Published in final edited form as:

Cell Signal. 2009 May ; 21(5): 685–694. doi:10.1016/j.cellsig.2009.01.005.

Interleukin-1 regulates keratinocyte expression of T cell targeting chemokines through interleukin-1 receptor associated kinase-1 (IRAK1) dependent and independent pathways

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Abstract

IL-1 is a potent pro-inflammatory cytokine that activates intracellular signaling cascades some of which may involve IL-1 receptor associated kinase-1 (IRAK1). Psoriasis is a T cell dependent chronic inflammatory condition of the skin of unknown cause. IL-1 has been implicated in psoriasis pathology, but the mechanism has not been elucidated. Interestingly, expression of IRAK1 is elevated in psoriatic skin. To identify a potential link between IL-1, keratinocytes and T cells in skin inflammation we employed pathway-focused microarrays to evaluate IL-1 dependent gene expression in keratinocytes. Several candidate mRNAs encoding known T cell chemoattractants were identified in primary keratinocytes and the stable keratinocyte cell line HaCaT. CCL5 and CCL20 mRNA and protein levels were confirmed up-regulated by IL-1 in concentration and time-dependent manners. Furthermore IL-1 synergized with IFN- γ and TNF- α . Expression of CXCL9, CXCL10 and CXCL11 mRNAs was also increased in response to IL-1, but protein could only be detected in medium from cells treated with IFN- γ alone or in combination with IL-1. Over-expression of IRAK1 led to increased constitutive and cytokine induced production of CCL5 and CCL20. Inhibition of IRAK1 activity through RNAi or expression of a dominant negative mutant blocked production of CCL5 and CCL20 but had no effect upon the IL-1 enhancement of IFN- γ induced CXCL9, CXCL10 and CXCL11 production. In conclusion IL-1 regulates T cell targeting chemokine production in keratinocytes through IRAK1 dependent and independent pathways. These pathways may contribute to acute and chronic skin inflammation.

Keywords

skin; inflammation; interleukin-1; interleukin-1 receptor associated kinase-1; T cell chemotaxis; gene expression; signaling

1. Introduction

Psoriasis is a chronic inflammatory condition of the skin that affects 0.3–3% of the world population depending on ethnicity [1]. The disease causes disfiguring thickened red skin

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covered with white/silvery scales and often severely affects life quality through psychological and emotional distress. It has been estimated that as many as 95% of psoriasis patients have psychiatric problems with depression and anxiety being the most common complaints [2]. The condition may be managed using anti-inflammatory agents such as corticosteroids, methotrexate and biologics such as the TNF- α neutralizing Etanercept with considerable variation in effectiveness. However, an actual cure for psoriasis remains to be developed.

The cause of psoriasis appears to be a multi-factorial process involving both genes and the environment leading to the dys-regulated skin structure and chronic inflammation (reviewed in [1,3]). Lesions often occur on areas of the skin exposed to most mechanical stress such as back, buttocks, knees and elbows. Keratinocytes, the primary constituents of the epidermis, hyper-proliferate and fail to undergo normal differentiation in affected skin. The disease pathology also includes elevated numbers of leukocytes and increased cytokine expression in the lesions themselves.

Historically the condition has been considered a T helper 1 polarized disease as levels of interleukin (IL)-2, interferon (IFN)- γ and TNF- α could readily be shown to be up-regulated in the affected skin [3]. However, more comprehensive analyses have revealed that a vast array of genes, including cytokines, growth factors, and differentiation markers is differentially regulated during the disease [4–9]. The scientific opinion pendulum has been swinging between keratinocytes and T cells as the cause of psoriasis. New insight into psoriasis pathology has been provided by studies demonstrating that activation of keratinocytes is sufficient to trigger psoriasis-like lesions in transgenic mice. Yet, this process is dependent upon the presence of T cells [10] demonstrating an intricate interplay between these different cell types. Additional recent reports have suggested a potential role for IL-1 in psoriasis pathology [8,9,11–16].

IL-1 plays a fundamental role in orchestrating inflammatory responses primarily through regulation of gene expression. There are two forms of IL-1, IL-1 α and IL-1 β , encoded by separate genes. IL-1 α is expressed as an intracellular protein and is released during mechanical stress or injury leading to local inflammation (reviewed in [17,18]). IL-1 β is synthesized as an inactive pro-molecule that is secreted into the surrounding tissue or bloodstream after cleavage by caspase-1 [17]. Both IL-1 α and IL-1 β bind to the IL-1 receptor type I (IL-1RI). In conjunction with the IL-1RI accessory protein (IL-1RAcP) IL-1RI activates intracellular signaling cascades leading to activation of the transcription factors AP-1 and NF- κ B (reviewed in [19]). These transcription factors in turn regulate expression of genes and gene products involved in many immunological responses, e.g. complement factors, cytokines and adhesion molecules. IL-1 signaling can also lead to mRNA stabilization ([20–22] and refs. therein).

The intracellular signaling pathways involved in relaying the message of receptor engagement to the nucleus and mRNA stabilization have predominantly been studied in leukocytes and kidney cells. IL-1R associated kinase-1 (IRAK1) is a serine/threonine kinase recruited to the intracellular domains of IL-1RI and IL-1RAcP following ligand binding. Activated IRAK1 dissociates from the receptor complex and activates down-stream signaling factors, e.g. tumor necrosis factor- β receptor associated factor-6 (TRAF6) and transforming growth factor- β activated protein kinase 1 (TAK1). These in turn regulate activation of NF- κ B [19]. The role of IRAK1 in AP-1 activation and mRNA stabilization is controversial. Dominant negative mutants of TAK1 can prevent the stabilization of the IL-8 mRNA and the activation of both NF- κ B and Jun amino-terminal kinase (JNK, an upstream activator of AP-1) ([20] and refs. therein). This suggests that the three sub-pathways diverge at, or after, TAK1. Curiously it has been demonstrated that IRAK1, but not TRAF6, is required for IL-1 α induced stabilization of the mouse chemokine KC [21]. In contrast other groups have found that IRAK1 cannot stabilize the IL-2 mRNA [22] and deletion mutants of IRAK1 have differential effects upon the different sub-pathways [23]. These latter studies imply branching of the pathways at, or before, IRAK1.

