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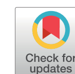
Keratinocyte-specific deletion of the IL-6RA exacerbates the inflammatory response during irritant contact dermatitis



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ABSTRACT

Irritant Contact Dermatitis (ICD) is the most common occupational skin disorder. During ICD, keratinocytes initiate the inflammatory cascade by producing cytokines including IL-6. This laboratory previously reported that IL-6 deficiency exacerbates skin inflammation during ICD, yet the role of the IL-6Rα in keratinocyte function has yet to be elucidated. To investigate how IL-6Rα function in keratinocytes influences the inflammatory response during ICD, keratinocyte-specific IL-6Rα KO (IL6ra$^{\Delta ker}$) and WT mice were exposed to two well-known occupational irritants; JP-8 jet fuel, and benzalkonium chloride (BKC), or acetone control for three days. Dermatitis lesions were collected and flow cytometric and immunohistochemical analyses revealed that IL6ra$^{\Delta ker}$ skin displayed increased populations of CD11b^{+}CD45^{+} and F4/80^{+} cells respectively relative to WT. However, IL6ra$^{\Delta ker}$ mouse skin contained reduced numbers of γδ T cells relative to WT. Furthermore, IL6ra$^{\Delta ker}$ skin expressed increased levels of pro-inflammatory cytokines including IL-1β, IL-22, and CCL4 but decreased levels of anti-inflammatory cytokines IL-4 and IL-10. These results indicate that epidermal keratinocyte IL-6Rα function modulates epidermal hyperplasia, immune cell infiltration into skin and cytokine expression during ICD and suggests that the previously reported protective effect of IL-6 during ICD might be mediated primarily by keratinocyte derived IL-6Rα.

1. Introduction

Contact dermatitis is a very common skin condition that can greatly affect the quality of life of affected individuals (Ale and Maibach, 2010; Lee et al., 2013a). CD is a highly prevalent disease with relevant socioeconomic impact; representing more than 90% of reported occupational disorders (Mathias, 1989). CD can be classified as allergic contact dermatitis (ACD) or irritant contact dermatitis (ICD) depending on whether it is of immunological origin or initiated by physical/chemical/mechanical damage, respectively. ICD is the most common form of CD and accounts for approximately 80% of cases (Sasseville, 2008). The pathophysiology of ICD involves epidermal barrier disruption, cellular changes in the epidermis and inflammatory cytokine release (Berardesca and Distante, 1995; Smith, Basketter et al. 2002; Lisby and Baadsgaard, 2006). The inflammatory response that occurs following irritant exposure leads to an activation of the skin immune system independent of antigen presentation (Gober and Gaspari, 2008). The extent of epidermal barrier disruption and inflammatory cell influx varies depending on the nature of the irritant and host factors (Ale and Maibach, 2010; Lee et al., 2013b).

IL-6 is a pleiotropic cytokine with well-delineated pro-inflammatory, anti-inflammatory and non-immune functions in biological tissues (Scheller et al., 2011; Tanaka, Narazaki et al. 2014; Rose-John, 2015). Numerous cell types including keratinocytes and fibroblasts as well as hematopoietic cells such as macrophages, neutrophils and T cells can produce IL-6 (Tanaka, Narazaki et al. 2014; Rose-John, 2015). The IL-6 signaling complex consists of IL-6, the non-signaling IL-6Rα, and the ubiquitously expressed gp130 subunit, which serves as the signal transduction component (Tanaka, Narazaki et al. 2014). On the cell, IL-6: IL-6Rα complex binds to two complexes of gp130 to form a hexamer initiating signal transduction which includes activation of JAK/STAT3, ERK and PI3K signal transduction pathways (Rose-John, 2015). Unlike gp130, membrane bound IL-6Rα is expressed at high levels by few cells including keratinocytes, megakaryocytes, hepatocytes and immune cells such as monocytes, macrophages and T cells (Yoshizaki, Nishimoto et al. 1990; Rose-John, Scheller et al. 2006). IL-6 can also affect cells which do not express appreciable levels of membrane bound IL-6Rα via the cleaved soluble IL-6Rα (sIL-6Rα). In humans sIL-6Rα is obtained through proteolytic cleavage of the membrane-bound form by ADAM 17 (Mülberg, Schooltink et al. 1993) or

Abbreviations: IL6ra$^{\Delta ker}$, keratinocyte-specific IL-6Rα knockout; BKC, Benzalkonium chloride; JP-8, Jet propellant 8 fuel; γδ T, gamma delta T cell

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alternative splicing (Müller-newen, Köhne et al. 1996). The occurrence of membrane-bound and soluble versions of IL-6Rα allow for two signaling pathways to exist for IL-6: Classical signaling via the membrane bound IL-6Rα, and trans-signaling via the sIL-6Rα. Classical signaling is said to be responsible for the anti-inflammatory effects of IL-6 whilst trans-signaling mediates the pro-inflammatory effects of IL-6 (Scheller et al., 2011).

IL-6 has also been shown to be essential for skin homeostasis and healing, where IL-6 knockout (IL-6KO) mice show delayed wound healing characterized by impaired re-epithelization, granulation tissue formation, angiogenesis, and macrophage infiltration (Gallucci, Simeonova et al. 2000; McFarland-Mancini, Funk et al. 2010). During ICD, IL-6 is predominantly produced by keratinocytes and has been shown to influence the pathogenesis of this type of dermatitis (Gröne, 2002; Bae, Shim et al. 2010; Lee et al., 2013b). Indeed, IL-6KO mice experience pronounced dermatoses characterized by increased epidermal thickening and immune cell infiltration relative to wild type following exposure to irritants (Lee et al., 2013a). However, how IL-6Rα function in epidermal keratinocytes influences skin toxicity during ICD is unknown.

