression in CLE keratinocytes in order to increase responsiveness of these cells to IL-22. While this work has shown that human dermal fibroblasts can respond to IFNλ with subsequent downstream expression of MxA, it would be interesting to investigate the differences observed in mRNA and protein regulation in the presence of MAPK inhibitors. Due to the important roles played by both STAT1 and MxA in the pathogenesis of CLE, it would be important to see how dermal fibroblasts from CLE patients behave with regards to STAT1 and MAPKs activation and signalling. It is possible that these cells will respond differently to cells obtained from healthy individuals. STAT1 activation is really important in the anti-proliferative effect of IFNs (Maher et al., 2008). It would be interesting to see if these cells will respond to IFNλ with subsequent expression of more ISGs apart from MxA. This study has shown that CLE keratinocytes have reduced expression of IL-22R1 and it would thus be of high interest to see if fibroblasts from CLE patients express IL-22R1 and investigate if they are responsive to IL-22 with the evidence of a functional outcome.

# Publications related to this work

**Alase A; El-Sherbiny Y; Vital E; Tobin D; Turner N; Wittmann M**. IFNλ Stimulates MxA Production in Human Dermal Fibroblasts Via A MAPK-Dependent STAT1-Independent Mechanism (2015). Journal of Investigative Dermatology doi: 10.1038/jid.2015.317.

**Alase A; Stacey M; Doble R; Goodfield M; Wittman M.** Regulation of IFN lambda production and response in the skin compartment, 2013 (Abstract, JID, doi:10.1038/jid.2013.108).

# Presentations

**Regulation of IFNλ production and response in the skin compartment: Implications for inflammatory skin diseases** (Postal presentation, International Investigative Dermatology meeting, Edinburgh, 2013)

**Regulation of IFNλ production and response in the skin compartment: Implications for inflammatory skin diseases** (Oral presentation, Yorkshire immunology meeting, University of Sheffield, 2013)

**Regulation of IFNλ production and response in the skin compartment: Implications for inflammatory skin diseases** (Postal presentation BSID meeting, Newcastle, 2014)

**IFNλ stimulates MxA production in human dermal fibroblasts via a MAPK-dependent STAT1-independent mechanism** (Oral presentation, Yorkshire and Northern Rheumatology meeting, York, 2014)

**The role of IL-10 family members in inflammatory skin diseases** (Oral presentation, CSS departmental seminar, University of Bradford, 2015)

**IFNλ stimulates MxA production in human dermal fibroblasts via a MAPK-dependent STAT1-independent mechanism** (Oral presentation, BSID meeting, Southampton, 2015)

**IFNλ stimulates MxA production in human dermal fibroblasts via a MAPK-dependent STAT1-independent mechanism** (Postgraduate mini-conference, University of Bradford, 2015)

# References

Abushahba, W., Balan, M., Castaneda, I., Yuan, Y., Reuhl, K., Raveche, E., de la Torre, A., Lasfar, A., and Kotenko, S.V. (2010). Antitumor activity of type I and type III interferons in BNL hepatoma model. Cancer Immunol Immunother *59*, 1059-1071.

Ahmad, U., Ali, R., Lebastchi, A.H., Qin, L., Lo, S.F., Yakimov, A.O., Khan, S.F., Choy, J.C., Geirsson, A., Pober, J.S.*, et al.* (2010). IFN-gamma primes intact human coronary arteries and cultured coronary smooth muscle cells to double-stranded RNA- and self-RNA-induced inflammatory responses by upregulating TLR3 and melanoma differentiation-associated gene 5. Journal of immunology *185*, 1283-1294.

Al-Qattan, M.M., Abd-Elwahed, M.M., Hawary, K., Arafah, M.M., and Shier, M.K. (2015). Myofibroblast expression in skin wounds is enhanced by collagen III suppression. Biomed Res Int *2015*, 958695.

Al-Refu, K., Edward, S., Ingham, E., and Goodfield, M. (2009). Expression of hair follicle stem cells detected by cytokeratin 15 stain: implications for pathogenesis of the scarring process in cutaneous lupus erythematosus. Br J Dermatol *160*, 1188-1196.

Alase, A., Stacey, M., Doble, R., Goodfield, M., Wittmann, M. (2013). Regulation of IFNλ production and response in the skin compartment: Implications for inflammatory skin diseases.

Albanesi, C., Cavani, A., and Girolomoni, G. (1998). Interferon-gamma-stimulated human keratinocytes express the genes necessary for the

production of peptide-loaded MHC class II molecules. J Invest Dermatol *110*, 138-142.

Albanesi, C., Fairchild, H.R., Madonna, S., Scarponi, C., De Pita, O., Leung, D.Y., and Howell, M.D. (2007). IL-4 and IL-13 negatively regulate TNF-alpha- and IFN-gamma-induced beta-defensin expression through STAT-6, suppressor of cytokine signaling (SOCS)-1, and SOCS-3. J Immunol *179*, 984-992.

Alexander, W.S. (2002). Suppressors of cytokine signalling (SOCS) in the immune system. Nat Rev Immunol *2*, 410-416.

Amoura, Z., Combadiere, C., Faure, S., Parizot, C., Miyara, M., Raphael, D., Ghillani, P., Debre, P., Piette, J.C., and Gorochov, G. (2003). Roles of CCR2 and CXCR3 in the T cell-mediated response occurring during lupus flares. Arthritis Rheum *48*, 3487-3496.

Andoh, A., Bamba, S., Fujiyama, Y., Brittan, M., and Wright, N.A. (2005). Colonic subepithelial myofibroblasts in mucosal inflammation and repair: contribution of bone marrow-derived stem cells to the gut regenerative response. Journal of gastroenterology *40*, 1089-1099.

Ank, N., Iversen, M.B., Bartholdy, C., Staeheli, P., Hartmann, R., Jensen, U.B., Dagnaes-Hansen, F., Thomsen, A.R., Chen, Z., Haugen, H.*, et al.* (2008). An important role for type III interferon (IFN-lambda/IL-28) in TLR-induced antiviral activity. J Immunol *180*, 2474-2485.

Ank, N., West, H., Bartholdy, C., Eriksson, K., Thomsen, A.R., and Paludan, S.R. (2006). Lambda interferon (IFN-lambda), a type III IFN, is induced by viruses and IFNs and displays potent antiviral activity against select virus infections in vivo. Journal of virology *80*, 4501-4509.

Aringer, M., Feierl, E., Steiner, G., Stummvoll, G.H., Hofler, E., Steiner, C.W., Radda, I., Smole, J.S., and Graninger, W.B. (2002). Increased bioactive TNF in human systemic lupus erythematosus: associations with cell death. Lupus *11*, 102-108.

Aringer, M., and Smolen, J.S. (2012). Therapeutic blockade of TNF in patients with SLE-promising or crazy? Autoimmun Rev *11*, 321-325.

Arrue, I., Saiz, A., Ortiz-Romero, P.L., and Rodriguez-Peralto, J.L. (2007). Lupus-like reaction to interferon at the injection site: report of five cases. J Cutan Pathol *34 Suppl 1*, 18-21.

Arthur, J.S., and Ley, S.C. (2013). Mitogen-activated protein kinases in innate immunity. Nat Rev Immunol *13*, 679-692.

Babon, J.J., Kershaw, N.J., Murphy, J.M., Varghese, L.N., Laktyushin, A., Young, S.N., Lucet, I.S., Norton, R.S., and Nicola, N.A. (2012). Suppression of cytokine signaling by SOCS3: characterization of the mode of inhibition and the basis of its specificity. Immunity *36*, 239-250.

Bachmann, M., Ulziibat, S., Hardle, L., Pfeilschifter, J., and Muhl, H. (2013). IFNalpha converts IL-22 into a cytokine efficiently activating STAT1 and its downstream targets. Biochem Pharmacol *85*, 396-403.

Balan, S., Ollion, V., Colletti, N., Chelbi, R., Montanana-Sanchis, F., Liu, H., Vu Manh, T.P., Sanchez, C., Savoret, J., Perrot, I.*, et al.* (2014). Human XCR1+ dendritic cells derived in vitro from CD34+ progenitors closely resemble blood dendritic cells, including their adjuvant responsiveness, contrary to monocyte-derived dendritic cells. J Immunol *193*, 1622-1635.

Barry, S.P., Townsend, P.A., Latchman, D.S., and Stephanou, A. (2007). Role of the JAK-STAT pathway in myocardial injury. Trends in molecular medicine *13*, 82-89.

Bensouilah, J., Buck, P., Tisserand, R., Avis, A. (2007). Aromadermatology: Aromatherapy in the Treatment and Care of Common Skin Conditions (the University of Michigan, Massachusett, USA: Radcliffe).

Bergamin, E., Wu, J., and Hubbard, S.R. (2006). Structural basis for phosphotyrosine recognition by suppressor of cytokine signaling-3. Structure *14*, 1285-1292.

Bird, L. (2015). Viral infection: Cytokine cooperation. Nat Rev Immunol.

Bleicher, L., de Moura, P.R., Watanabe, L., Colau, D., Dumoutier, L., Renauld, J.C., and Polikarpov, I. (2008). Crystal structure of the IL-22/IL-22R1 complex and its implications for the IL-22 signaling mechanism. FEBS Lett *582*, 2985-2992.

Bode, J.G., Ludwig, S., Ehrhardt, C., Albrecht, U., Erhardt, A., Schaper, F., Heinrich, P.C., and Haussinger, D. (2003). IFN-alpha antagonistic activity of HCV core protein involves induction of suppressor of cytokine signaling-3. FASEB J *17*, 488-490.