Although IL-1 is required for an effective immune response, excessive levels of IL-1 may lead to tissue damage. An important regulator of IL-1 activity is the IL-1 receptor antagonist (IL-1RA). IL-1RA acts as a classical antagonist by binding to the IL-1RI without triggering the intracellular signaling cascade and thereby prevents binding and cellular activation by the agonist IL-1. It has recently been observed that mice lacking IL-1RA develop skin lesions strongly resembling the human psoriasis pathology [11]. This indirectly suggests a role for IL-1 in driving the reported phenotype. Interestingly, expression of IL-1 β [8,24–26] and IRAK1 [4–6] is up-regulated in affected areas of skin from psoriasis patients. In contrast, IL-1 α expression is either unaffected or down-regulated in psoriatic skin [4,8,24,25]. Microarray studies have demonstrated correlations between the transcriptome from psoriasis biopsies with the expression profiles of keratinocytes stimulated with IL-1 α [8,9,27]. IL-1 has been implicated in the psoriasis-associated hyperproliferation of keratinocytes [12–16]. However, a link between IL-1 and the psoriasis pathology pre-requisite interaction between keratinocytes and T cells ([10] and refs. therein) has not been established. While some studies have reported IL-1 regulated chemokine production by keratinocytes [28–30] these observations could not be reproduced by others [8,9]. The involvement of IRAK1 in IL-1 signaling in keratinocytes and the potential role that elevated IRAK1 levels may have in psoriasis pathology have never been explored. In the present study we demonstrate that IL-1 stimulated keratinocytes express elevated levels of Cys-Cys (CC) and Cys-X-Cys (CXC) motif chemokines involved in T cell recruitment. Furthermore, IL-1 can act in synergy with TNF- α and IFN- γ . While IL-1 regulated CC chemokine production is IRAK1 dependent the IL-1 effect upon IFN- γ driven CXC chemokine expression is IRAK1 independent.

2. Materials and methods

2.1. Cell cultures and treatments

Human primary keratinocytes (neonatal and adult) were obtained from Invitrogen (Carlsbad, CA) and maintained in Defined Keratinocyte Serum Free Medium supplemented with 50 μ g/ml gentamicin (Invitrogen). The human keratinocyte cell line HaCaT was generously provided by Dr. Meenhard Herlyn (Wistar Institute, Philadelphia, PA) and maintained in Dulbecco's modified Eagle's medium with glutamax-1 (L-alanyl-L-glutamine, Invitrogen) supplemented with 10% (vol./vol.) fetal calf serum (Sigma, St. Louis, MO) and 50 μ g/ml gentamicin. Cells were grown to approximately 70–80% confluence and treated with medium only, IL-1 α (National Cancer Institute, Frederick, MD), IL-1 β (PeproTech, Rocky Hill, NJ), IFN- γ (PeproTech) or TNF- α (Zeneca Pharmaceuticals, Macclesfield, UK) as indicated. All experiments were performed at least three times with similar outcomes.

2.2. RNA isolation and array hybridization

Total RNA was extracted using the RNeasy purification system according to the manufacturer's instructions (Qiagen, Valencia, CA). Synthesis of cDNA and generation of biotinylated cRNA was performed using TrueLabeling-AMP 2.0 (SuperArray Bioscience Corp., Frederick, MD) according to the manufacturer's instructions. Briefly, 3 μ g RNA per sample was used for cDNA synthesis followed by *o*/night *in vitro* transcription in the presence of biotin-16-UTP (Roche, Indianapolis, IN). The cRNA was purified using ArrayGrade cRNA Cleanup Kit (SuperArray Bioscience Corp.) according to the manufacturer's instructions and quantified by UV spectrometry (yield 10–15 μ g). Biotin-labeled cRNAs (2 μ g per array) were hybridized to the Oligo GEArray® Human Inflammatory Cytokines & Receptors Microarray (SuperArray Bioscience Corp.) *o*/night at 60°C, 10 rpm in a hybridization oven. After washing, biotinylated cRNA was detected using alkaline phosphatase streptavidin and ECL Plus Western blotting detection reagents (GE Healthcare, Piscataway, NJ). Short and intermediate exposures of arrays (Fig. 1) were analyzed using the GEArray Expression Analysis Suite 2.0 (<http://geasuite.sabiosciences.com/>). Gene expression was standardized against GAPDH.

2.3. Real-time RT-PCR

Reverse transcription of 1 µg total RNA was performed at 37°C for 1.5 hour using AMV reverse transcriptase (Promega, Madison, WI) and oligo(dN)6 primer (GE Healthcare) in the presence of RNAGuard Ribonuclease Inhibitor (GE Healthcare). AMV was inactivated at 95°C for 10 min. Primer pairs specific for individual mRNA/cDNAs were designed such that PCR products (80–100 bp) span exon-exon junctions thereby preventing amplification of genomic DNA. Amplification of GAPDH cDNA was used for normalization. Real-time RT-PCR was performed using RT² Real-Time SYBR Green PCR Master Mix (SuperArray Bioscience Corp.) on an Opticon2 instrument (Bio-Rad, Hercules, CA). Forty cycles of amplification were performed involving sequential denaturation at 95°C for 20 sec, annealing at 63°C for 30 sec, plate reading, and extension at 72°C for 30 sec. Assays were validated using serial dilutions and confirmation of equal amplification efficiencies of the cDNA of interest and the GAPDH cDNA. Fold differences in expression were calculated using the Comparative C_T method [31] by standardizing against GAPDH expression and comparing expression in cytokine treated cells to expression in cells treated with medium only.

The following gene specific primers were used: CCL5-F, 5'-GCTGTCATCCTCATTGCTAC-3'; CCL5-R, 5'-AATGTAGGCAAAGCAGCAGG-3'; CCL20-F, 5'-TGATGTCAGTGCTGCTACTC-3'; CCL20-R, 5'-ATGTCACAGCCTTCATTGGC-3'; CXCL9-F, 5'-GCATCATCTTGCTGGTTCTG-3'; CXCL9-R, 5'-TGTAGGTGGATAGTCCCTTG-3'; CXCL10-F, 5'-CTGCCATTCTGATTTGCTGC-3'; CXCL10-R, 5'-CGTACAGTTCTAGAGAGAGG-3'; CXCL11-F, 5'-TGTGAAGGGCATGGCTATAG-3'; CXCL11-R2, 5'-TTGAACATGGGGAAGCCTTG-3'; GAPDH-SF2, 5'-GGTCGGAGTCAACGGATTTG-3'; GAPDH-SR2, 5'-TGGGTGGAATCATATTGGAAC-3'.

2.4. ELISA

CCL5 concentrations in culture medium from stimulated cells were determined using the Human CCL5 ELISA Development Kit (PeproTech). Levels of CCL20, CXCL9, CXCL10 and CXCL11 were determined using the appropriate DuoSet ELISA Development Kit (R&D Systems, Minneapolis, MN).

2.5. Over-expression of full-length IRAK1 or dominant negative mutant and knockdown by RNAi

Full-length IRAK1 and the dominant negative deletion mutant IRAK1(1–217) are described elsewhere [32,33]. The cloning plasmid pCI-Neo (Promega) was used to transfect control cells. Validated IRAK1 specific shRNA expression plasmid was obtained from SuperArray. Control plasmid containing a scrambled shRNA sequence was also obtained from SuperArray. All plasmids encode resistance to neomycin. Plasmids (40 µg) were transfected into 1–2 million HaCaT cells using 2 square pulses of 8 msec at 500 Volt/cm in an ECM 830 ElectroSquarePorator (Harvard Apparatus, Holliston, MA). Cells were allowed to recover overnight in basic medium as described above after which medium was replaced with basic medium supplemented with 800 µg Geneticin (Invitrogen). After 48 hours cells were treated with trypsin and plated for cytokine treatments. The following day cells were treated with cytokines as described above. Medium and cells were harvested after 24 hours.