Keratinocytes are the primary cell type in the epidermis and play a predominant role in skin inflammation (Mckenzie and Sauder, 1990; Nickoloff and Naidu, 1994). Keratinocytes produce IL-6 and express the membrane-bound IL-6Rα and thus can respond through the classical pathway (Yoshizaki et al., 1990). Following irritant exposure, keratinocytes release not only IL-6 but a host of other pro-inflammatory cytokines including IL-1α, IL-1β, TNF-α which can influence the infiltration of immune cells into the epidermis and dermis (Wood, Elias et al. 1996; Gröne, 2002; Lee et al., 2013b). These cytokines also have consequences on epidermal barrier function, and keratinocyte proliferation and differentiation (Hänel et al., 2013).

Therefore, it was sought to investigate how IL-6Rα function in these cells could influence the inflammatory response during ICD. To this end, mice with a keratinocyte-specific conditional knockout of the IL-6Rα were generated. Herein, it is reported that ablation of IL-6Rα in epidermal keratinocytes exacerbated skin toxicity following irritant exposure. This was characterized by increased pro-inflammatory leukocyte infiltration into skin, epidermal hyperplasia and greatly altered inflammatory cytokine expression after irritant exposure in knockout relative to littermate control mice. These findings provide novel insights into the role of IL-6Rα function in epidermal keratinocytes and how this can influence skin toxicity following exposure to occupational irritants. Evaluation of the association between IL-6Rα function in epidermal keratinocytes and how this impacts individual susceptibility to ICD could lead to better risk assessment of high risk populations.

2. Methods

2.1. Generation of mice with a keratinocyte-specific knockout of the IL-6Rα

Mice were generated with a keratin 14 conditional knockout of IL-6Rα in epidermal keratinocytes as homozygous genotype (IL6ra$^{fl/fl}$ K14-Cre$^{Tg/wt}$; IL6ra$^{\Delta ker}$). Littermates homozygous for loxP-flanked Il6ra alleles but lacking the K14-Cre transgene (IL6ra$^{fl/fl}$; called WT here) were used as control. Tamoxifen-inducible keratin 14 Cre-mediated recombination deleted exons 4–6 of the IL-6Rα gene in basal keratinocytes (Gierut, Jacks et al. 2014; Gunschmann, Chiticariu et al. 2014). Cre-mediated deletion of the IL-6Rα gene was confirmed by PCR analysis of genomic DNA extracted from mouse tails and knockout efficiency was characterized by RT-PCR of whole skin. All animal experiments were conducted in accordance with protocols approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Oklahoma Health Sciences Center.

2.2. Dermal irritant exposure

Mice at the age of 6–8 weeks were used for all experiments. A murine model of ICD was utilized as previously described (Gallucci et al., 2004; Lee et al., 2013a). Mice were sedated with Isoflurane and ˜5 cm of hair was removed via shaving on the dorsal side of the animals 24 h prior to initial irritant exposure. 50 µl of 2% benzalkonium chloride (BKC) (Sigma), filtered Jet Propellant 8 (JP-8) fuel ((Air Force Research Laboratory (AFRL/HEPB), Wright-Patterson Air Force Base and the Air Force Office for Scientific Research (AFOSR)), or acetone control was applied to the shaved back skin of mice daily for 2 h. This exposure was repeated for three (3) consecutive days. Mice were euthanized 24 h after the last day of irritant exposure and lesional skin was harvested and used for histological, multiplex or flow cytometric analysis.

2.3. Skin histology and immunohistochemistry

Cryosections were prepared as 8 mm skin cross-sections and Hematoxylin and Eosin (H&E) stained. Digital images of the skin histopathology were acquired with a Leica 4000b microscope (Leica Microsystems, Buffalo Grove, IL). Analysis of epidermal thickness was conducted with ImageJ (NIH). Immunohistochemistry sections were fixed with 4% paraformaldehyde and stained essentially as previously described (Lee et al., 2013a). Bound primary antibody (F4/80, clone BM8, BioLegend, San Diego, CA) were detected by incubation with Alexa Fluo 488 conjugated secondary antibody (Invitrogen), followed by counterstaining with DAPI (Vector Labs, Burlingame, CA).

2.4. Flow cytometry

After irritant exposure, single cell suspensions of immune cells were isolated from pooled skin samples (4 mice per group) as previously described (Calhoun, Luckett-Chastain et al. 2018). Samples were blocked with anti-mouse CD16/CD32 (BioLegend, San Diego, CA) and stained with conjugated antibodies for 30 min at 4 ° C protected from light. Fluorochrome-conjugated antibodies: anti-mouse monoclonal antibodies to APC CD45 (30-F11), PE CD11b (M1/70), Alexa Fluor® 488 F4/80 (BM8), APC γδ TCR (GL3) and Alexa Fluor 488 CD3 (17A2) were purchased from BioLegend (San Diego, CA). Data were acquired from 10,000 gated-events on Stratedigm S100EXi flow cytometer and analyzed using FlowJo_V10 software.

2.5. Multiplex immunoasssays

Total protein from lesional skin was prepared by homogenizing skin samples in PBS (10 mM sodium phosphate, 150 mM NaCl, pH 7.3) containing protease inhibitor cocktail (#P8340 Sigma) as previously described (Calhoun, Luckett-Chastain et al. 2018). The expression of inflammatory cytokines and chemokines were determined by a commercially available multiplex immunoassay (Product Number #EPX360-26092-901; eBioscience). Data are presented relative to total protein determined by a Bradford assay (BioRad, Hercules, CA).