Bolen, C.R., Ding, S., Robek, M.D., and Kleinstein, S.H. (2014). Dynamic expression profiling of type I and type III interferon-stimulated hepatocytes reveals a stable hierarchy of gene expression. Hepatology *59*, 1262-1272.

Boniface, K., Bernard, F.X., Garcia, M., Gurney, A.L., Lecron, J.C., and Morel, F. (2005). IL-22 inhibits epidermal differentiation and induces proinflammatory gene expression and migration of human keratinocytes. J Immunol *174*, 3695-3702.

Boniface, K., Guignouard, E., Pedretti, N., Garcia, M., Delwail, A., Bernard, F.X., Nau, F., Guillet, G., Dagregorio, G., Yssel, H.*, et al.* (2007). A role for T cell-derived interleukin 22 in psoriatic skin inflammation. Clin Exp Immunol *150*, 407-415.

Bonni, A., Brunet, A., West, A.E., Datta, S.R., Takasu, M.A., and Greenberg, M.E. (1999). Cell survival promoted by the Ras-MAPK signaling pathway by transcription-dependent and -independent mechanisms. Science *286*, 1358-1362.

Bos, J.D., and Kapsenberg, M.L. (1986). The Skin Immune-System - Its Cellular-Constituents and Their Interactions. Immunology Today *7*, 235-240.

Brand, S., Beigel, F., Olszak, T., Zitzmann, K., Eichhorst, S.T., Otte, J.M., Diepolder, H., Marquardt, A., Jagla, W., Popp, A.*, et al.* (2006). IL-22 is increased in active Crohn's disease and promotes proinflammatory gene expression and intestinal epithelial cell migration. Am J Physiol Gastrointest Liver Physiol *290*, G827-838.

Brand, S., Zitzmann, K., Dambacher, J., Beigel, F., Olszak, T., Vlotides, G., Eichhorst, S.T., Goke, B., Diepolder, H., and Auernhammer, C.J. (2005). SOCS-1 inhibits expression of the antiviral proteins 2',5'-OAS and MxA induced by the novel interferon-lambdas IL-28A and IL-29. Biochem Biophys Res Commun *331*, 543-548.

Brender, C., Nielsen, M., Ropke, C., Nissen, M.H., Svejgaard, A., Billestrup, N., Geisler, C., and Odum, N. (2001). Interferon-alpha induces transient suppressors of cytokine signalling expression in human T cells. Experimental and clinical immunogenetics *18*, 80-85.

Bruun, C., Heding, P.E., Ronn, S.G., Frobose, H., Rhodes, C.J., Mandrup-Poulsen, T., and Billestrup, N. (2009). Suppressor of cytokine signalling-3 inhibits Tumor necrosis factor-alpha induced apoptosis and signalling in beta cells. Molecular and cellular endocrinology *311*, 32-38.

Capaldo, C.T., Beeman, N., Hilgarth, R.S., Nava, P., Louis, N.A., Naschberger, E., Sturzl, M., Parkos, C.A., and Nusrat, A. (2012). IFN-gamma and TNF-alpha-induced GBP-1 inhibits epithelial cell proliferation through suppression of beta-catenin/TCF signaling. Mucosal Immunol *5*, 681-690.

Carow, B., and Rottenberg, M.E. (2014). SOCS3, a Major Regulator of Infection and Inflammation. Frontiers in immunology *5*, 58.

Carrion, M., Juarranz, Y., Martinez, C., Gonzalez-Alvaro, I., Pablos, J.L., Gutierrez-Canas, I., and Gomariz, R.P. (2013). IL-22/IL-22R1 axis and S100A8/A9 alarmins in human osteoarthritic and rheumatoid arthritis synovial fibroblasts. Rheumatology (Oxford) *52*, 2177-2186.

Casciola-Rosen, L., and Rosen, A. (1997). Ultraviolet light-induced keratinocyte apoptosis: a potential mechanism for the induction of skin lesions and autoantibody production in LE. Lupus *6*, 175-180.

Chai, Y., Huang, H.L., Hu, D.J., Luo, X., Tao, Q.S., Zhang, X.L., and Zhang, S.Q. (2011). IL-29 and IFN-alpha regulate the expression of MxA, 2',5'-OAS and PKR genes in association with the activation of Raf-MEK-ERK and PI3K-AKT signal pathways in HepG2.2.15 cells. Mol Biol Rep *38*, 139-143.

Chang, L., and Karin, M. (2001). Mammalian MAP kinase signalling cascades. Nature *410*, 37-40.

Chen, C.Z., and Raghunath, M. (2009). Focus on collagen: in vitro systems to study fibrogenesis and antifibrosis state of the art. Fibrogenesis Tissue Repair *2*, 7.

Chen, X.W., Shen, Y., Sun, C.Y., Wu, F.X., Chen, Y., and Yang, C.D. (2011). Anti-class a scavenger receptor autoantibodies from systemic lupus erythematosus patients impair phagocytic clearance of apoptotic cells by macrophages in vitro. Arthritis Res Ther *13*, R9.

Chen, Y.Q., Mauviel, A., Ryynanen, J., Sollberg, S., and Uitto, J. (1994). Type VII collagen gene expression by human skin fibroblasts and keratinocytes in culture: influence of donor age and cytokine responses. J Invest Dermatol *102*, 205-209.

Chen, Z., Laurence, A., Kanno, Y., Pacher-Zavisin, M., Zhu, B.M., Tato, C., Yoshimura, A., Hennighausen, L., and O'Shea, J.J. (2006). Selective regulatory function of Socs3 in the formation of IL-17-secreting T cells. Proc Natl Acad Sci U S A *103*, 8137-8142.

Clark, A.R., Dean, J.L., and Saklatvala, J. (2003). Post-transcriptional regulation of gene expression by mitogen-activated protein kinase p38. FEBS Lett *546*, 37-44.

Cobleigh, M.A., and Robek, M.D. (2013). Protective and pathological properties of IL-22 in liver disease: implications for viral hepatitis. Am J Pathol *182*, 21-28.

Contet-Audonneau, J.L., Jeanmaire, C., and Pauly, G. (1999). A histological study of human wrinkle structures: comparison between sun-exposed areas of the face, with or without wrinkles, and sun-protected areas. Br J Dermatol *140*, 1038-1047.

Cordiali-Fei, P., Bianchi, L., Bonifati, C., Trento, E., Ruzzetti, M., Francesconi, F., Bultrini, S., D'Agosto, G., Bordignon, V., Francavilla, V.*, et al.* (2014). Immunologic biomarkers for clinical and therapeutic management of psoriasis. Mediators Inflamm *2014*, 236060.

Cowin, A.J., Holmes, T.M., Brosnan, P., and Ferguson, M.W. (2001). Expression of TGF-beta and its receptors in murine fetal and adult dermal wounds. Eur J Dermatol *11*, 424-431.

Croker, B.A., Krebs, D.L., Zhang, J.G., Wormald, S., Willson, T.A., Stanley, E.G., Robb, L., Greenhalgh, C.J., Forster, I., Clausen, B.E.*, et al.* (2003). SOCS3 negatively regulates IL-6 signaling in vivo. Nat Immunol *4*, 540-545.

Crowson, A.N., and Magro, C. (2001). The cutaneous pathology of lupus erythematosus: a review. J Cutan Pathol *28*, 1-23.

Cuadrado, A., and Nebreda, A.R. (2010). Mechanisms and functions of p38 MAPK signalling. The Biochemical journal *429*, 403-417.

Cuevas, B.D., Abell, A.N., and Johnson, G.L. (2007). Role of mitogen-activated protein kinase kinase kinases in signal integration. Oncogene *26*, 3159-3171.

Dai, J., Megjugorac, N.J., Gallagher, G.E., Yu, R.Y., and Gallagher, G. (2009). IFN-lambda1 (IL-29) inhibits GATA3 expression and suppresses Th2 responses in human naive and memory T cells. Blood *113*, 5829-5838.

Dalpke, A., Heeg, K., Bartz, H., and Baetz, A. (2008). Regulation of innate immunity by suppressor of cytokine signaling (SOCS) proteins. Immunobiology *213*, 225-235.

DaSilva, J., Xu, L., Kim, H.J., Miller, W.T., and Bar-Sagi, D. (2006). Regulation of sprouty stability by Mnk1-dependent phosphorylation. Mol Cell Biol *26*, 1898-1907.

Dauer, D.J., Ferraro, B., Song, L., Yu, B., Mora, L., Buettner, R., Enkemann, S., Jove, R., and Haura, E.B. (2005). Stat3 regulates genes common to both wound healing and cancer. Oncogene *24*, 3397-3408.

Dey-Rao, R., and Sinha, A.A. (2015). Genome-wide transcriptional profiling of chronic cutaneous lupus erythematosus (CCLE) peripheral blood identifies systemic alterations relevant to the skin manifestation. Genomics *105*, 90-100.

Dey-Rao, R., Smith, J.R., Chow, S., and Sinha, A.A. (2014). Differential gene expression analysis in CCLE lesions provides new insights regarding the genetics basis of skin vs. systemic disease. Genomics *104*, 144-155.

Dhanasekaran, D.N., and Reddy, E.P. (2008). JNK signaling in apoptosis. Oncogene *27*, 6245-6251.

Dickensheets, H., Sheikh, F., Park, O., Gao, B., and Donnelly, R.P. (2013). Interferon-lambda (IFN-lambda) induces signal transduction and gene expression in human hepatocytes, but not in lymphocytes or monocytes. J Leukoc Biol *93*, 377-385.

Dokladda, K., Green, K.A., Pan, D.A., and Hardie, D.G. (2005). PD98059 and U0126 activate AMP-activated protein kinase by increasing the cellular AMP:ATP ratio and not via inhibition of the MAP kinase pathway. FEBS Lett *579*, 236-240.