2.6. Western blotting

Cells from RNAi experiments were lysed in PBS with 2 M urea and 0.85% SDS. Proteins were separated in PAGER Gold 4–20% Tris-Glycine SDS-PAGE gels (Lonza, Rockland, ME) and transferred to PVDF membranes. Expression of IRAK1 and GAPDH was examined by

Western blotting using anti-IRAK1 (H-273) and anti-GAPDH (FL-335) (Santa Cruz Biotechnology, Santa Cruz, CA), respectively. Proteins were visualized using ECL.

2.7. Statistical analyses

Data are shown as average values and standard deviations from one representative experiment of at least three experiments. Data were analyzed using the Student's *t* test when appropriate.

3. Results

3.1. IL-1 β stimulated keratinocytes express T cell targeting CC chemokine mRNAs present in psoriatic lesions

Psoriatic lesions are characterized by increased leukocyte infiltration, primarily by T cells [1, 3,34]. Recent studies have independently demonstrated an essential link between activated keratinocytes and T cells [10], and suggested involvement of IL-1 in psoriasis pathology [8, 9,11–16]. To search for a potential biological role of IL-1 in linking keratinocytes to T cells we searched for IL-1 regulated cytokines expressed by keratinocytes. Primary keratinocytes were treated with medium only or 10 ng/ml IL-1 β for 6 hours. Total RNA was isolated and used for hybridization to the Oligo GEArray Human Inflammatory Cytokines & Receptors Microarray including oligo targets for 113 cytokines and receptors (Fig. 1). Several mRNAs were found to be up-regulated in the presence of IL-1 (Table 1). Interestingly, the mRNAs encoding the known T cell chemoattractants CCL5 and CCL20 were noticeably elevated in IL-1 treated cells compared to cells treated with medium only (Fig. 1 and Table 1). These latter two mRNAs have previously been reported to be up-regulated in psoriatic skin compared to normal skin ([6,7,35,36] and refs. therein) and could theoretically provide a mechanistic link between IL-1, keratinocytes, and T cells in psoriasis pathology.

To validate the array data, time-course experiments involving increasing concentrations of IL-1 β were performed using both primary keratinocytes and the established stable keratinocyte cell line HaCaT. Levels of CCL5 and CCL20 mRNA were determined using real-time RT-PCR and the comparative C_T method [31]. Expression levels of the CCL5 and CCL20 mRNAs were found to be IL-1 concentration dependent in both primary keratinocytes (Fig. 2A) and HaCaT cells (Fig. 2B). Interestingly, the kinetics of the observed expression changes differed for the two mRNAs. CCL5 mRNA levels gradually changed and peaked after 3–6 hours. At the 3-hour time-point CCL5 mRNA levels were approximately 8-fold higher ($p < 0.01$) in 5 ng/ml IL-1 β treated primary keratinocytes than in primary cells treated with medium only (Fig. 2A). In the keratinocyte cell line HaCaT CCL5 mRNA levels peaked after 6 hours and an approximately 15-fold induction ($p < 0.01$) in mRNA levels was observed in 5 ng/ml IL-1 β treated cells compared to untreated cells (Fig. 2B). In contrast to the gradual changes in CCL5 mRNA levels a rapid burst in CCL20 mRNA levels was observed in both primary keratinocytes and HaCaT cells after only 1.5 hour (Fig. 2A and B). Approximately 50-fold and 140-fold increases ($p < 0.01$) in CCL20 mRNA expression were observed in 5 ng/ml IL-1 β stimulated primary and HaCaT keratinocytes compared to untreated cells after 1.5 hour. Levels of CCL20 mRNA gradually declined at later time-points (Fig. 2A and B).

Studies using IL-1 α revealed CCL5 and CCL20 mRNA expression profiles similar to those induced by IL-1 β (data not shown).

3.2. IL-1 β up-regulates CC chemokine secretion by keratinocytes

If IL-1 stimulated keratinocytes are to communicate with leukocytes through chemokines the keratinocytes must express the chemokines into the surrounding tissue or medium. Therefore we examined if IL-1 β stimulated keratinocytes and/or HaCaT cells secrete detectable levels of chemokines using ELISA. We were unable to detect CCL5 after 1.5 hours. At later time-points

CCL5 levels increased in an IL-1 β concentration dependent manner in the culture medium from both primary (Fig. 2C) and HaCaT (Fig. 2D) keratinocytes throughout the duration of the experiment. Approximately 7-fold increases ($p < 0.01$) in CCL5 protein levels in the culture medium were observed after 24-hours in both primary and HaCaT 5 ng/ml IL-1 β stimulated keratinocytes compared to untreated cells (Fig. 2 C and D). CCL5 protein levels reached approximately 150 pg/ml after 24 hours (not shown). In comparison CCL5 secretion by cells treated with medium only reached approximately 50 pg/ml after 24 hours (not shown).

Levels of CCL20 protein also increased in an IL-1 β concentration dependent manner (Fig. 2 C and D). At 6–24 hours levels of CCL20 protein secreted into the medium was approximately 15-fold higher ($p < 0.01$) from 5 ng/ml IL-1 β treated primary and HaCaT cells than from untreated cells (Fig. 2 C and D). Maximum induction of CCL20 proteins levels was reached faster than CCL5 proteins levels (Fig. 2 C and D). This is in agreement with the immediate induction of the CCL20 mRNA compared to the more gradual changes in CCL5 mRNA levels. Levels of CCL20 protein secreted into the culture medium ranged from approximately 40 to 600 pg/ml in HaCaT cells treated with medium only and 5 ng/ml IL-1 β , respectively (not shown). In medium from primary keratinocytes treated with medium only or 5 ng/ml IL-1 β for 24 hours, CCL20 levels were approximately 250 pg/ml and 4000 pg/ml, respectively (not shown). Similar observations were made when using IL-1 α (data not shown).

3.3. IL-1 β up-regulates CXC chemokine mRNA levels but does not lead to protein secretion

It has been demonstrated that the IL-1 related cytokine IL-18 enhances production of CXCL9, CXCL10 and CXCL11 expression in keratinocytes [37]. These CXC chemokines are up-regulated in psoriatic skin compared to normal skin ([6] and refs therein). In the array screening we observed strong signals indicative of high levels of CXCL10 mRNA expression (Fig. 1, intermediate exposure). Signal intensities from the medium only and the IL-1 β treated samples did not differ in shorter exposures (Fig. 1). In longer exposures (Fig. 1) weak signals could be detected for the CXCL11 mRNA. The signal was slightly up-regulated in the IL-1 β treated sample compared to medium only. The CXCL9 mRNA could not be detected. To further explore CXC expression we employed real-time RT-PCR analyses as described above. Both the CXCL9 and CXCL11 mRNAs were up-regulated in time and concentration dependent manners in both primary keratinocytes and HaCaT cells (Fig. 3). Maximum induction by IL-1 β was observed at the 9-hour time-point at which levels were 4- to 8-fold ($p < 0.05$) higher than in medium only treated cells (Fig. 3). Surprisingly, the CXCL10 mRNA was dramatically up-regulated in response to IL-1 β (Fig. 3). Induction was time and IL-1 β -concentration dependent. In primary cells the greatest increase (21-fold, $p < 0.01$) was observed after 9-hours whereas in HaCaT cells maximum levels (29-fold, $p < 0.01$) were found at the 3-hour time-point (Fig. 3).