2.6. Statistical analysis

All experiments were replicated at least three times and representative findings are shown. Statistical significance between groups was determined by two-way ANOVA followed by Tukey's multiple comparison test. P values of 0.05 or less were considered statistically significant.

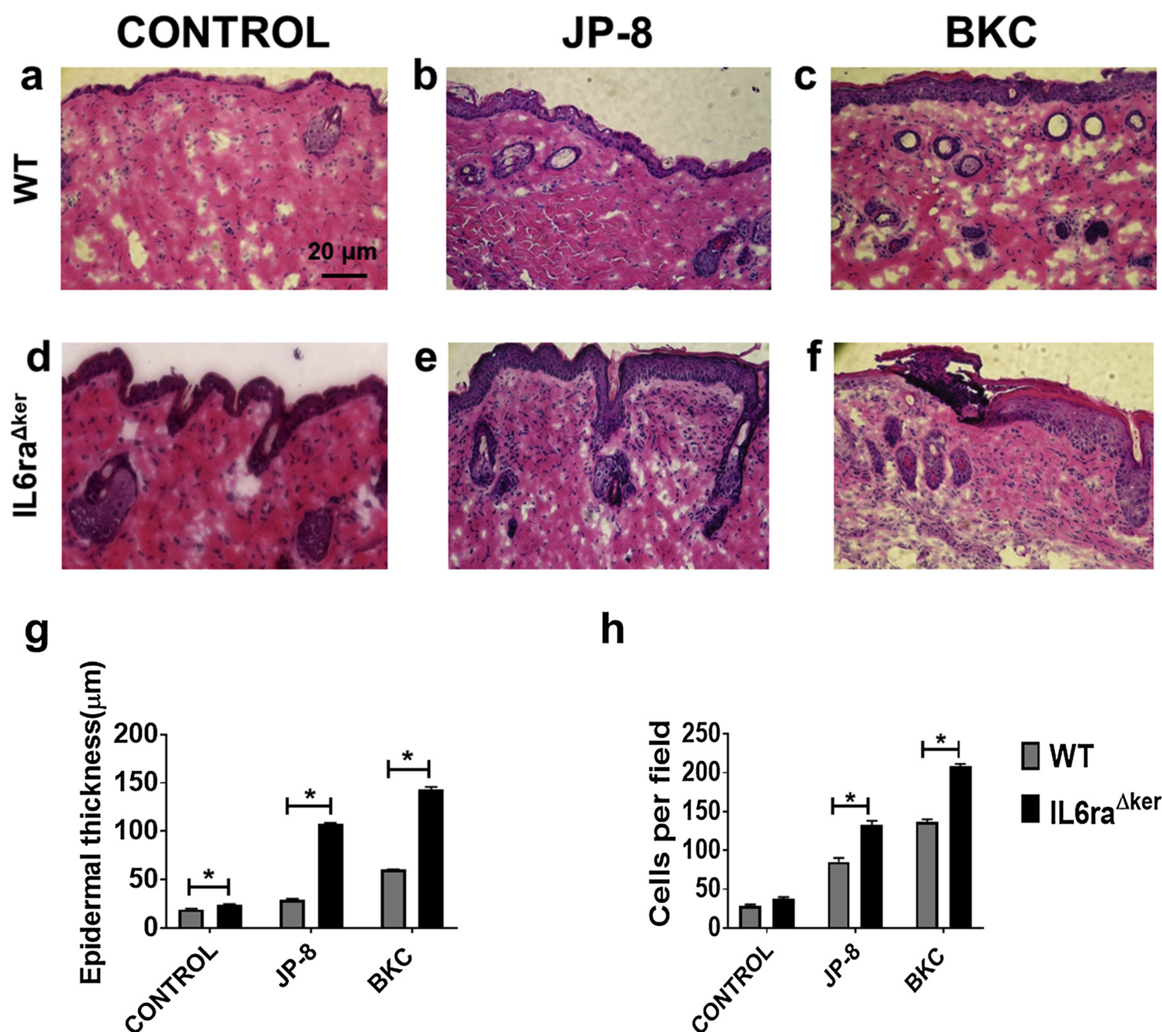

Fig. 1. Loss of IL-6Rα in epidermal keratinocytes leads to increased epidermal hyperplasia and immune cell infiltration during ICD.
WT and IL6ra$^{\Delta ker}$ mice were exposed to BKC, JP-8 or control for three (3) consecutive days to induce ICD. 24 h after irritant exposure, ˜8 mm biopsies of lesional skin were collected and embedded in O.C.T compound for histological analysis. Skin samples were cross-sectioned, and then Hematoxylin and eosin (H&E) stained. Representative H&E stains from WT (a–c) and IL6ra$^{\Delta ker}$ (d–f) are shown. Quantification of epidermal thickness (g) or cellular infiltration (h) as determined by Image J (NIH) is presented. Data is presented as mean ± SD (n = 15 mice per treatment per genotype). *P ≤ 0.05

3. Results

3.1. IL6ra$^{\Delta ker}$ mice show increased epidermal hyperplasia and immune cell infiltration following irritant exposure