Domina, A.M., Vrana, J.A., Gregory, M.A., Hann, S.R., and Craig, R.W. (2004). MCL1 is phosphorylated in the PEST region and stabilized upon ERK

activation in viable cells, and at additional sites with cytotoxic okadaic acid or taxol. Oncogene *23*, 5301-5315.

Donnelly, R.P., Sheikh, F., Kotenko, S.V., and Dickensheets, H. (2004). The expanded family of class II cytokines that share the IL-10 receptor-2 (IL-10R2) chain. J Leukoc Biol *76*, 314-321.

Dorner, T., Hucko, M., Mayet, W.J., Trefzer, U., Burmester, G.R., and Hiepe, F. (1995). Enhanced membrane expression of the 52 kDa Ro(SS-A) and La(SS-B) antigens by human keratinocytes induced by TNF alpha. Ann Rheum Dis *54*, 904-909.

Duhen, T., Geiger, R., Jarrossay, D., Lanzavecchia, A., and Sallusto, F. (2009). Production of interleukin 22 but not interleukin 17 by a subset of human skin-homing memory T cells. Nat Immunol *10*, 857-863.

Duong, F.H., Trincucci, G., Boldanova, T., Calabrese, D., Campana, B., Krol, I., Durand, S.C., Heydmann, L., Zeisel, M.B., Baumert, T.F.*, et al.* (2014). IFN-lambda receptor 1 expression is induced in chronic hepatitis C and correlates with the IFN-lambda3 genotype and with nonresponsiveness to IFN-alpha therapies. J Exp Med *211*, 857-868.

Egli, A., Levin, A., Santer, D.M., Joyce, M., O'Shea, D., Thomas, B.S., Lisboa, L.F., Barakat, K., Bhat, R., Fischer, K.P.*, et al.* (2014). Immunomodulatory Function of Interleukin 28B during primary infection with cytomegalovirus. J Infect Dis *210*, 717-727.

Eickelberg, O., Pansky, A., Koehler, E., Bihl, M., Tamm, M., Hildebrand, P., Perruchoud, A.P., Kashgarian, M., and Roth, M. (2001). Molecular mechanisms of TGF-(beta) antagonism by interferon (gamma) and cyclosporine A in lung fibroblasts. FASEB J *15*, 797-806.

Eloranta, M.L., Alm, G.V., and Ronnblom, L. (2013). Disease mechanisms in rheumatology--tools and pathways: plasmacytoid dendritic cells and their role in autoimmune rheumatic diseases. Arthritis Rheum *65*, 853-863.

Eriksen, K.W., Woetmann, A., Skov, L., Krejsgaard, T., Bovin, L.F., Hansen, M.L., Gronbaek, K., Billestrup, N., Nissen, M.H., Geisler, C.*, et al.* (2010). Deficient SOCS3 and SHP-1 expression in psoriatic T cells. J Invest Dermatol *130*, 1590-1597.

Eyerich, S., Eyerich, K., Pennino, D., Carbone, T., Nasorri, F., Pallotta, S., Cianfarani, F., Odorisio, T., Traidl-Hoffmann, C., Behrendt, H.*, et al.* (2009). Th22 cells represent a distinct human T cell subset involved in epidermal immunity and remodeling. J Clin Invest *119*, 3573-3585.

Fabbrocini, G., Annunziata, M.C., D'Arco, V., De Vita, V., Lodi, G., Mauriello, M.C., Pastore, F., and Monfrecola, G. (2010). Acne scars: pathogenesis, classification and treatment. Dermatol Res Pract *2010*, 893080.

Farkas, L., Beiske, K., Lund-Johansen, F., Brandtzaeg, P., and Jahnsen, F.L. (2001). Plasmacytoid dendritic cells (natural interferon- alpha/beta-producing cells) accumulate in cutaneous lupus erythematosus lesions. Am J Pathol *159*, 237-243.

Federici, M., Giustizieri, M.L., Scarponi, C., Girolomoni, G., and Albanesi, C. (2002). Impaired IFN-gamma-dependent inflammatory responses in human keratinocytes overexpressing the suppressor of cytokine signaling 1. J Immunol *169*, 434-442.

Fujita, H. (2013). The role of IL-22 and Th22 cells in human skin diseases. J Dermatol Sci *72*, 3-8.

George, P.M., Badiger, R., Alazawi, W., Foster, G.R., and Mitchell, J.A. (2012). Pharmacology and therapeutic potential of interferons. Pharmacol Ther *135*, 44-53.

Ghodsi, S.Z., Orawa, H., and Zouboulis, C.C. (2009). Prevalence, severity, and severity risk factors of acne in high school pupils: a community-based study. J Invest Dermatol *129*, 2136-2141.

Ghosh, A.K., Yuan, W., Mori, Y., Chen, S., and Varga, J. (2001). Antagonistic regulation of type I collagen gene expression by interferon-gamma and transforming growth factor-beta. Integration at the level of p300/CBP transcriptional coactivators. J Biol Chem *276*, 11041-11048.

Glennie, N.D., Yeramilli, V.A., Beiting, D.P., Volk, S.W., Weaver, C.T., and Scott, P. (2015). Skin-resident memory CD4+ T cells enhance protection against Leishmania major infection. J Exp Med.

Glim, J.E., van Egmond, M., Niessen, F.B., Everts, V., and Beelen, R.H. (2013). Detrimental dermal wound healing: what can we learn from the oral mucosa? Wound Repair Regen *21*, 648-660.

Goh, K.C., Haque, S.J., and Williams, B.R. (1999). p38 MAP kinase is required for STAT1 serine phosphorylation and transcriptional activation induced by interferons. EMBO J *18*, 5601-5608.

Gough, D.J., Levy, D.E., Johnstone, R.W., and Clarke, C.J. (2008). IFNgamma signaling-does it mean JAK-STAT? Cytokine Growth Factor Rev *19*, 383-394.

Griffiths, C.E., and Barker, J.N. (2007). Pathogenesis and clinical features of psoriasis. Lancet *370*, 263-271.

Han, J., Lee, J.D., Bibbs, L., and Ulevitch, R.J. (1994). A MAP kinase targeted by endotoxin and hyperosmolarity in mammalian cells. Science *265*, 808-811.

Hao, J.Q. (2014). Targeting interleukin-22 in psoriasis. Inflammation *37*, 94-99.

Hari, A., Flach, T.L., Shi, Y., and Mydlarski, P.R. (2010). Toll-like receptors: role in dermatological disease. Mediators of inflammation *2010*, 437246.

Hasegawa, T., Nakao, A., Sumiyoshi, K., Tsuboi, R., and Ogawa, H. (2003). IFN-gamma fails to antagonize fibrotic effect of TGF-beta on keloid-derived dermal fibroblasts. J Dermatol Sci *32*, 19-24.

Heim, M.H. (2012). Interferons and hepatitis C virus. Swiss Med Wkly *142*, w13586.

Hernandez, P.P., Mahlakoiv, T., Yang, I., Schwierzeck, V., Nguyen, N., Guendel, F., Gronke, K., Ryffel, B., Holscher, C., Dumoutier, L.*, et al.* (2015). Interferon-lambda and interleukin 22 act synergistically for the induction of interferon-stimulated genes and control of rotavirus infection. Nat Immunol *16*, 698-707.

Herrmann, M., Voll, R.E., and Kalden, J.R. (2000). Etiopathogenesis of systemic lupus erythematosus. Immunol Today *21*, 424-426.

Hertzog, P., Forster, S., and Samarajiwa, S. (2011). Systems biology of interferon responses. J Interferon Cytokine Res *31*, 5-11.

Holland, D.B., Jeremy, A.H., Roberts, S.G., Seukeran, D.C., Layton, A.M., and Cunliffe, W.J. (2004). Inflammation in acne scarring: a comparison of the responses in lesions from patients prone and not prone to scar. Br J Dermatol *150*, 72-81.

Hong, F., Jaruga, B., Kim, W.H., Radaeva, S., El-Assal, O.N., Tian, Z., Nguyen, V.A., and Gao, B. (2002). Opposing roles of STAT1 and STAT3 in T cell-mediated hepatitis: regulation by SOCS. J Clin Invest *110*, 1503-1513.

Inagaki-Ohara, K., Kondo, T., Ito, M., and Yoshimura, A. (2013). SOCS, inflammation, and cancer. Jak-Stat *2*, e24053.

Ivashkiv, L.B., and Donlin, L.T. (2014). Regulation of type I interferon responses. Nat Rev Immunol *14*, 36-49.

Jabbari, A., Suarez-Farinas, M., Fuentes-Duculan, J., Gonzalez, J., Cueto, I., Franks, A.G., Jr., and Krueger, J.G. (2014). Dominant Th1 and minimal Th17 skewing in discoid lupus revealed by transcriptomic comparison with psoriasis. J Invest Dermatol *134*, 87-95.

Ji, Y., Yang, X., Li, J., Lu, Z., Li, X., Yu, J., and Li, N. (2014). IL-22 promotes the migration and invasion of gastric cancer cells via IL-22R1/AKT/MMP-9 signaling. International journal of clinical and experimental pathology *7*, 3694-3703.

Jo, D., Liu, D., Yao, S., Collins, R.D., and Hawiger, J. (2005). Intracellular protein therapy with SOCS3 inhibits inflammation and apoptosis. Nature medicine *11*, 892-898.

Johnson, G.L., and Nakamura, K. (2007). The c-jun kinase/stress-activated pathway: regulation, function and role in human disease. Biochim Biophys Acta *1773*, 1341-1348.