Using CXCL9, CXCL10 and CXCL11 specific ELISAs we were unable to detect secretion of any of the CXC chemokines (data not shown). The detection limits of the assays were less than 75, 25 and 10 pg/ml, respectively.

3.4. IL-1 β amplifies chemokine production induced by IFN- γ and TNF- α

IFN- γ and TNF- α have been linked to psoriasis pathology [1,3]. However, the actual tissue milieu in psoriatic skin contains a complex mixture of factors including IL-1 β , in addition to IFN- γ and TNF- α . To examine the potential effects of IL-1 β on keratinocyte responses to IFN- γ and TNF- α , primary keratinocytes and HaCaT cells were treated with medium only, IL-1 β , IFN- γ or TNF- α . Cells were also co-treated with combinations of cytokines. Expression of CC and CXC chemokine mRNA and protein was examined using real-time RT-PCR and ELISA. Similar results were observed in primary keratinocytes (below) and HaCaT cells (not shown).

CCL20 was not significantly regulated by IFN- γ (Fig. 4). As expected, stimulation of cells with IL-1 β or IL-1 β +IFN- γ yielded similar degrees of CCL20 induction. In contrast, CCL5 expression was induced by IL-1 β and IFN- γ individually (Fig. 4). While IL-1 β induced approximately 7- and 3-fold ($p < 0.01$) increases in CCL5 mRNA and protein levels, respectively, IFN- γ increased both CCL5 mRNA and protein levels approximately 2-fold ($p < 0.01$ and $p < 0.05$, respectively). Combination treatment led to synergistic induction of both CCL5 mRNA and protein (Fig. 4). CCL5 mRNA and protein levels were approximately 20- (Fig. 4A) and 8-fold (Fig. 4B) higher following co-treatment ($p < 0.01$ comparing combination treatment to individual treatment).

Both CCL5 and CCL20 expression was regulated by TNF- α (Fig. 4). Approximately 30-fold increases ($p < 0.01$) in both mRNA levels were observed (Fig. 4A). CCL5 and CCL20 protein levels were approximately 12- and 20-fold ($p < 0.01$) higher, respectively, in medium from cells treated with TNF- α than cells treated with medium only for 6-hours (Fig. 4B). For CCL5 mRNA and protein expression a synergistic activity of IL-1 β and TNF- α was observed in co-treated cells compared to cells treated with either agent alone (Fig. 4). Approximately 70- and 25-fold ($p < 0.01$) increases in CCL5 mRNA and protein levels, respectively, were found in cells treated with both IL-1 β and TNF- α compared to cells treated with medium only (Fig. 4).

Co-treatment of cells with IL-1 β and TNF- α had an approximately additive effect upon CCL20 protein expression levels (Fig. 4B) whereas co-treatment had a greater than additive effect upon the CCL20 mRNA levels (Fig. 4A). Both IL-1 β and TNF- α induced CCL20 mRNA approximately 30-fold ($p < 0.01$). Combination treatment led to an approximately 100-fold ($p < 0.01$ compared to cells treated with medium only) increase in CCL20 mRNA levels ($p < 0.01$ comparing individual treatment to combination treatment). Individually, IL-1 β and TNF- α stimulated 17- and 20-fold ($p < 0.01$) increases in CCL20 secretion, respectively, whereas in combination they induced a 34-fold increase (Fig. 4B, $p < 0.05$ comparing individual treatment to combination treatment).

Expression of the CXC chemokine mRNAs was dramatically induced by IFN- γ . Six hours post-treatment CXCL9, CXCL10 and CXCL11 mRNAs were 15,278-, 572- and 745-fold increased ($p < 0.01$, Fig. 5A). At the 6-hour time-point IL-1 β induced these mRNAs 2-, 11- and 4-fold, respectively (Fig. 5A). When cells were co-treated with IFN- γ and IL-1 β CXCL9, CXCL10 and CXCL11 mRNA levels were increased 34,530-, 2919- and 1473-fold ($p < 0.01$), respectively, compared to cells treated with medium only (Fig. 5A). These levels were significantly higher ($p < 0.05$) than the corresponding levels in cells treated with IFN- γ only. CXC chemokines could only be detected in medium from cells treated with IFN- γ alone or IFN- γ with IL-1 β . At the 9-hour time-point medium from cells treated with IFN- γ contained 460 pg/ml CXCL9. CXCL10 and CXCL11 could be detected already at the 6-hour time-point at which levels were 252 and 960 pg/ml, respectively (data not shown). At the same time-points IL-1 β significantly ($p < 0.05$) increased protein secretion to 546, 827 and 1968 pg/ml CXCL9, CXCL10 or CXCL11, respectively (data not shown). CXC protein levels continued to rise throughout the duration of the time-course experiments. At the 24-hour time-point levels of each CXC chemokine were greater than those observed for CCL20 (4 ng/ml, data not shown).

TNF- α treatment of cells increased CXCL9, CXCL10 and CXCL11 mRNA levels 8-, 12- and 19-fold, respectively ($p < 0.05$, Fig. 5B). Similar degrees of CXCL9 and CXCL11 mRNA induction were observed when cells were additionally treated with IL-1 β (Fig. 5B). In contrast, IL-1 β acted in synergy with TNF- α to induce CXCL10 mRNA levels 44 times above levels in cells treated with medium only ($p < 0.01$, Fig. 5B). Although the CXC chemokine mRNAs were significantly elevated when cells were treated with TNF- α alone or in combination with IL-1 β the CXC chemokines could not be detected in the culture medium throughout 24-hour time-course experiments (data not shown).

3.5. Elevated IRAK1 levels enhance chemokine expression

IRAK1 levels are increased in psoriatic skin compared to normal skin [4–6]. To examine how this may affect chemokine production by keratinocytes an IRAK1 expression construct or empty control vector was transfected into HaCaT cells. Cultures were enriched for transfected cells with geneticin and treated with cytokines for 24 hours. Secretion of chemokines into the medium was determined using ELISA. Over-expression of IRAK1 led to significant ($p < 0.01$) constitutive secretion of both CCL5 (Fig. 6A) and CCL20 (Fig. 6B). In medium from cells transfected with the IRAK1 expression construct levels of CCL5 and CCL20 were respectively 6- and 19-fold higher than in medium from cells transfected with the control plasmid (Fig. 6). When cells were treated with IL-1 β or TNF- α levels of CCL5 and CCL20 secretion were consistently 1.5–2-fold higher ($p < 0.05$) in medium from cells transfected with the IRAK1 expression construct than in medium from control cells treated with the same cytokine (Fig. 6). IFN- γ induced CCL5 secretion by control cells 4-fold but stimulated a significantly higher (7-fold, $p < 0.05$) production in cells over-expressing IRAK1 (Fig. 6A).