Histopathology of ICD lesions show moderate epidermal thickening and infiltration by leukocytes (Krasteva et al., 1999, Eberhard et al., 2004), where the extent of epidermal hyperplasia and immune infiltration varies depending on the irritant and extent of exposure (Slodownik et al., 2008). To investigate how IL-6Rα influences epidermal hyperplasia and leukocyte infiltration during ICD, mice with a keratinocyte-specific knockout of the IL-6Rα (IL6ra$^{\Delta ker}$) and littermate control (WT) mice were exposed to benzalkonium chloride (BKC) and Jet Propellant 8 (JP-8) fuel, two well-characterized occupational irritants or acetone control for a period of three (3) consecutive days. Lesional skin was harvested from irritant-exposed mice and used for histological analysis as described (Lee et al., 2013a; Calhoun et al., 2018). Quantitative image analysis of Hematoxylin and eosin (H&E) stained lesional skin from mice revealed that IL6ra$^{\Delta ker}$ mice presented with an exaggerated response to BKC and JP-8 fuel relative to WT (Fig. 1a–f) characterized by increased epidermal hyperplasia (Fig. 1g), and infiltration of leukocytes cells into lesional skin (Fig. 1h). This occurrence was more pronounced following BKC exposure relative to JP-8 and control exposures (Fig. 1g, h black versus grey bars).

3.2. Loss of IL6Rα in epidermal keratinocytes alters immune cell infiltration into skin during ICD

Irritant exposure leads to the infiltration of leukocytes into skin (Willis, Young et al. 1986; Avnstorp, Ralfkiaer et al. 1987). Multiple immune cells including macrophages, neutrophils, dendritic cells and NK cells have been shown to migrate into lesional skin during ICD (Corsini and Galli, 2000; Calhoun et al., 2018). To investigate how IL-6Rα function in epidermal keratinocytes influences immune cell infiltration during ICD, immunohistochemistry and flow cytometry were employed. Immunohistochemistry revealed higher numbers of macrophages (F4/80) in both BKC and JP-8 exposed skin from IL6ra$^{\Delta ker}$ mice (Fig. 2a–f). Flow cytometric analysis of single cell suspensions from lesional skin also showed IL6ra$^{\Delta ker}$ mouse skin had higher numbers of macrophages (CD11b$^+$ F4/80$^+$ cells) and CD11b$^+$ CD45$^+$ cells relative to WT mice after both BKC and JP-8 exposures (Fig. 2g–m, Fig. 3a–h).

Flow cytometric analysis further revealed significantly lower numbers of γδ T cells (CD3$^+$ γδ TCR$^+$) in IL6ra$^{\Delta ker}$ mouse skin relative to WT after both BKC and JP-8 exposure (Fig. 4a–g).