Johnson, J.R., Nishioka, M., Chakir, J., Risse, P.A., Almaghlouth, I., Bazarbashi, A.N., Plante, S., Martin, J.G., Eidelman, D., and Hamid, Q. (2013). IL-22 contributes to TGF-beta1-mediated epithelial-mesenchymal transition in asthmatic bronchial epithelial cells. Respiratory research *14*, 118.

Joshi, S., Kaur, S., Kroczynska, B., and Platanias, L.C. (2010). Mechanisms of mRNA translation of interferon stimulated genes. Cytokine *52*, 123-127.

Kajita, A., Morizane, S., Takiguchi, T., Yamamoto, T., Yamada, M., and Iwatsuki, K. (2015). Interferon-Gamma Enhances TLR3 Expression and Anti-Viral Activity in Keratinocytes. J Invest Dermatol *135*, 2005-2011.

Kim, B.S. (2015). Innate lymphoid cells in the skin. J Invest Dermatol *135*, 673-678.

Kim, I.H., West, C.E., Kwatra, S.G., Feldman, S.R., and O'Neill, J.L. (2012). Comparative efficacy of biologics in psoriasis: a review. Am J Clin Dermatol *13*, 365-374.

Kim, K., Cho, S.K., Sestak, A., Namjou, B., Kang, C., and Bae, S.C. (2009). Interferon-gamma gene polymorphisms associated with susceptibility to systemic lupus erythematosus. Ann Rheum Dis *69*, 1247-1250.

Klein, R., Moghadam-Kia, S., Taylor, L., Coley, C., Okawa, J., LoMonico, J., Chren, M.M., and Werth, V.P. (2011). Quality of life in cutaneous lupus erythematosus. J Am Acad Dermatol *64*, 849-858.

Klimpel, G.R., Infante, A.J., Patterson, J., Hess, C.B., and Asuncion, M. (1990). Virus-induced interferon alpha/beta (IFN-alpha/beta) production by T cells and by Th1 and Th2 helper T cell clones: a study of the immunoregulatory actions of IFN-gamma versus IFN-alpha/beta on functions of different T cell populations. Cell Immunol *128*, 603-618.

Kotenko, S.V., Gallagher, G., Baurin, V.V., Lewis-Antes, A., Shen, M., Shah, N.K., Langer, J.A., Sheikh, F., Dickensheets, H., and Donnelly, R.P. (2003). IFN-lambdas mediate antiviral protection through a distinct class II cytokine receptor complex. Nat Immunol *4*, 69-77.

Krzyzowska, M., Swiatek, W., Fijalkowska, B., Niemialtowski, M., and Schollenberger, A. (2010). The Role of Map Kinases in Immune Response. Advances in Cell Biology *2*.

Kubiczkova, L., Sedlarikova, L., Hajek, R., and Sevcikova, S. (2012). TGF-beta - an excellent servant but a bad master. Journal of translational medicine *10*, 183.

Kubo, M. (2013). Therapeutic hope for psoriasis offered by SOCS (suppressor of cytokine signaling) mimetic peptide. Eur J Immunol *43*, 1702-1705.

Kuhn, A., and Bijl, M. (2008). Pathogenesis of cutaneous lupus erythematosus. Lupus *17*, 389-393.

Kuhn, A., Herrmann, M., Kleber, S., Beckmann-Welle, M., Fehsel, K., Martin-Villalba, A., Lehmann, P., Ruzicka, T., Krammer, P.H., and Kolb-Bachofen, V. (2006). Accumulation of apoptotic cells in the epidermis of patients with cutaneous lupus erythematosus after ultraviolet irradiation. Arthritis Rheum *54*, 939-950.

Kuhn, A., Richter-Hintz, D., Oslislo, C., Ruzicka, T., Megahed, M., and Lehmann, P. (2000). Lupus erythematosus tumidus--a neglected subset of cutaneous Lupus erythematosus: report of 40 cases. Arch Dermatol *136*, 1033-1041.

Kuhn, A., Sigges, J., Biazar, C., Ruland, V., Patsinakidis, N., Landmann, A., Amler, S., Bonsmann, G., and coauthors, E. (2014). Influence of smoking on disease severity and antimalarial therapy in cutaneous lupus erythematosus: analysis of 1002 patients from the EUSCLE database. Br J Dermatol *171*, 571-579.

Kumar, P., Rajasekaran, K., Palmer, J.M., Thakar, M.S., and Malarkannan, S. (2013). IL-22: An Evolutionary Missing-Link Authenticating the Role of the Immune System in Tissue Regeneration. Journal of Cancer *4*, 57-65.

Lande, R., Gregorio, J., Facchinetti, V., Chatterjee, B., Wang, Y.H., Homey, B., Cao, W., Su, B., Nestle, F.O., Zal, T.*, et al.* (2007). Plasmacytoid dendritic cells sense self-DNA coupled with antimicrobial peptide. Nature *449*, 564-569.

Lasfar, A., Abushahba, W., Balan, M., and Cohen-Solal, K.A. (2011). Interferon lambda: a new sword in cancer immunotherapy. Clin Dev Immunol *2011*, 349575.

Leask, A., and Abraham, D.J. (2004). TGF-beta signaling and the fibrotic response. FASEB J *18*, 816-827.

Lee, H.M., Mima, T., Sugino, H., Aoki, C., Adachi, Y., Yoshio-Hoshino, N., Matsubara, K., and Nishimoto, N. (2009). Interactions among type I and type II interferon, tumor necrosis factor, and beta-estradiol in the regulation of immune response-related gene expressions in systemic lupus erythematosus. Arthritis Res Ther *11*, R1.

Lee, J.C., Laydon, J.T., McDonnell, P.C., Gallagher, T.F., Kumar, S., Green, D., McNulty, D., Blumenthal, M.J., Heys, J.R., Landvatter, S.W.*, et al.* (1994). A protein kinase involved in the regulation of inflammatory cytokine biosynthesis. Nature *372*, 739-746.

Lei, K., and Davis, R.J. (2003). JNK phosphorylation of Bim-related members of the Bcl2 family induces Bax-dependent apoptosis. Proc Natl Acad Sci U S A *100*, 2432-2437.

Lejeune, D., Dumoutier, L., Constantinescu, S., Kruijer, W., Schuringa, J.J., and Renauld, J.C. (2002). Interleukin-22 (IL-22) activates the JAK/STAT,

ERK, JNK, and p38 MAP kinase pathways in a rat hepatoma cell line. Pathways that are shared with and distinct from IL-10. J Biol Chem *277*, 33676-33682.

Lew, W., Bowcock, A.M., and Krueger, J.G. (2004). Psoriasis vulgaris: cutaneous lymphoid tissue supports T-cell activation and "Type 1" inflammatory gene expression. Trends Immunol *25*, 295-305.

Li, J., Tomkinson, K.N., Tan, X.Y., Wu, P., Yan, G., Spaulding, V., Deng, B., Annis-Freeman, B., Heveron, K., Zollner, R.*, et al.* (2004a). Temporal associations between interleukin 22 and the extracellular domains of IL-22R and IL-10R2. Int Immunopharmacol *4*, 693-708.

Li, S.P., Junttila, M.R., Han, J., Kahari, V.M., and Westermarck, J. (2003). p38 Mitogen-activated protein kinase pathway suppresses cell survival by inducing dephosphorylation of mitogen-activated protein/extracellular signal-regulated kinase kinase1,2. Cancer research *63*, 3473-3477.

Li, W., Lewis-Antes, A., Huang, J., Balan, M., and Kotenko, S.V. (2008). Regulation of apoptosis by type III interferons. Cell Prolif *41*, 960-979.

Li, X., Leung, S., Qureshi, S., Darnell, J.E., Jr., and Stark, G.R. (1996). Formation of STAT1-STAT2 heterodimers and their role in the activation of IRF-1 gene transcription by interferon-alpha. The Journal of biological chemistry *271*, 5790-5794.

Li, Y., Sassano, A., Majchrzak, B., Deb, D.K., Levy, D.E., Gaestel, M., Nebreda, A.R., Fish, E.N., and Platanias, L.C. (2004b). Role of p38alpha Map kinase in Type I interferon signaling. J Biol Chem *279*, 970-979.

Liang, M., Wang, J., Chu, H., Zhu, X., He, H., Liu, Q., Qiu, J., Zhou, X., Guan, M., Xue, Y.*, et al.* (2013). Interleukin-22 inhibits bleomycin-induced pulmonary fibrosis. Mediators of inflammation *2013*, 209179.

Lim, C., and Savan, R. (2014). The role of the IL-22/IL-22R1 axis in cancer. Cytokine Growth Factor Rev *25*, 257-271.

Liu, B.S., Janssen, H.L., and Boonstra, A. (2011). IL-29 and IFNalpha differ in their ability to modulate IL-12 production by TLR-activated human macrophages and exhibit differential regulation of the IFNgamma receptor expression. Blood *117*, 2385-2395.

Lodish H, B.A., Zipursky SL, et al. (2000). Collagen: The Fibrous Proteins of the Matrix. (New York: W. H. Freeman).

Longman, R.S., Diehl, G.E., Victorio, D.A., Huh, J.R., Galan, C., Miraldi, E.R., Swaminath, A., Bonneau, R., Scherl, E.J., and Littman, D.R. (2014). CX(3)CR1(+) mononuclear phagocytes support colitis-associated innate lymphoid cell production of IL-22. J Exp Med *211*, 1571-1583.

Lovgren, T., Eloranta, M.L., Bave, U., Alm, G.V., and Ronnblom, L. (2004). Induction of interferon-alpha production in plasmacytoid dendritic cells by immune complexes containing nucleic acid released by necrotic or late apoptotic cells and lupus IgG. Arthritis Rheum *50*, 1861-1872.