Expression of CCL20 is not significantly regulated by IFN- γ (Fig. 4B). Unsurprisingly similar levels of CCL20 were found in medium from cells transfected with the same expression construct and treated with medium only or IFN- γ (Fig. 6B). Elevated IRAK1 levels did not significantly affect expression of the CXC chemokines (data not shown).

3.6. IL-1 induced CC chemokine expression is dependent upon IRAK1

To further explore the involvement of IRAK1 in chemokine production we examined the consequence(s) of blocking or eliminating the activity of IRAK1. To knockdown IRAK1 expression plasmids encoding a scrambled negative control sequence and a validated IRAK1 specific shRNA were separately transfected into HaCaT cells. Cells expressing shRNA were selected with geneticin and treated with medium only, IL-1 β , TNF- α , IFN- γ or cytokine combinations for 2 or 24 hours. Expression of IRAK1 was examined by Western blotting. As expected IRAK1 levels were significantly reduced in cells expressing the IRAK1 specific shRNA compared to cells expressing the scrambled negative control sequence (Fig. 7). Secreted levels of CCL5 (Fig. 8A) and CCL20 (Fig. 8B) were next determined by ELISA. Levels of CCL5 in medium from IL-1 β treated IRAK1 targeted cells were 62% ($p < 0.05$) of levels observed from IL-1 β treated cells expressing the negative control shRNA (Fig. 8A). CCL20 levels in medium from the IL-1 β treated and IRAK1 targeted cells were decreased to 33% ($p < 0.05$) of levels secreted by cells expressing the negative control shRNA and treated with IL-1 β (Fig. 8B).

A modest non-significant ($p > 0.05$) increase in CCL5 expression in TNF- α treated cells was observed when cells expressed the IRAK1 specific shRNA compared to cells expressing the negative control shRNA (Fig. 8A). No difference in CCL20 expression was observed between the TNF- α treated control and IRAK1 targeted cells (Fig. 8B) demonstrating specificity of the IRAK1 shRNA. The ability of IL-1 to enhance CCL5 and CCL20 expression induced by TNF- α and IFN- γ was inhibited in a similar manner (data not shown).

To complement the knockdown study a dominant negative IRAK1 deletion mutant, IRAK1 (1-217), comprising amino acids 1-217 of IRAK1 was over-expressed in HaCaT cells. Control cells transfected with an empty expression vector and cells expressing IRAK1(1-217) were selected with geneticin and expression of CCL5 and CCL20 examined as described above.

The results summarized in Fig. 8C identified significantly lower levels (61%, $p < 0.05$) of CCL5 in medium from IL-1 β treated cells expressing IRAK1(1-217) than in cells transfected with the empty vector and treated with IL-1 β . Similarly levels of CCL20 in medium from the IRAK1(1-217) transfected and IL-1 β treated cells were only 55% ($p < 0.05$) of levels from the

corresponding control cells (Fig. 8D). The expression levels of CCL5 and CCL20 in TNF- α treated cells were similar irrespective of the transfected plasmid (Fig. 8C and D) demonstrating that IRAK1(1-217) specifically inhibited the IL-1 β induction of CC chemokine production. In summary the data demonstrates that IL-1 β regulates CCL5 and CCL20 expression through IRAK1 dependent pathways.

3.7. IL-1 effect on IFN- γ induced CXC chemokine expression is IRAK1 independent

Our studies above established that the CXC chemokines CXCL9, CXCL10 and CXCL11 are dramatically regulated by IFN- γ and that IL-1 β alone has a limited effect upon CXC chemokine production. However, IL-1 β can significantly enhance IFN- γ induced CXC gene expression. To examine if this IL-1 β effect is dependent upon IRAK1 medium only, IFN- γ and IFN- γ + IL-1 β treated samples from experiments described above were examined. At the 2 hour time-point IFN- γ alone increased the CXCL9, CXCL10 and CXCL11 mRNA levels 4000-, 4- and 50-fold, respectively (not shown). Co-treatment with IL-1 β significantly ($p < 0.05$) increased CXC mRNA levels approximately an additional 1.5-, 3.5- and 2.5-fold, respectively, in cells expressing the negative control shRNA (Fig. 9A) or the empty expression vector (Fig. 9B). Although the IRAK1 specific shRNA and IRAK1(1-217) diminished IL-1 β -induced CC chemokine production (Fig. 8) these agents did not inhibit the IL-1 β enhancement of CXC expression (Fig. 9). Similar observations (data not shown) were made when examining CXC protein expression by ELISA. While a trend appeared, that IL-1 β enhanced IFN- γ responses more in IRAK1 targeted cells than in control cells, this effect was only significant ($p < 0.05$) for CXCL11 expression in the IRAK1(1-217) expressing cells (Fig. 9B, #). A similar effect was not observed when protein expression was examined (data not shown). Overall the data suggest that IL-1 β enhances IFN- γ induced CXC expression through an IRAK1 independent mechanism(s).

4. Discussion

IL-1 is a potent pro-inflammatory cytokine which regulates gene expression and cellular processes through stabilization of some mRNAs and activation of the transcription factors NF- κ B and AP-1 [19]. Little is known about IL-1 signaling in keratinocytes and the involvement of IRAK1 has not previously been examined. Here we demonstrate for the first time that IL-1 regulates chemokine expression in keratinocytes through IRAK1 dependent pathways (Fig. 6 and Fig. 8). We also demonstrate to the best of our knowledge for the first time that IL-1 can enhance chemokine gene expression induced by TNF- α and IFN- γ (Fig. 4 and Fig. 5). Interestingly, the latter activity is, in part, IRAK1 independent. IRAK1 is required for IL-1 enhancement of TNF- α or IFN- γ induced CCL5 and CCL20 expression. However, IL-1 augmentation of IFN- γ driven CXCL9, CXCL10 and CXCL11 expression is independent of IRAK1 (Fig. 9). Our data suggest the presence of an important IL-1 signaling branch-point before IRAK1.

Expression of CXCL9, CXCL10 and CXCL11 is regulated via mRNA stabilization, NF- κ B, STAT1 and/or IFN-regulatory factor-1 (IRF-1) ([38,39] and refs. therein). Involvement of IRAK1 in NF- κ B activation induced by IL-1 is well established [19]. Apparently conflicting data has been obtained on the role of IRAK1 in mRNA stabilization. IRAK1 is required for IL-1 stabilization of the KC mRNA in HeLa cells [21], but appears not to be involved in IL-1 mediated stabilization of the IL-2 mRNA in the murine thymoma cell line EL4.NOB-1 [22]. It is possible that different mechanisms are involved in regulating stability of different chemokine and cytokine mRNA. Some of these mechanisms may be IRAK1 dependent and others IRAK1 independent. Furthermore, distinct cell types may regulate signaling pathways through different mechanisms. Previously we demonstrated that the 3' untranslated region of the IL-1RAcP mRNA confers mRNA instability in liver cells but not in kidney cells [40]. The