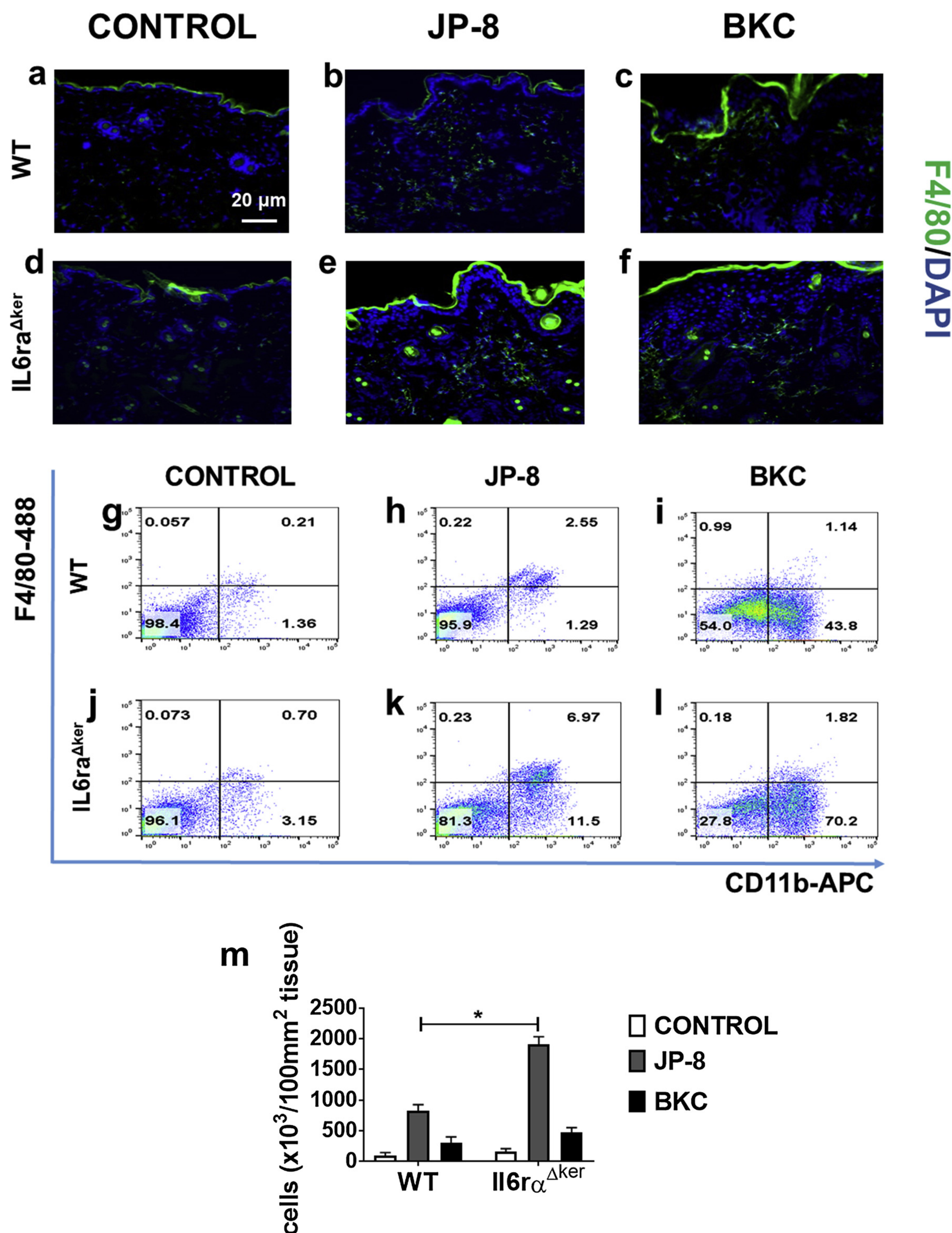

Fig. 2. Analysis of macrophage expression in irritant-exposed skin.
IL6ra$^{\Delta ker}$ and WT mice were exposed to BKC, JP-8 or control for 3 consecutive days. Sections of lesional skin from irritant-exposed mice were stained for the expression of F4/80 (green) and counterstained with DAPI. Representative fluorescent images are shown. WT (a–c), IL6ra$^{\Delta ker}$ (d–f). Lesional skin biopsies were obtained and the number of macrophages (CD11b$^+$F4/80$^+$) cells were determined with flow cytometry. Representative scatter plots of three independent experiments are shown. WT (g–i), IL6ra$^{\Delta ker}$ (j–l). Absolute cell counts of the different populations are shown (m). Data is presented as mean ± SD.*$P \leq 0.05$. (n = 4 mice per treatment per genotype). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

3.3. Irritants induce increased inflammatory cytokine and chemokine expression in IL6ra$^{\Delta ker}$ mouse skin

ICD is characterized by increased inflammatory cytokine/chemokine expression by keratinocytes and other cells (Frosch and John, 2006; de Jongh et al., 2007). Indeed the expression of pro-inflammatory cytokines IL-1α, IL-1β and TNF-α have been reported to be upregulated in lesional skin following exposure to chemical irritants (de