Lowes, M.A., Suarez-Farinas, M., and Krueger, J.G. (2014). Immunology of psoriasis. Annual review of immunology *32*, 227-255.

Lu, Z., and Xu, S. (2006). ERK1/2 MAP kinases in cell survival and apoptosis. IUBMB life *58*, 621-631.

Lutfalla, G., Holland, S.J., Cinato, E., Monneron, D., Reboul, J., Rogers, N.C., Smith, J.M., Stark, G.R., Gardiner, K., Mogensen, K.E.*, et al.* (1995). Mutant

U5A cells are complemented by an interferon-alpha beta receptor subunit generated by alternative processing of a new member of a cytokine receptor gene cluster. EMBO J *14*, 5100-5108.

MacMicking, J.D. (2012). Interferon-inducible effector mechanisms in cell-autonomous immunity. Nat Rev Immunol *12*, 367-382.

Madonna, S., Scarponi, C., Pallotta, S., Cavani, A., and Albanesi, C. (2012). Anti-apoptotic effects of suppressor of cytokine signaling 3 and 1 in psoriasis. Cell death & disease *3*, e334.

Mah, W., Jiang, G., Olver, D., Cheung, G., Kim, B., Larjava, H., and Hakkinen, L. (2014). Human gingival fibroblasts display a non-fibrotic phenotype distinct from skin fibroblasts in three-dimensional cultures. PLoS One *9*, e90715.

Maher, S.G., Sheikh, F., Scarzello, A.J., Romero-Weaver, A.L., Baker, D.P., Donnelly, R.P., and Gamero, A.M. (2008). IFNalpha and IFNlambda differ in their antiproliferative effects and duration of JAK/STAT signaling activity. Cancer Biol Ther *7*, 1109-1115.

McGee, H.M., Schmidt, B.A., Booth, C.J., Yancopoulos, G.D., Valenzuela, D.M., Murphy, A.J., Stevens, S., Flavell, R.A., and Horsley, V. (2013). IL-22 promotes fibroblast-mediated wound repair in the skin. J Invest Dermatol *133*, 1321-1329.

McGrath, J.A., Eady, R. A. J. and Pope, F. M. (2008). Anatomy and Organization of Human Skin. In Rook's Textbook of Dermatology, S.B. T. Burns, N. Cox and C. Griffiths, ed. (Malden, Massachusetts, USA: Blackwell Publishing, Inc.).

Meister, M., Tomasovic, A., Banning, A., and Tikkanen, R. (2013). Mitogen-Activated Protein (MAP) Kinase Scaffolding Proteins: A Recount. International journal of molecular sciences *14*, 4854-4884.

Meller, S., Winterberg, F., Gilliet, M., Muller, A., Lauceviciute, I., Rieker, J., Neumann, N.J., Kubitza, R., Gombert, M., Bunemann, E.*, et al.* (2005). Ultraviolet radiation-induced injury, chemokines, and leukocyte recruitment: An amplification cycle triggering cutaneous lupus erythematosus. Arthritis Rheum *52*, 1504-1516.

Mennechet, F.J., and Uze, G. (2006). Interferon-lambda-treated dendritic cells specifically induce proliferation of FOXP3-expressing suppressor T cells. Blood *107*, 4417-4423.

Meyer, O. (2009). Interferons and autoimmune disorders. Joint Bone Spine *76*, 464-473.

Michalak-Stoma, A., Bartosinska, J., Kowal, M., Juszkiewicz-Borowiec, M., Gerkowicz, A., and Chodorowska, G. (2013). Serum levels of selected Th17 and Th22 cytokines in psoriatic patients. Disease markers *35*, 625-631.

Miknis, Z.J., Magracheva, E., Li, W., Zdanov, A., Kotenko, S.V., and Wlodawer, A. (2010). Crystal structure of human interferon-lambda1 in complex with its high-affinity receptor interferon-lambdaR1. J Mol Biol *404*, 650-664.

Milner, J.D., Brenchley, J.M., Laurence, A., Freeman, A.F., Hill, B.J., Elias, K.M., Kanno, Y., Spalding, C., Elloumi, H.Z., Paulson, M.L.*, et al.* (2008). Impaired T(H)17 cell differentiation in subjects with autosomal dominant hyper-IgE syndrome. Nature *452*, 773-776.

Mitra, A., Raychaudhuri, S.K., and Raychaudhuri, S.P. (2012a). Functional role of IL-22 in psoriatic arthritis. Arthritis Res Ther *14*, R65.

Mitra, A., Raychaudhuri, S.K., and Raychaudhuri, S.P. (2012b). IL-22 induced cell proliferation is regulated by PI3K/Akt/mTOR signaling cascade. Cytokine *60*, 38-42.

Morizane, S., and Gallo, R.L. (2012). Antimicrobial peptides in the pathogenesis of psoriasis. J Dermatol *39*, 225-230.

Morrison, D.K. (2012). MAP kinase pathways. Cold Spring Harbor perspectives in biology *4*.

Moustou, A.E., Matekovits, A., Dessinioti, C., Antoniou, C., Sfikakis, P.P., and Stratigos, A.J. (2009). Cutaneous side effects of anti-tumor necrosis factor biologic therapy: a clinical review. J Am Acad Dermatol *61*, 486-504.

Muhl, H., Scheiermann, P., Bachmann, M., Hardle, L., Heinrichs, A., and Pfeilschifter, J. (2013). IL-22 in tissue-protective therapy. Br J Pharmacol *169*, 761-771.

Munoz, L.E., Janko, C., Schulze, C., Schorn, C., Sarter, K., Schett, G., and Herrmann, M. (2010). Autoimmunity and chronic inflammation - two clearance-related steps in the etiopathogenesis of SLE. Autoimmun Rev *10*, 38-42.

Nagalakshmi, M.L., Murphy, E., McClanahan, T., and de Waal Malefyt, R. (2004). Expression patterns of IL-10 ligand and receptor gene families provide leads for biological characterization. Int Immunopharmacol *4*, 577-592.

Naschberger, E., Croner, R.S., Merkel, S., Dimmler, A., Tripal, P., Amann, K.U., Kremmer, E., Brueckl, W.M., Papadopoulos, T., Hohenadl, C.*, et al.*

(2008). Angiostatic immune reaction in colorectal carcinoma: Impact on survival and perspectives for antiangiogenic therapy. Int J Cancer *123*, 2120-2129.

Naschberger, E., Wenzel, J., Kretz, C.C., Herrmann, M., Sturzl, M., and Kuhn, A. (2010). Increased expression of guanylate binding protein-1 in lesional skin of patients with cutaneous lupus erythematosus. Exp Dermatol *20*, 102-106.

Nguyen, V.A., Chen, J., Hong, F., Ishac, E.J., and Gao, B. (2000). Interferons activate the p42/44 mitogen-activated protein kinase and JAK-STAT (Janus kinase-signal transducer and activator transcription factor) signalling pathways in hepatocytes: differential regulation by acute ethanol via a protein kinase C-dependent mechanism. The Biochemical journal *349*, 427-434.

Nickoloff, B.J., Xin, H., Nestle, F.O., and Qin, J.Z. (2007). The cytokine and chemokine network in psoriasis. Clinics in dermatology *25*, 568-573.

Nograles, K.E., Zaba, L.C., Guttman-Yassky, E., Fuentes-Duculan, J., Suarez-Farinas, M., Cardinale, I., Khatcherian, A., Gonzalez, J., Pierson, K.C., White, T.R.*, et al.* (2008). Th17 cytokines interleukin (IL)-17 and IL-22 modulate distinct inflammatory and keratinocyte-response pathways. Br J Dermatol *159*, 1092-1102.

Nordstrom, I., and Eriksson, K. (2012). HHV-6B induces IFN-lambda1 responses in cord plasmacytoid dendritic cells through TLR9. PLoS One *7*, e38683.

Novick, D., Cohen, B., and Rubinstein, M. (1994). The human interferon alpha/beta receptor: characterization and molecular cloning. Cell *77*, 391-400.

Nyberg, F., Fransson, J., and Stephansson, E. (2000). Proliferation and effects of UVA irradiation in cultured fibroblasts from lesions in cutaneous lupus erythematosus. Exp Dermatol *9*, 53-57.

Nystrom, A., Velati, D., Mittapalli, V.R., Fritsch, A., Kern, J.S., and Bruckner-Tuderman, L. (2013). Collagen VII plays a dual role in wound healing. J Clin Invest *123*, 3498-3509.

Occleston, N.L., Metcalfe, A.D., Boanas, A., Burgoyne, N.J., Nield, K., O'Kane, S., and Ferguson, M.W. (2010). Therapeutic improvement of scarring: mechanisms of scarless and scar-forming healing and approaches to the discovery of new treatments. Dermatol Res Pract *2010*.

Ohl, K., and Tenbrock, K. (2011). Inflammatory cytokines in systemic lupus erythematosus. J Biomed Biotechnol *2011*, 432595.

Oliveira, G.V., Hawkins, H.K., Chinkes, D., Burke, A., Tavares, A.L., Ramos-e-Silva, M., Albrecht, T.B., Kitten, G.T., and Herndon, D.N. (2009). Hypertrophic versus non hypertrophic scars compared by immunohistochemistry and laser confocal microscopy: type I and III collagens. Int Wound J *6*, 445-452.

Olsen, D.R., Peltonen, J., Jaakkola, S., Chu, M.L., and Uitto, J. (1989). Collagen gene expression by cultured human skin fibroblasts. Abundant steady-state levels of type VI procollagen messenger RNAs. J Clin Invest *83*, 791-795.