here reported IRAK1 independent IL-1 amplification of IFN- γ induced CXCL9, CXCL10 and CXCL11 expression may be mediated through IRAK1 independent IL-1 activated mRNA stabilization. An alternative scenario is that IL-1 enhances stabilization of the CXC chemokines through cross-talk with the IFN- γ signaling cascade. MyD88, an upstream activator of IRAK1 [19], has recently been demonstrated to also be involved in IFN- γ signaling in macrophages, specifically IFN- γ induced stabilization of the CXCL10 and TNF- α mRNAs [38]. We observed no effects upon IFN- γ alone or IFN- γ plus IL-1 β induced gene expression when IRAK1 activity was inhibited with a dominant negative mutant or RNAi mediated expression knockdown (Fig. 9). Given the involvement of MyD88 in both IL-1 and IFN- γ signaling [19,38] MyD88 activated by IL-1 may through an unknown mechanism enter or enhance the IFN- γ signaling cascade and thereby lead to amplification of the pathway. Further studies specifically designed to identify the mechanism(s) whereby IL-1 amplifies IFN- γ induced CXCL9, CXCL10 or CXCL11 expression in an IRAK1 independent manner will be required to definitively address these interesting hypotheses.

Psoriasis is a chronic inflammatory condition of the skin involving increased infiltration by leukocytes, primarily T cells, and hyperproliferating keratinocytes. Whether the condition is caused by a defect involving keratinocytes or T cells is a highly controversial subject. However, infiltration by T cells usually precedes hyperproliferation [1] and a recent study convincingly demonstrated that constitutive STAT3 activation in keratinocytes is sufficient to trigger a psoriasis-like condition in immunocompetent mice [10]. Furthermore, the latter observed phenotype is dependent upon T cells [10]. IL-1 β and IRAK1 are up-regulated in psoriatic skin [4–6,8,24–26] and the potential role of IL-1 in psoriasis pathology has recently regained the interest of the scientific community [8,9,11]. While early studies indicated that IL-1 β was inactive in psoriatic skin [41], later studies have found this to be incorrect [42,43]. The apparent inactivity of IL-1 β may have been due to co-purification of one or more of the many known IL-1 β antagonists and inhibitors many of which have only recently been identified ([32,44, 45] and refs. therein). A mechanism linking IL-1 and IRAK1 to T cell recruitment in skin inflammation and psoriasis has not been identified.

Expression of the CCL5, CCL20, CXCL9, CXCL10 and CXCL11 mRNAs is known to be up-regulated in actively inflamed skin from patients with psoriasis ([6,7,35,36] and refs. therein). Microarray studies have examined the remarkable correlation between IL-1 α induced changes in the keratinocyte transcriptome to the psoriatic skin expression profile. These latter studies did not observe IL-1 α dependent differential expression of chemokines [8,9]. However, we found that both CC and CXC chemokine mRNAs are rapidly up-regulated in a transient manner following either IL-1 α or IL-1 β treatment of both primary and HaCaT cells (Fig. 1–3 and data not shown). At the 24-hour time-point CCL5 and CCL20 mRNA levels are already significantly reduced compared to the peak induction observed at earlier time-points (Fig. 1–3). This transient expression pattern may explain why Mee *et al.* failed to identify an chemokine encoding genes as targets of IL-1 α mediated gene regulation [8,9]. An additional microarray based study of IL-1 α 's effect upon the keratinocyte transcriptome has been published [27]. This study identified CCL5, CCL20 and CXCL10 mRNA levels induced by IL-1 α alone in the array screening. However, these observations were not fully, if at all, validated by real-time RT-PCR or analyses of protein expression [27]. IL-1 α regulation of the CXCL10 mRNA was confirmed by real-time PCR but protein expression was not examined. CCL5 and CCL20 expression and interactions between IL-1 and IFN- γ signaling was not examined either [27]. Our study emphasizes the importance of validating data from microarray studies before conclusions are made. Expression levels of the CXCL10 mRNA were found to be constitutively high and not induced by IL-1 β in our microarray analyses (Fig. 1). However, RT-PCR analyses revealed that CXCL10 mRNA levels are in fact up-regulated by IL-1 in keratinocytes (Fig. 3). It is possible that the array analyses failed to identify differential expression in response to IL-1 due to saturation of probe targets on the array. Of even greater importance are our observations

that although IL-1 induced high levels of CXC chemokine mRNAs (Fig. 3) comparable to the levels of the CCL5 and CCL20 mRNAs (Fig. 1 and data not shown) CXC chemokines were not secreted at significant levels by keratinocytes treated with IL-1 alone. Analyses of the functional messenger(s), here secreted chemokines, are essential before conclusions, or new hypotheses, on involvement in physiological processes can be made. While chemokine mRNA expression is transient in our studies, chemokine protein levels continued to rise throughout our experiments. This difference likely reflects mRNA and protein specific stabilities, but is also suggestive of the potency of even transient changes in mRNA expression.

Our data demonstrate that IL-1 increases keratinocyte production of chemokines involved in T cell chemotaxis. Psoriasis lesions often occur in areas exposed to the most mechanical stress, e.g. lower back, elbows and knees [1]. Disruption of the skin barrier function through tape-stripping of the stratum corneum in mice leads to release of pre-formed pools of IL-1 α [46]. Based on our observations, the mechanisms whereby IL-1 contributes to psoriasis pathology may be as follows: In normal/unaffected skin mechanical injury to the skin releases IL-1 α into the surrounding tissue. This will stimulate CCL5 and CCL20 production by keratinocytes (Fig. 1–2) and subsequent T cell recruitment. In a healthy individual this may lead to sub-clinical inflammation which is rapidly resolved. In psoriasis patients, however, the inflammation becomes chronic due to one or more currently unknown defects in the immune response. It should be noted that spontaneous resolution of psoriasis lesions can occur. In the developing lesion IL-1 α and IRAK1 become up-regulated. IL-1 β , the activity of which is enhanced by the elevated IRAK1 levels, subsequently contributes to maintaining or worsening the inflammation through further chemokine production (Fig. 1–3) and hence T cell recruitment. The synergy between IL-1 β and IFN- γ and TNF- α (Fig. 4–5) leads to further amplification and/or sustained pro-inflammatory responses.

Our data suggest roles for IL-1 as both a trigger of new lesions and as an amplifier of ongoing inflammation. However, IL-1 is likely to have additional pathological effects in chronically inflamed skin as recent reports have shown wide IL-1 α effects upon the keratinocyte transcriptome after 24 hours [8,9,27]. Functional studies of IL-1 have previously demonstrated that it stimulates expression of keratinocyte growth factor [12] and keratinocyte proliferation [14–16]. This suggests a potential pathological involvement of IL-1 in the epidermal hyperplasia which follows T cell infiltration in psoriatic lesions.