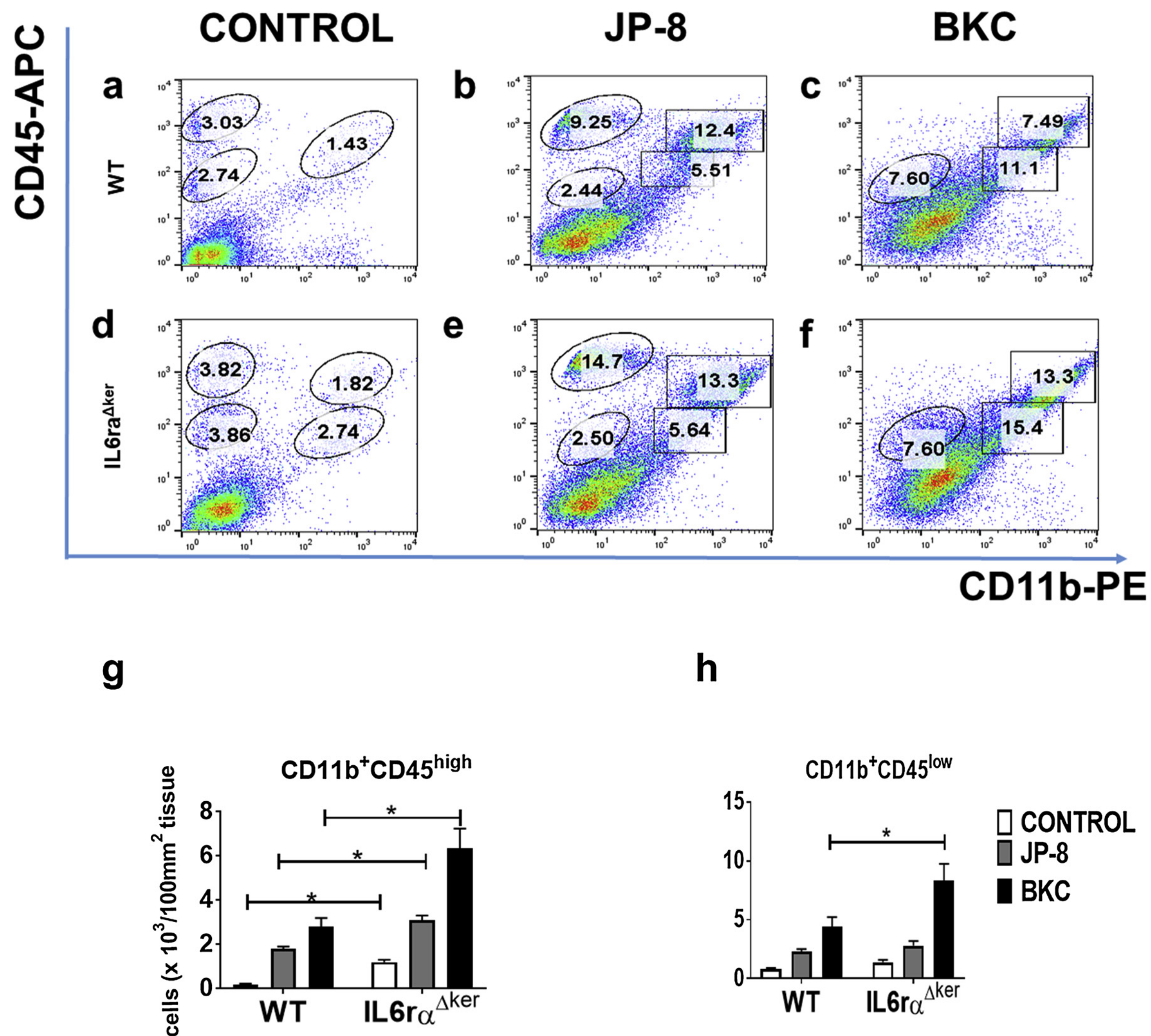

Fig. 3. IL-6Rα deficiency in epidermal keratinocytes alters infiltrating leukocyte composition during ICD.
ICD was established in WT and IL6ra$^{\Delta ker}$ mice using irritants as described. Lesional skin biopsies were obtained and the number of infiltrating monocytic leukocytes ($CD11b^+$ $CD45^+$) cells were determined with flow cytometry. Representative scatter plots of three independent experiments are shown. WT (a–c), IL6ra$^{\Delta ker}$ (d–f). Absolute cell counts of the different populations are shown. $CD11b^+$ $CD45^{high}$ (g), $CD11b^+$ $CD45^{low}$ (h). (n = 4 mice per treatment per genotype). Data presented as mean ± SD of percent positive. *$P \leq 0.05$

Jongh et al., 2007; Lee et al., 2013b). To determine what role IL-6Rα plays in influencing the inflammatory cytokine milieu, multiplex immunoasssays of skin lesion extract was performed (Fig. 5a). Protein expression data from BKC-exposed skin revealed higher levels of pro-inflammatory cytokines IL-1β, IL-1α, IL-6, and IL-22 in IL6ra$^{\Delta ker}$ mouse skin relative to WT (Fig. 5b–e black bars). Also, the expression of chemokine proteins CCL3-4, CXCL2 and CXCL10 were significantly higher in lesional skin from IL6ra$^{\Delta ker}$ mice relative to WT following BKC exposure (Fig. 5 f–i black bars). Conversely, following JP-8 exposure, only the pro-inflammatory cytokine IL-1α was significantly increased in IL6ra$^{\Delta ker}$ mice relative to WT (Fig. 5c grey bars).

Additionally, lesional skin from IL6ra$^{\Delta ker}$ mice showed a marked reduction in the expression of the anti-inflammatory cytokines, IL-10 and IL-4 following both JP-8 and BKC exposure (Fig. 5 j, k grey and black bars). Table 1 presents all the 36 cytokines/chemokines analyzed with multiplex immunoassay kit.

4. Discussion

Keratinocytes play an important role in the initiation of the inflammatory response during ICD. Disruption of the skin barrier by irritants leads to the release of inflammatory mediators by keratinocytes which in turn perpetuate the inflammatory response during ICD (Wood, Elias et al. 1996; Effendy, Löffler et al. 2000). Dermatitis lesions present with moderate epidermal thickening (acanthosis) and influx of immune cells (Krasteva, Kehren et al. 1999; Eberhard, Ortiz et al. 2004). Indeed, H&E stained histological sections of skin taken from 7 day JP-8 and BKC exposed skin showed thickened epidermis and increased immune cell infiltration relative to control (Gallucci et al., 2004). Previous studies have shown that IL-6 deficiency led to greater immune cell infiltration into lesional skin during ICD (Lee et al., 2013a). However, no study has examined what role IL-6Rα function in epidermal keratinocytes plays in modulating skin toxicity following exposure to common occupational irritants. In the present study, it is demonstrated that a conditional ablation of the IL-6Rα in epidermal keratinocytes increases susceptibility to ICD following irritant exposure. These results suggests the utility of assessing differential expression of IL-6Rα in populations with potential exposure as a method of risk assessment.

Macrophages are phagocytes that can assume a pro-inflammatory or anti-inflammatory phenotype. Lesional skin from IL6ra$^{\Delta ker}$ mice displayed significantly higher levels of F4/80 and $CD11b^+$ $F4/80^+$ expressing cells relative to WT (Fig. 2). It is also of interest to note that IL6ra$^{\Delta ker}$ mouse skin presented with significantly higher levels of the macrophage chemoattractants, CCL-3 and CCL-4 relative to WT (Fig.5).