Paget, C., Ivanov, S., Fontaine, J., Renneson, J., Blanc, F., Pichavant, M., Dumoutier, L., Ryffel, B., Renauld, J.C., Gosset, P.*, et al.* (2012). Interleukin-22 is produced by invariant natural killer T lymphocytes during influenza A

virus infection: potential role in protection against lung epithelial damages. J Biol Chem *287*, 8816-8829.

Palucka, A.K., Blanck, J.P., Bennett, L., Pascual, V., and Banchereau, J. (2005). Cross-regulation of TNF and IFN-alpha in autoimmune diseases. Proc Natl Acad Sci U S A *102*, 3372-3377.

Pan, H.F., Li, X.P., Zheng, S.G., and Ye, D.Q. (2013). Emerging role of interleukin-22 in autoimmune diseases. Cytokine Growth Factor Rev *24*, 51-57.

Pan, H.F., Zhao, X.F., Yuan, H., Zhang, W.H., Li, X.P., Wang, G.H., Wu, G.C., Tang, X.W., Li, W.X., Li, L.H.*, et al.* (2009). Decreased serum IL-22 levels in patients with systemic lupus erythematosus. Clin Chim Acta *401*, 179-180.

Piette, E.W., Foering, K.P., Chang, A.Y., Okawa, J., Ten Have, T.R., Feng, R., and Werth, V.P. (2012). Impact of smoking in cutaneous lupus erythematosus. Arch Dermatol *148*, 317-322.

Platanias, L.C. (2003). The p38 mitogen-activated protein kinase pathway and its role in interferon signaling. Pharmacol Ther *98*, 129-142.

Popovic, K., Ek, M., Espinosa, A., Padyukov, L., Harris, H.E., Wahren-Herlenius, M., and Nyberg, F. (2005). Increased expression of the novel proinflammatory cytokine high mobility group box chromosomal protein 1 in skin lesions of patients with lupus erythematosus. Arthritis Rheum *52*, 3639-3645.

Postal, M., and Appenzeller, S. (2011). The role of Tumor Necrosis Factor-alpha (TNF-alpha) in the pathogenesis of systemic lupus erythematosus. Cytokine *56*, 537-543.

Radaeva, S., Sun, R., Pan, H.N., Hong, F., and Gao, B. (2004). Interleukin 22 (IL-22) plays a protective role in T cell-mediated murine hepatitis: IL-22 is a survival factor for hepatocytes via STAT3 activation. Hepatology *39*, 1332-1342.

Rana, A., Minz, R.W., Aggarwal, R., Anand, S., Pasricha, N., and Singh, S. (2012). Gene expression of cytokines (TNF-alpha, IFN-gamma), serum profiles of IL-17 and IL-23 in paediatric systemic lupus erythematosus. Lupus *21*, 1105-1112.

Raut, A.S., Prabhu, R.H., and Patravale, V.B. (2013). Psoriasis clinical implications and treatment: a review. Critical reviews in therapeutic drug carrier systems *30*, 183-216.

Regis, G., Pensa, S., Boselli, D., Novelli, F., and Poli, V. (2008). Ups and downs: the STAT1:STAT3 seesaw of Interferon and gp130 receptor signalling. Seminars in cell & developmental biology *19*, 351-359.

Ren, X., Hu, B., and Colletti, L.M. (2010). IL-22 is involved in liver regeneration after hepatectomy. Am J Physiol Gastrointest Liver Physiol *298*, G74-80.

Rhodes, B., and Vyse, T.J. (2008). The genetics of SLE: an update in the light of genome-wide association studies. Rheumatology (Oxford) *47*, 1603-1611.

Ripple, M.O., Kim, N., and Springett, R. (2013). Acute mitochondrial inhibition by mitogen-activated protein kinase/extracellular signal-regulated kinase kinase (MEK) 1/2 inhibitors regulates proliferation. J Biol Chem *288*, 2933-2940.

Roberts, A.B., Sporn, M.B., Assoian, R.K., Smith, J.M., Roche, N.S., Wakefield, L.M., Heine, U.I., Liotta, L.A., Falanga, V., Kehrl, J.H.*, et al.* (1986). Transforming growth factor type beta: rapid induction of fibrosis and angiogenesis in vivo and stimulation of collagen formation in vitro. Proc Natl Acad Sci U S A *83*, 4167-4171.

Roberts, P.J., and Der, C.J. (2007). Targeting the Raf-MEK-ERK mitogen-activated protein kinase cascade for the treatment of cancer. Oncogene *26*, 3291-3310.

Ronnblom, L., and Eloranta, M.L. (2013). The interferon signature in autoimmune diseases. Curr Opin Rheumatol *25*, 248-253.

Roskoski, R., Jr. (2010). RAF protein-serine/threonine kinases: structure and regulation. Biochem Biophys Res Commun *399*, 313-317.

Roskoski, R., Jr. (2012a). ERK1/2 MAP kinases: structure, function, and regulation. Pharmacological research : the official journal of the Italian Pharmacological Society *66*, 105-143.

Roskoski, R., Jr. (2012b). MEK1/2 dual-specificity protein kinases: structure and regulation. Biochem Biophys Res Commun *417*, 5-10.

Sabat, R., Ouyang, W., and Wolk, K. (2014). Therapeutic opportunities of the IL-22-IL-22R1 system. Nature reviews Drug discovery *13*, 21-38.

Sadler, A.J., and Williams, B.R. (2008). Interferon-inducible antiviral effectors. Nat Rev Immunol *8*, 559-568.

Scarponi, C., Butturini, E., Sestito, R., Madonna, S., Cavani, A., Mariotto, S., and Albanesi, C. (2014). Inhibition of inflammatory and proliferative responses of human keratinocytes exposed to the sesquiterpene lactones dehydrocostuslactone and costunolide. PLoS One *9*, e107904.

Scotton, C.J., and Chambers, R.C. (2007). Molecular targets in pulmonary fibrosis: the myofibroblast in focus. Chest *132*, 1311-1321.

Sestito, R., Madonna, S., Scarponi, C., Cianfarani, F., Failla, C.M., Cavani, A., Girolomoni, G., and Albanesi, C. (2011). STAT3-dependent effects of IL-22 in human keratinocytes are counterregulated by sirtuin 1 through a direct inhibition of STAT3 acetylation. FASEB J *25*, 916-927.

Sharma, N.L., Mahajan, V.K., Gupta, N., Ranjan, N., and Lath, A. (2009). Ehlers-Danlos syndrome--vascular type (ecchymotic variant): cutaneous and dermatopathologic features. J Cutan Pathol *36*, 486-492.

Sheppard, P., Kindsvogel, W., Xu, W., Henderson, K., Schlutsmeyer, S., Whitmore, T.E., Kuestner, R., Garrigues, U., Birks, C., Roraback, J.*, et al.* (2003). IL-28, IL-29 and their class II cytokine receptor IL-28R. Nature immunology *4*, 63-68.

Shi, Y., and Massague, J. (2003). Mechanisms of TGF-beta signaling from cell membrane to the nucleus. Cell *113*, 685-700.

Shouda, T., Yoshida, T., Hanada, T., Wakioka, T., Oishi, M., Miyoshi, K., Komiya, S., Kosai, K., Hanakawa, Y., Hashimoto, K.*, et al.* (2001). Induction of the cytokine signal regulator SOCS3/CIS3 as a therapeutic strategy for treating inflammatory arthritis. J Clin Invest *108*, 1781-1788.

Siren, J., Pirhonen, J., Julkunen, I., and Matikainen, S. (2005). IFN-alpha regulates TLR-dependent gene expression of IFN-alpha, IFN-beta, IL-28, and IL-29. Journal of immunology *174*, 1932-1937.

Soh, J., Donnelly, R.J., Kotenko, S., Mariano, T.M., Cook, J.R., Wang, N., Emanuel, S., Schwartz, B., Miki, T., and Pestka, S. (1994). Identification and

sequence of an accessory factor required for activation of the human interferon gamma receptor. Cell *76*, 793-802.

Sommereyns, C., Paul, S., Staeheli, P., and Michiels, T. (2008). IFN-lambda (IFN-lambda) is expressed in a tissue-dependent fashion and primarily acts on epithelial cells in vivo. PLoS Pathog *4*, e1000017.

Song, L.L., Ponomareva, L., Shen, H., Duan, X., Alimirah, F., and Choubey, D. (2010). Interferon-inducible IFI16, a negative regulator of cell growth, down-regulates expression of human telomerase reverse transcriptase (hTERT) gene. PLoS One *5*, e8569.

Sonkoly, E., Wei, T., Janson, P.C., Saaf, A., Lundeberg, L., Tengvall-Linder, M., Norstedt, G., Alenius, H., Homey, B., Scheynius, A.*, et al.* (2007). MicroRNAs: novel regulators involved in the pathogenesis of psoriasis? PLoS One *2*, e610.

Sontheimer, R.D., Maddison, P.J., Reichlin, M., Jordon, R.E., Stastny, P., and Gilliam, J.N. (1982). Serologic and HLA associations in subacute cutaneous lupus erythematosus, a clinical subset of lupus erythematosus. Ann Intern Med *97*, 664-671.

Sorensen, O.E., Cowland, J.B., Theilgaard-Monch, K., Liu, L., Ganz, T., and Borregaard, N. (2003). Wound healing and expression of antimicrobial peptides/polypeptides in human keratinocytes, a consequence of common growth factors. J Immunol *170*, 5583-5589.

Steen, H.C., and Gamero, A.M. (2010). Interferon-lambda as a potential therapeutic agent in cancer treatment. J Interferon Cytokine Res *30*, 597-602.

Stephanou, A. (2004). Role of STAT-1 and STAT-3 in ischaemia/reperfusion injury. Journal of cellular and molecular medicine *8*, 519-525.