IL-1 α and IL-1 β are generally believed to have similar biological functions, but differences have been reported ([47,48] and refs. therein). The unique activities of IL-1 α may be specifically related to its intracellular localization. Interestingly, *IL1A* and *IL1B* are differentially regulated in psoriatic skin. While expression of the IL-1 β mRNA is up-regulated [8,24–26], IL-1 α mRNA expression is often down-regulated [4,8,24,25]. Our studies indicate that extracellular IL-1 α and IL-1 β have the same effect upon chemokine expression by keratinocytes (data not shown). While IL-1 α may play a role in T cell recruitment in pre-psoriatic skin following extracellular release triggered by mechanical stress, IL-1 α may play a role in the maintenance and/or progression of inflamed lesions. Whether IL-1 β and extracellular and intracellular IL-1 α have similar and/or distinct functions in skin physiology and pathology remains to be determined.

CCL5 and its receptor CCR5 have been evaluated as novel targets for new therapies for psoriasis. Unfortunately, clinical trials have been unsuccessful [49]. Given the redundancy in T cell targeting chemokines produced by activated keratinocytes (CCL5, CCL20, CXCL9, CXCL10 and CXCL11 as reported here), this outcome may not be surprising. Targeted neutralization of an upstream regulator may represent a more suitable strategy for the development of novel therapies. Inhibition of TNF- α has proven very successful at relieving psoriasis, yet, this approach leads to significant immune suppression with associated risks of

infections [50]. Treatment with etanercept (soluble TNF-R) leads to rapid and complete reduction in IL-1 β levels in psoriatic skin. This is followed by reduction in other cytokine levels, including CCL20, and numbers of T cells [26]. Inhibition of IL-1 or IRAK1 activity may represent suitable alternative targets for novel anti-inflammatory therapies with increased efficacy for conditions such as psoriasis.

Conclusions

This report demonstrates that both IL-1 α and IL-1 β can increase expression of CCL5 and CCL20 by keratinocytes. Furthermore, IL-1 can synergize with IFN- γ and TNF- γ and increase production of CCL5, CCL20, CXCL9, CXCL10 and CXCL11 by keratinocytes. IL-1 induced regulation of CCL5 and CCL20 is dependent upon IRAK1. In contrast IL-1 enhancement of IFN- γ signaling is IRAK1 independent. The data imply that the IL-1 signaling cascade branches before IRAK1 into pathways leading to IRAK1 dependent and independent regulatory mechanisms. Future studies may determine the mechanism(s) and pathway(s) involved in IL-1 enhancement of IFN- γ signaling. The involved pathways may play important roles in acute and chronic inflammation in the skin.

Acknowledgements

This work was in part supported by National Institutes of Health Grant AR053672, the Transdisciplinary Program in Translational Medicine and Therapeutics University of Pennsylvania, and a grant from the American Heart Association.

Abbreviations

ABCF1	ATP-binding cassette sub-family F (GCN20) member 1
ACTB	beta-actin
AS	artificial sequence
AS-R	artificial sequence related
B2M	beta-2-microglobulin
BAS	biotinylated artificial sequence
BCL6	B-cell CLL
BLR1	Burkitt lymphoma 6
CC	Cys-Cys chemokine
CRP	C-reactive protein
CXC	

	Cys-X-Cys chemokine
EDG3	endothelial differentiation sphingolipid G-protein-coupled receptor 3
HSP90AB1	heat shock 90kDa protein 1 beta
ICEBERG	ICEBERG caspase-1 inhibitor
IL	interleukin
IL-1RI	IL-1 receptor type I
IL-1RAcP	IL-1 receptor accessory protein
IL-1RA	IL-1 receptor antagonist
IL-R	interleukin receptor
IL6ST	IL-6 signal transducer
LTA	lymphotoxin alpha
LTB	lymphotoxin beta
LTB4R	leukotriene B4 receptor
MIF	macrophage migration inhibitory factor
PF4	platelet factor 4
PUC18	pUC18 plasmid DNA
RPS27A	ribosomal protein S27a
SCYE1	small inducible cytokine subfamily E member 1
SPP1	secreted phosphoprotein 1
TAK1	transforming growth factor- β activated protein kinase 1

TNF	tumor necrosis factor
TNFRSF1A	TNF receptor superfamily member 1A
TNFRSF1B	TNF receptor superfamily member 1B
TRAF6	TNF receptor associated factor-6
TOLLIP	Toll-like receptor interacting protein
XCL1	chemokine (C motif) ligand 1
XCR1	chemokine (C motif) receptor 1

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Fig. 1. IL-1 β induces expression of CC and CXC chemokine mRNAs. Hybridization of biotin-16-UTP labeled cRNA to the Oligo GEArray Human Inflammatory Cytokines & Receptors Microarray (SuperArray Bioscience Corp.) was performed. The cRNA samples were derived from primary keratinocytes treated with medium only (*left panels*) or 10 ng/ml IL-1 β (*right panels*) for 6 hours. Positions of the CCL5 and CCL20 chemokine cRNA probe targets are indicated with arrows aiming to the right (upper CCL20, lower CCL5). The CXCL9, CXCL10 and CXCL11 targets are indicated with arrows aiming left (strong signal: CXCL10, weak signals: lower CXCL9, upper CXCL11). The full panel of examined genes involved from left to right and top to bottom: RPS27A, ABCF1, BCL6, BLR1, C3, C4A, C5, CCL1, CCL11,

CCL13, CCL15, CCL16, CCL17, CCL18, CCL19, CCL2, CCL20, CCL21, CCL23, CCL24, CCL25, CCL26, CCL3, CCL4, CCL5, CCL7, CCL8, CCR1, CCR2, CCR3, CCR4, CCR5, CCR6, CCR7, CCR8, CCR9, C/EBP-B, CRP, CX3CL1, CX3CR1, CXCL1, CXCL10, CXCL11, CXCL12, CXCL13, CXCL14, CXCL2, CXCL3, CXCL5, CXCL6, CXCL9, CXCR4, EDG3, ICEBERG, IFNA2, IFNG, IL10, IL10RA, IL10RB, IL11, IL11RA, IL12A, IL12B, IL12RB1, IL12RB2, IL13, IL13RA1, IL13RA2, IL15, IL15RA, IL16, IL17A, IL17C, IL17RA, IL18, IL18R1, IL1A, IL1B, IL1R1, IL1R2, IL1RN, IL2, IL20, IL21, IL22, IL2RA, IL2RB, IL2RG, IL3, IL4, IL5, IL5RA, IL6, IL6R, IL6ST, IL8, IL8RA, IL8RB, IL9, IL9R, LTA, LTB, LTB4R, MIF, PF4, SCYE1, SPP1, TNF, TNFRSF1A, TNFRSF1B, CD40L, TOLLIP, XCL1, XCR1, PUC18, Blank, Blank, AS1R2, AS1R1, AS1, GAPDH, B2M, HSP90AB1, HSP90AB1, ACTB, ACTB, BAS2C, BAS2C.

Fig. 2.

Levels of CCL5 and CCL20 mRNA and protein expression are time and IL-1 concentration dependent. Primary keratinocytes (A and C) and HaCaT cells (B and D) were treated with medium only or increasing concentrations of IL-1 β as indicated. Total RNA and culture medium were collected after 1.5, 3, 6, 9 and 24 hours. CCL5 and CCL20 mRNA levels were determined using real-time RT-PCR and the comparative C_T method (A and B). CCL5 and CCL20 mRNA levels were normalized against GAPDH mRNA levels and are graphically represented as fold change in comparison to cells treated with medium only. CCL5 and CCL20 protein levels in the culture medium were determined using ELISA (C and D). Protein levels are graphically represented as fold change in comparison to cells treated with medium only. # Not detected.