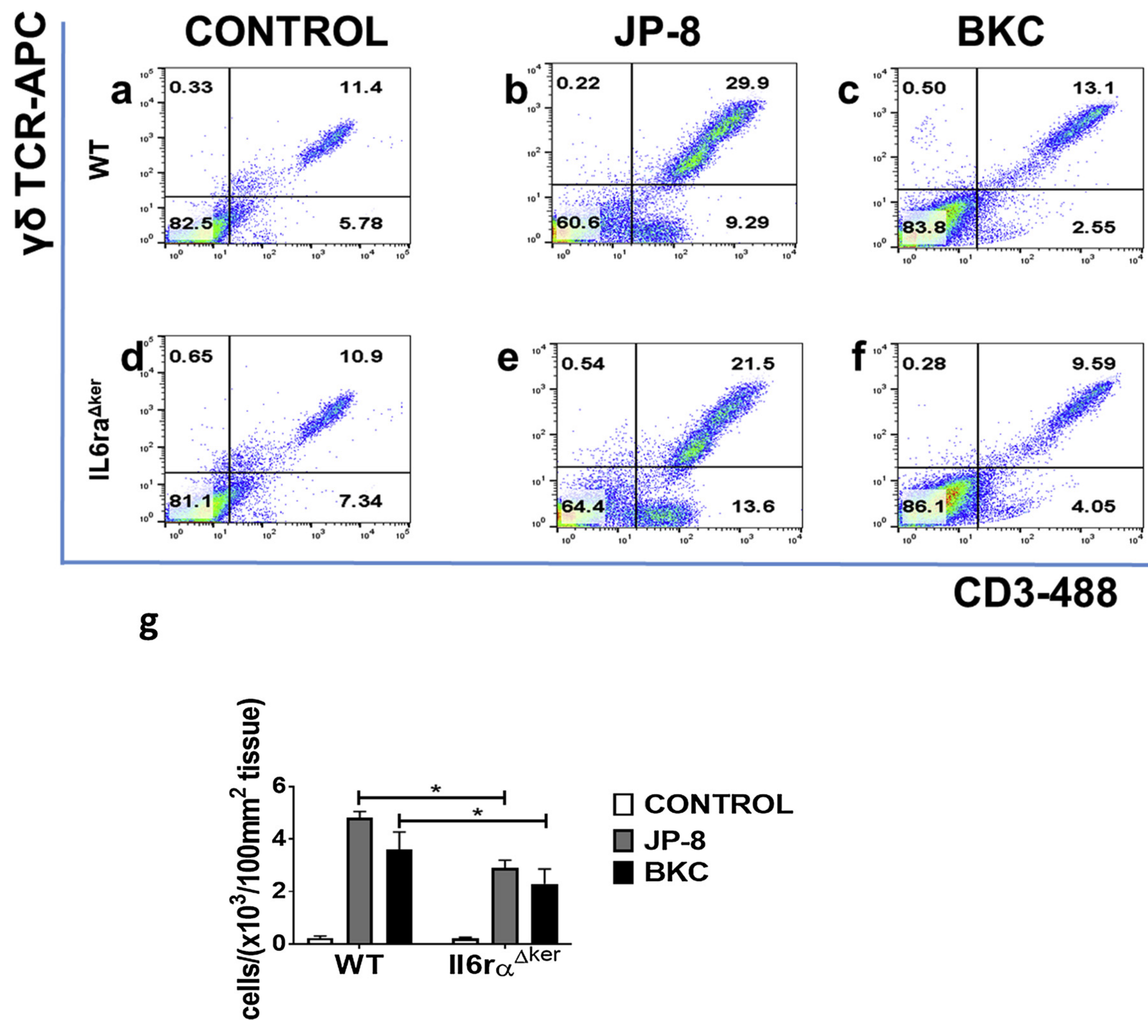

Fig. 4. IL-6Rα deficiency in epidermal keratinocytes modifies Gamma Delta (γδ) T cell population in irritant exposed skin.
WT and IL6ra$^{\Delta ker}$ mice were exposed to BKC, JP-8 or control for three (3) consecutive days to induce ICD. Lesional skin biopsies were collected from mice 24 h post irritant-exposure and the number of infiltrating γδ T cell populations were determined with flow cytometry. Representative scatter plots of three independent experiments are shown WT (a–c), IL6ra$^{\Delta ker}$ (d–f). Absolute cell counts of the different populations are shown (g). (n = 4 mice per treatment per genotype). Data presented as mean ± SD of percent positive. *P ≤ 0.05

Altered levels of CCL-3 and CCL-4 between IL6ra$^{\Delta ker}$ and WT skin correlated with alterations in the populations of macrophages in both skin types (Fig. 2). These results taken together suggest that IL-6Rα activation in epidermal keratinocytes functions to limit the release of CCL-3 and CCL-4 chemokines ultimately reducing the influx of macrophages into lesional skin. Macrophages are a source of pro-inflammatory cytokines including IL-1α, IL-1β, TNF-α during ICD (Smith, Basketter et al. 2002; Lee et al., 2013b). It was shown following BKC exposure, that lesional skin from IL6ra$^{\Delta ker}$ mice expressed significantly higher levels of pro-inflammatory cytokine proteins relative to WT (Fig. 5). Thus, an additional function of IL-6Rα in epidermal keratinocytes may be to limit the expression of pro-inflammatory cytokines by limiting the influx of macrophages. Furthermore, lower levels of the anti-inflammatory cytokines, IL-10 and IL-4, were observed in IL6ra$^{\Delta ker}$ mice relative to WT following JP-8 and BKC exposure suggesting that IL-6Rα function in epidermal keratinocytes promotes an anti-inflammatory response. When comparing irritants, it was observed that BKC led to a higher inflammatory response relative to JP8. This occurrence might be explained by the chemical composition of these two irritants. BKC is a cationic surfactant and has been shown to cause skin barrier disruption (Beltrani, 2003; Lee et al., 2013a). JP-8 on the other hand is a weak irritant known to cause relatively milder dermatosis (Lee et al., 2013a). These results affirms the conclusion that different chemicals use different inflammatory pathways to induce skin inflammation (Patrick, Burkhalter et al. 1987). It will be of interest to conduct these studies with other occupational irritants like sodium lauryl sulfate.