Stephanou, A., Brar, B.K., Knight, R.A., and Latchman, D.S. (2000). Opposing actions of STAT-1 and STAT-3 on the Bcl-2 and Bcl-x promoters. Cell death and differentiation *7*, 329-330.

Szoka, L., Karna, E., Morka, R.P., and Palka, J.A. (2015). Enalapril stimulates collagen biosynthesis through prolidase-dependent mechanism in cultured fibroblasts. Naunyn Schmiedebergs Arch Pharmacol.

Takekawa, M., Kubota, Y., Nakamura, T., and Ichikawa, K. (2011). Regulation of stress-activated MAP kinase pathways during cell fate decisions. Nagoya journal of medical science *73*, 1-14.

Takekoshi, T., Wu, X., Mitsui, H., Tada, Y., Kao, M.C., Sato, S., Dwinell, M.B., and Hwang, S.T. (2013). CXCR4 negatively regulates keratinocyte proliferation in IL-23-mediated psoriasiform dermatitis. J Invest Dermatol *133*, 2530-2537.

ten Dijke, P., and Hill, C.S. (2004). New insights into TGF-beta-Smad signalling. Trends in biochemical sciences *29*, 265-273.

Tiedtke, J.a.M., O (2007). Stimulation of collagen production in human dermal fibroblasts. Costmetic Sci Tech, 15-18.

Tissari, J., Siren, J., Meri, S., Julkunen, I., and Matikainen, S. (2005). IFN-alpha enhances TLR3-mediated antiviral cytokine expression in human endothelial and epithelial cells by up-regulating TLR3 expression. Journal of immunology *174*, 4289-4294.

Tohyama, M., Hanakawa, Y., Shirakata, Y., Dai, X., Yang, L., Hirakawa, S., Tokumaru, S., Okazaki, H., Sayama, K., and Hashimoto, K. (2009). IL-17 and IL-22 mediate IL-20 subfamily cytokine production in cultured keratinocytes via increased IL-22 receptor expression. Eur J Immunol *39*, 2779-2788.

Tohyama, M., Yang, L., Hanakawa, Y., Dai, X., Shirakata, Y., and Sayama, K. (2012). IFN-alpha enhances IL-22 receptor expression in keratinocytes: a possible role in the development of psoriasis. J Invest Dermatol *132*, 1933-1935.

Tomasello, E., Pollet, E., Vu Manh, T.P., Uze, G., and Dalod, M. (2014). Harnessing Mechanistic Knowledge on Beneficial Versus Deleterious IFN-I Effects to Design Innovative Immunotherapies Targeting Cytokine Activity to Specific Cell Types. Frontiers in immunology *5*, 526.

Tomita, S., Ishibashi, K., Hashimoto, K., Sugino, T., Yanagida, T., Kushida, N., Shishido, K., Aikawa, K., Sato, Y., Suzutani, T.*, et al.* (2011). Suppression of SOCS3 increases susceptibility of renal cell carcinoma to interferon-alpha. Cancer science *102*, 57-63.

Trifari, S., Kaplan, C.D., Tran, E.H., Crellin, N.K., and Spits, H. (2009). Identification of a human helper T cell population that has abundant production of interleukin 22 and is distinct from T(H)-17, T(H)1 and T(H)2 cells. Nat Immunol *10*, 864-871.

Uddin, S., Lekmine, F., Sharma, N., Majchrzak, B., Mayer, I., Young, P.R., Bokoch, G.M., Fish, E.N., and Platanias, L.C. (2000). The Rac1/p38 mitogen-activated protein kinase pathway is required for interferon alpha-dependent transcriptional activation but not serine phosphorylation of Stat proteins. J Biol Chem *275*, 27634-27640.

Uitto, J., and Christiano, A.M. (1992). Molecular genetics of the cutaneous basement membrane zone. Perspectives on epidermolysis bullosa and other blistering skin diseases. J Clin Invest *90*, 687-692.

van Boxel-Dezaire, A.H., Rani, M.R., and Stark, G.R. (2006). Complex modulation of cell type-specific signaling in response to type I interferons. Immunity *25*, 361-372.

van der Rest, M., and Garrone, R. (1991). Collagen family of proteins. FASEB J *5*, 2814-2823.

Van Maele, L., Carnoy, C., Cayet, D., Ivanov, S., Porte, R., Deruy, E., Chabalgoity, J.A., Renauld, J.C., Eberl, G., Benecke, A.G.*, et al.* (2014). Activation of Type 3 innate lymphoid cells and interleukin 22 secretion in the lungs during Streptococcus pneumoniae infection. J Infect Dis *210*, 493-503.

Vanbervliet, B., Bendriss-Vermare, N., Massacrier, C., Homey, B., de Bouteiller, O., Briere, F., Trinchieri, G., and Caux, C. (2003). The inducible CXCR3 ligands control plasmacytoid dendritic cell responsiveness to the constitutive chemokine stromal cell-derived factor 1 (SDF-1)/CXCL12. J Exp Med *198*, 823-830.

Vermi, W., Lonardi, S., Morassi, M., Rossini, C., Tardanico, R., Venturini, M., Sala, R., Tincani, A., Poliani, P.L., Calzavara-Pinton, P.G.*, et al.* (2009). Cutaneous distribution of plasmacytoid dendritic cells in lupus erythematosus. Selective tropism at the site of epithelial apoptotic damage. Immunobiology *214*, 877-886.

Vilcek, J. (2006). Fifty years of interferon research: aiming at a moving target. Immunity *25*, 343-348.

Villanova, F., Flutter, B., Tosi, I., Grys, K., Sreeneebus, H., Perera, G.K., Chapman, A., Smith, C.H., Di Meglio, P., and Nestle, F.O. (2014). Characterization of innate lymphoid cells in human skin and blood

demonstrates increase of NKp44+ ILC3 in psoriasis. J Invest Dermatol *134*, 984-991.

Volk, S.W., Wang, Y., Mauldin, E.A., Liechty, K.W., and Adams, S.L. (2011). Diminished type III collagen promotes myofibroblast differentiation and increases scar deposition in cutaneous wound healing. Cells Tissues Organs *194*, 25-37.

Vuorio, E., and de Crombrugghe, B. (1990). The family of collagen genes. Annu Rev Biochem *59*, 837-872.

Wang, D., Drenker, M., Eiz-Vesper, B., Werfel, T., and Wittmann, M. (2008). Evidence for a pathogenetic role of interleukin-18 in cutaneous lupus erythematosus. Arthritis Rheum *58*, 3205-3215.

Wang, J.F., Jiao, H., Stewart, T.L., Shankowsky, H.A., Scott, P.G., and Tredget, E.E. (2007). Fibrocytes from burn patients regulate the activities of fibroblasts. Wound Repair Regen *15*, 113-121.

Wang, Y., Wu, T.R., Cai, S., Welte, T., and Chin, Y.E. (2000). Stat1 as a component of tumor necrosis factor alpha receptor 1-TRADD signaling complex to inhibit NF-kappaB activation. Mol Cell Biol *20*, 4505-4512.

Wauson, E.M., Guerra, M.L., Barylko, B., Albanesi, J.P., and Cobb, M.H. (2013). Off-target effects of MEK inhibitors. Biochemistry *52*, 5164-5166.

Wen, Z., Liao, Q., Zhao, J., Hu, Y., You, L., Lu, Z., Jia, C., Wei, Y., and Zhao, Y. (2014). High expression of interleukin-22 and its receptor predicts poor prognosis in pancreatic ductal adenocarcinoma. Annals of surgical oncology *21*, 125-132.

Wenzel, J., Bauer, R., Bieber, T., and Boehm, I. (2000a). Presence of antinuclear antibodies in patients with lupus erythematosus is correlated with diminished T-helper cells. Br J Dermatol *143*, 1100-1101.

Wenzel, J., Bauer, R., Bieber, T., and Bohm, I. (2000b). Autoantibodies in patients with Lupus erythematosus: spectrum and frequencies. Dermatology *201*, 282-283.

Wenzel, J., and Tuting, T. (2008). An IFN-associated cytotoxic cellular immune response against viral, self-, or tumor antigens is a common pathogenetic feature in "interface dermatitis". J Invest Dermatol *128*, 2392-2402.

Wenzel, J., Uerlich, M., Worrenkamper, E., Freutel, S., Bieber, T., and Tuting, T. (2005a). Scarring skin lesions of discoid lupus erythematosus are characterized by high numbers of skin-homing cytotoxic lymphocytes associated with strong expression of the type I interferon-induced protein MxA. Br J Dermatol *153*, 1011-1015.

Wenzel, J., Worenkamper, E., Freutel, S., Henze, S., Haller, O., Bieber, T., and Tuting, T. (2005b). Enhanced type I interferon signalling promotes Th1-biased inflammation in cutaneous lupus erythematosus. J Pathol *205*, 435-442.

Wenzel, J., Zahn, S., Mikus, S., Wiechert, A., Bieber, T., and Tuting, T. (2007). The expression pattern of interferon-inducible proteins reflects the characteristic histological distribution of infiltrating immune cells in different cutaneous lupus erythematosus subsets. Br J Dermatol *157*, 752-757.

Wenzel, J., Zahn, S., and Tuting, T. (2010). Pathogenesis of cutaneous lupus erythematosus: common and different features in distinct subsets. Lupus *19*, 1020-1028.

Werth, V.P., Zhang, W., Dortzbach, K., and Sullivan, K. (2000). Association of a promoter polymorphism of tumor necrosis factor-alpha with subacute cutaneous lupus erythematosus and distinct photoregulation of transcription. J Invest Dermatol *115*, 726-730.