Fig. 3.

IL-1 up-regulates CXCL9, CXCL10 and CXCL11 mRNA levels in concentration and time dependent manners. Primary keratinocytes (A) and HaCaT cells (B) were treated with medium only or increasing concentrations of IL-1 β as indicated. Total RNA was collected after 1.5, 3, 6, 9 and 24 hours. The CXC chemokine CXCL9, CXCL10 and CXCL11 mRNA levels were determined using real-time RT-PCR and the comparative C_T method. CXC chemokine mRNA levels were normalized against GAPDH mRNA levels and are graphically represented as fold change in comparison to cells treated with medium only. * $p < 0.05$ compared to cells treated with medium only at the same time-point. ** $p < 0.01$ compared to cells treated with medium only at the same time-point.

Fig. 4.

IL-1 synergy with IFN- γ and TNF- α enhances CCL5 and CCL20 production. Primary keratinocytes were treated with medium only, 10 ng/ml IL-1 β , 50 ng/ml TNF- α and/or 20 ng/ml IFN- γ for 6 hours. Total RNA and culture medium was collected and expression of CCL5 (open bars) and CCL20 (filled bars) mRNA (A) and protein (B) examined using real-time RT-PCR and ELISA. Expression levels are graphically represented as fold change in comparison to cells treated with medium only. * $p < 0.05$ compared to cells treated with medium only. ** $p < 0.01$ compared to cells treated with medium only. # $p < 0.05$ compared to cells treated with individual cytokine only. ## $p < 0.01$ compared to cells treated with individual cytokine only.

Fig. 5.

IL-1 synergizes differentially with IFN- γ and TNF- α to enhance CXC chemokine expression. Primary keratinocytes were treated with medium only, 10 ng/ml IL-1 β and/or 20 ng/ml IFN- γ (A) or 50 ng/ml TNF- α (B) for 6 hours. Total RNA was collected and expression of CXCL9 (open bars), CXCL10 (gray bars) and CXCL11 (black bars) mRNA examined using real-time RT-PCR. Expression levels are graphically represented as fold change in comparison to cells treated with medium only. * $p < 0.05$ compared to cells treated with medium only. ** $p < 0.01$ compared to cells treated with medium only. # $p < 0.05$ compared to cells treated with individual cytokine only. ## $p < 0.01$ compared to cells treated with individual cytokine only.

Fig. 6.

Over-expression of IRAK1 leads to elevated CC chemokine secretion. HaCaT cells were transfected with either empty (open bars) or IRAK1 (filled bars) expression constructs. Geneticin selected cells were treated with medium only, 10 ng/ml IL-1 β , 50 ng/ml TNF- α or 20 ng/ml IFN- γ for 24 hours. Secretion of CCL5 (A) and CCL20 (B) was determined by ELISA. Expression levels are graphically represented as fold change in comparison to cells transfected with the empty expression construct and treated with medium only. * $p < 0.05$ compared to control cells transfected with empty vector and receiving the same treatment. ** $p < 0.01$ compared to control cells transfected with empty vector and receiving the same treatment.

Fig. 7.

RNAi mediated down-regulation of IRAK1 expression. HaCaT cells were transfected with expression constructs encoding a scrambled negative control shRNA sequence or an IRAK1 specific shRNA. Geneticin selected cells were treated with medium only (Med) or 10 ng/ml IL-1 β . After 24 hours cells were lysed in PBS with 2 M urea and 0.85% SDS and proteins examined by Western blotting. IRAK1 and GAPDH were detected using polyclonal anti-IRAK1 and anti-GAPDH rabbit antibodies, respectively.

Fig. 8.

IRAK1 is required for CC chemokine secretion. (A and B), HaCaT cells were transfected with expression constructs encoding a scrambled negative control shRNA sequence (open bars) or IRAK1 specific shRNA (filled bars). (C and D), cells were transfected with either an empty (open bars) or IRAK1(1-217) (filled bars) expression construct. Geneticin selected cells were treated with medium only, 10 ng/ml IL-1 β or 50 ng/ml TNF- α for 24 hours. Secretion of CCL5 (A and C) and CCL20 (B and D) was determined by ELISA. Expression of CCL5 and CCL20 in the control cells (open bars, scrambled shRNA (A and B) or empty vector (C and D)) was defined as 100 pct. Expression of CCL5 and CCL20 in the IRAK1 targeted cells (filled bars, IRAK1 specific shRNA (A and B) or IRAK1(1-217) expression vector (C and D)) is graphically represented relative to the control cells. * $p < 0.05$ compared to control cells transfected with scrambled shRNA plasmid (A and B) or empty vector (C and D) and receiving the same treatment.

Fig. 9.

IL-1 β enhancement of IFN- γ induced CXC chemokine expression is IRAK1 independent. **A**, HaCaT cells were transfected with expression constructs encoding a scrambled negative control shRNA sequence (open bars) or IRAK1 specific shRNA (filled bars). **B**, cells were transfected with either an empty (open bars) or IRAK1(1-217) (filled bars) expression construct. Geneticin selected cells were treated with 20 ng/ml IFN- γ or 20 ng/ml IFN- γ plus 10 ng/ml IL-1 β for 2 hours. Expression of CXC chemokine mRNAs was examined using real-time RT-PCR. Expression of the individual CXC chemokine mRNA in response to co-treatment with IFN- β + IL-1 β is graphically represented relative to expression induced by IFN- γ alone. * $p < 0.05$ compared to cells transfected with the same vector and treated with IFN- γ only. ** $p < 0.01$ compared to cells transfected with the same vector and treated with IFN- γ only. # $p < 0.05$ compared to control cells transfected with empty vector and receiving the same treatment.

Table 1Genes up-regulated by IL-1 β

GenBank	Gene	Description	Fold increase
NM_000064	C3	Complement component 3	1.40
NM_002982	CCL2	Chemokine (CC motif) ligand 2	1.41
NM_004591	CCL20	Chemokine (CC motif) ligand 20	4.67
NM_002985	CCL5	Chemokine (CC motif) ligand 5	5.30
NM_006273	CCL7	Chemokine (CC motif) ligand 7	1.61
NM_001511	CXCL1	Chemokine (CXC motif) ligand 1	4.37
NM_002089	CXCL2	Chemokine (CXC motif) ligand 2	3.43
NM_002189	IL15RA	Interleukin 15 receptor α	1.67
NM_000575	IL1A	Interleukin 1 α	1.48
NM_000576	IL1B	Interleukin 1 β	1.94
NM_004633	IL1R2	Interleukin 1 receptor, type II	2.50
NM_000577	IL1RN	Interleukin 1 receptor antagonist	2.35
NM_000600	IL6	Interleukin 6	2.48
NM_000594	TNF	Tumor necrosis factor- α	1.48