Williams, V.L., and Cohen, P.R. (2011). TNF alpha antagonist-induced lupus-like syndrome: report and review of the literature with implications for treatment with alternative TNF alpha antagonists. Int J Dermatol *50*, 619-625.

Wilson, N.J., Boniface, K., Chan, J.R., McKenzie, B.S., Blumenschein, W.M., Mattson, J.D., Basham, B., Smith, K., Chen, T., Morel, F.*, et al.* (2007). Development, cytokine profile and function of human interleukin 17-producing helper T cells. Nat Immunol *8*, 950-957.

Witte, K., Gruetz, G., Volk, H.D., Looman, A.C., Asadullah, K., Sterry, W., Sabat, R., and Wolk, K. (2009). Despite IFN-lambda receptor expression, blood immune cells, but not keratinocytes or melanocytes, have an impaired response to type III interferons: implications for therapeutic applications of these cytokines. Genes Immun *10*, 702-714.

Witte, K., Witte, E., Sabat, R., and Wolk, K. (2010). IL-28A, IL-28B, and IL-29: promising cytokines with type I interferon-like properties. Cytokine Growth Factor Rev *21*, 237-251.

Wittmann, M., and Goodfield, M. (2011). Cytokines in cutaneous lupus erythematosus. Expert review in dermatology *6*, 381-394.

Wittmann, M., Doble, R., Bachmann, M., Pfeilschifter, J., Werfel, T., and Muhl, H. (2012). IL-27 Regulates IL-18 binding protein in skin resident cells. PLoS One *7*, e38751.

Wolff, K., Goldsmith, L. A., Katz, S. I., Gilchrest, B. A., Paller, A., Leffell, D. J. (2008). Fitzpatrick's Dermatology In General Medicine Vol 1 (USA: McGraw-Hill Companies, Inc.).

Wolk, K., Haugen, H.S., Xu, W., Witte, E., Waggie, K., Anderson, M., Vom Baur, E., Witte, K., Warszawska, K., Philipp, S.*, et al.* (2009a). IL-22 and IL-20 are key mediators of the epidermal alterations in psoriasis while IL-17 and IFN-gamma are not. J Mol Med (Berl) *87*, 523-536.

Wolk, K., Kunz, S., Witte, E., Friedrich, M., Asadullah, K., and Sabat, R. (2004). IL-22 increases the innate immunity of tissues. Immunity *21*, 241-254.

Wolk, K., Witte, E., Hoffmann, U., Doecke, W.D., Endesfelder, S., Asadullah, K., Sterry, W., Volk, H.D., Wittig, B.M., and Sabat, R. (2007). IL-22 induces lipopolysaccharide-binding protein in hepatocytes: a potential systemic role of IL-22 in Crohn's disease. J Immunol *178*, 5973-5981.

Wolk, K., Witte, E., Reineke, U., Witte, K., Friedrich, M., Sterry, W., Asadullah, K., Volk, H.D., and Sabat, R. (2005). Is there an interaction between interleukin-10 and interleukin-22? Genes Immun *6*, 8-18.

Wolk, K., Witte, E., Wallace, E., Docke, W.D., Kunz, S., Asadullah, K., Volk, H.D., Sterry, W., and Sabat, R. (2006). IL-22 regulates the expression of genes responsible for antimicrobial defense, cellular differentiation, and mobility in keratinocytes: a potential role in psoriasis. Eur J Immunol *36*, 1309-1323.

Wolk, K., Witte, E., Warszawska, K., Schulze-Tanzil, G., Witte, K., Philipp, S., Kunz, S., Docke, W.D., Asadullah, K., Volk, H.D., *et al.* (2009b). The Th17 cytokine IL-22 induces IL-20 production in keratinocytes: a novel immunological cascade with potential relevance in psoriasis. Eur J Immunol *39*, 3570-3581.

Wolk, K., Witte, K., Witte, E., Raftery, M., Kokolakis, G., Philipp, S., Schonrich, G., Warszawska, K., Kirsch, S., Prosch, S., *et al.* (2013). IL-29 is produced by T(H)17 cells and mediates the cutaneous antiviral competence in psoriasis. Sci Transl Med *5*, 204ra129.

Wong, C.K., Wong, P.T., Tam, L.S., Li, E.K., Chen, D.P., and Lam, C.W. (2009). Activation profile of Toll-like receptors of peripheral blood lymphocytes in patients with systemic lupus erythematosus. Clin Exp Immunol *159*, 11-22.

Wu, Q., Yang, Q., Lourenco, E., Sun, H., and Zhang, Y. (2011). Interferon-lambda1 induces peripheral blood mononuclear cell-derived chemokines secretion in patients with systemic lupus erythematosus: its correlation with disease activity. Arthritis Res Ther *13*, R88.

Xie, M.H., Aggarwal, S., Ho, W.H., Foster, J., Zhang, Z., Stinson, J., Wood, W.I., Goddard, A.D., and Gurney, A.L. (2000). Interleukin (IL)-22, a novel human cytokine that signals through the interferon receptor-related proteins CRF2-4 and IL-22R. J Biol Chem *275*, 31335-31339.

Xu, J., Lamouille, S., and Derynck, R. (2009). TGF-beta-induced epithelial to mesenchymal transition. Cell Res *19*, 156-172.

Yang, Y., Kim, S.C., Yu, T., Yi, Y.S., Rhee, M.H., Sung, G.H., Yoo, B.C., and Cho, J.Y. (2014). Functional roles of p38 mitogen-activated protein kinase in

macrophage-mediated inflammatory responses. Mediators Inflamm *2014*, 352371.

Yin, Z., Dai, J., Deng, J., Sheikh, F., Natalia, M., Shih, T., Lewis-Antes, A., Amrute, S.B., Garrigues, U., Doyle, S.*, et al.* (2012). Type III IFNs are produced by and stimulate human plasmacytoid dendritic cells. J Immunol *189*, 2735-2745.

Yokota, S., Yokosawa, N., Okabayashi, T., Suzutani, T., and Fujii, N. (2005). Induction of suppressor of cytokine signaling-3 by herpes simplex virus type 1 confers efficient viral replication. Virology *338*, 173-181.

Yoneyama, M., Kikuchi, M., Natsukawa, T., Shinobu, N., Imaizumi, T., Miyagishi, M., Taira, K., Akira, S., and Fujita, T. (2004). The RNA helicase RIG-I has an essential function in double-stranded RNA-induced innate antiviral responses. Nat Immunol *5*, 730-737.

Yoshimura, A., Naka, T., and Kubo, M. (2007). SOCS proteins, cytokine signalling and immune regulation. Nat Rev Immunol *7*, 454-465.

Yu, B., Guan, M., Peng, Y., Shao, Y., Zhang, C., Yue, X., Zhang, J., Yang, H., Zou, H., Ye, W.*, et al.* (2011). Copy number variations of interleukin-17F, interleukin-21, and interleukin-22 are associated with systemic lupus erythematosus. Arthritis Rheum *63*, 3487-3492.

Yu, C., Chang, C., and Zhang, J. (2013). Immunologic and genetic considerations of cutaneous lupus erythematosus: a comprehensive review. J Autoimmun *41*, 34-45.

Zahn, S., Rehkamper, C., Ferring-Schmitt, S., Bieber, T., Tuting, T., and Wenzel, J. (2011). Interferon-alpha stimulates TRAIL expression in human keratinocytes and peripheral blood mononuclear cells: implications for the

pathogenesis of cutaneous lupus erythematosus. Br J Dermatol *165*, 1118-1123.

Zahn, S., Rehkamper, C., Kummerer, B.M., Ferring-Schmidt, S., Bieber, T., Tuting, T., and Wenzel, J. (2010). Evidence for a pathophysiological role of keratinocyte-derived type III interferon (IFNlambda) in cutaneous lupus erythematosus. J Invest Dermatol *131*, 133-140.

Zeitvogel, J., Dalpke, A., Eiz-Vesper, B., Kracht, M., Dittrich-Breiholz, O., Werfel, T., and Wittmann, M. (2012). Human primary keratinocytes show restricted ability to up-regulate suppressor of cytokine signaling (SOCS)3 protein compared with autologous macrophages. J Biol Chem *287*, 9923-9930.

Zenewicz, L.A., and Flavell, R.A. (2011). Recent advances in IL-22 biology. Int Immunol *23*, 159-163.

Zenewicz, L.A., Yancopoulos, G.D., Valenzuela, D.M., Murphy, A.J., Karow, M., and Flavell, R.A. (2007). Interleukin-22 but not interleukin-17 provides protection to hepatocytes during acute liver inflammation. Immunity *27*, 647-659.

Zhang, Y., and Dong, C. (2007). Regulatory mechanisms of mitogen-activated kinase signaling. Cell Mol Life Sci *64*, 2771-2789.

Zhang, Y.L., and Dong, C. (2005). MAP kinases in immune responses. Cellular & molecular immunology *2*, 20-27.

Zhou, X., Tang, J., Cao, H., Fan, H., and Li, B. (2015). Tissue resident regulatory T cells: novel therapeutic targets for human disease. Cellular & molecular immunology.

Zimmerer, J.M., Lesinski, G.B., Kondadasula, S.V., Karpa, V.I., Lehman, A., Raychaudhury, A., Becknell, B., and Carson, W.E., 3rd (2007). IFN-alpha-induced signal transduction, gene expression, and antitumor activity of immune effector cells are negatively regulated by suppressor of cytokine signaling proteins. J Immunol *178*, 4832-4845.

Zitzmann, K., Brand, S., Baehs, S., Goke, B., Meinecke, J., Spottl, G., Meyer, H., and Auernhammer, C.J. (2006). Novel interferon-lambdas induce antiproliferative effects in neuroendocrine tumor cells. Biochem Biophys Res Commun *344*, 1334-1341.