IL-6Rα deficiency in epidermal keratinocytes resulted in significantly higher number of cells expressing CD11b following both BKC and JP-8 exposures. Indeed, IL6ra$^{\Delta ker}$ mice presented with higher numbers of CD11b$^+$CD45$^+$ cells (Fig. 3) in lesional skin. It is of interest to note that different populations of CD11b$^+$CD45$^+$ cells existed across the BKC and JP-8 exposures for both mouse genotypes. Specifically, IL6ra$^{\Delta ker}$ mouse skin expressed higher numbers of CD11b$^+$CD45^{high} cells relative to WT following both BKC and JP-8 exposures. This cell type has been described as infiltrating peripheral leukocytes with a pro-inflammatory phenotype (Badie and Schartner, 2000). Additionally, CD11b$^+$CD45^{high} cells have been shown to produce high levels of pro-inflammatory cytokines such as TNF-α, IL-1α, IL-1β and IL-6 (Brandenburg, Turkowski et al. 2017). Thus, the apparent greater population of these cells may be associated with the high levels of pro-inflammatory cytokine expression that was observed in lesional skin from IL6ra$^{\Delta ker}$ mice relative to WT (Fig.5). Thus, it may be that the loss of the IL-6Rα in epidermal keratinocytes leads to increased influx of pro-inflammatory immune cells following irritant exposure. Moreover, IL6ra$^{\Delta ker}$ mouse skin displayed higher numbers of CD11b$^+$CD45^{low}

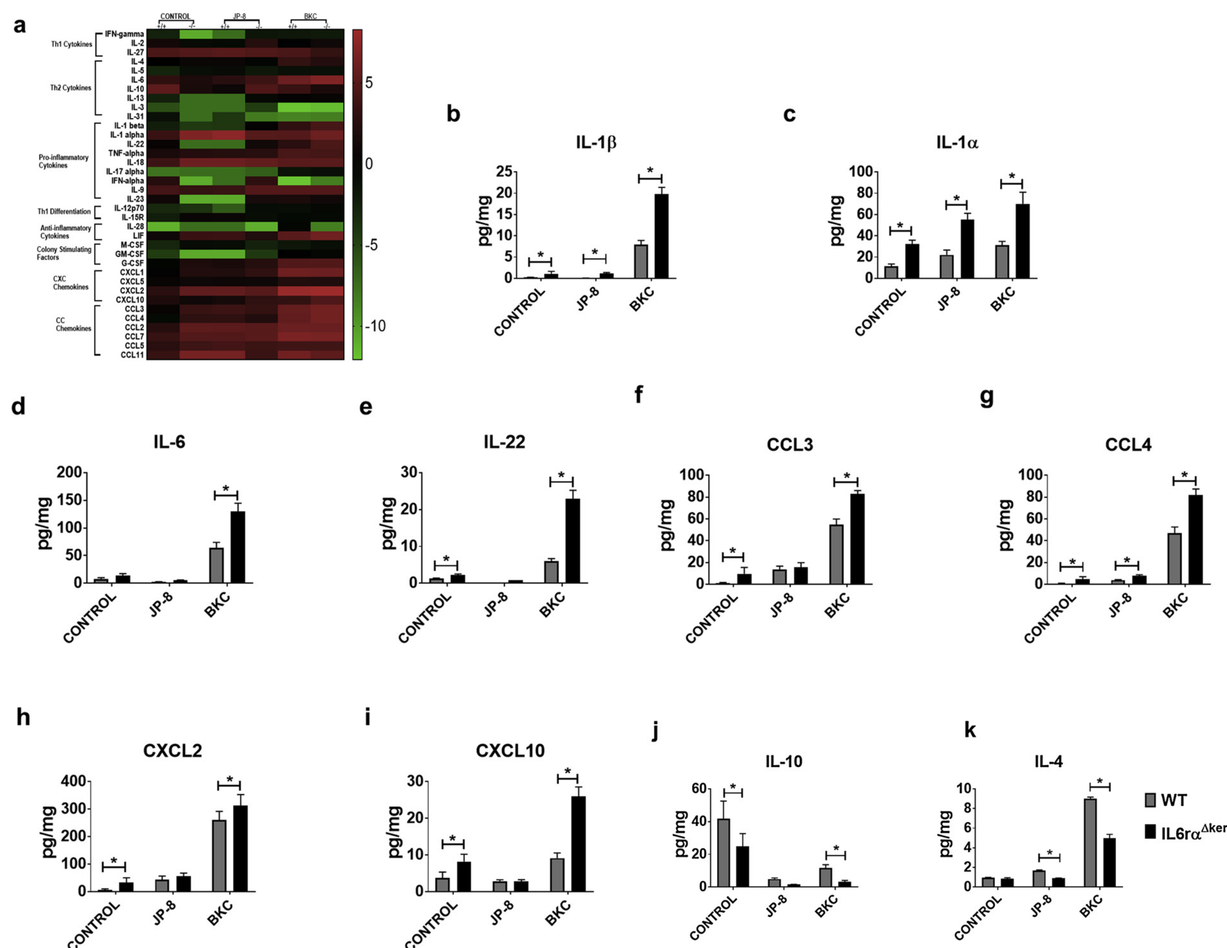

Fig. 5. Increased inflammatory cytokine and chemokine expression in IL6ra$^{\Delta ker}$ mouse skin.
IL6ra$^{\Delta ker}$ and WT mice were exposed to BKC, JP-8 or control for 3 consecutive days. Lesional skin was harvested from each genotype and inflammatory cytokine protein expression was determined by multiplex immunoassay (a). Lesional skin from Il6ra$^{\Delta ker}$ mice show increased expression of pro-inflammatory cytokines/ chemokines (b–i).Lesional skin from Il6ra$^{\Delta ker}$ mice show reduced expression of anti-inflammatory cytokines (j and k). Data is presented as mean ± SD (n = 15 per treatment group per genotype). *P ≤ 0.05. Wild type (WT, +/+), keratinocyte IL-6Rα KO (IL6ra$^{\Delta ker}$, -/-).

expressing cells after BKC exposure relative to WT (Fig. 3). Although little is known about these cells, it has been proposed that CD45^{low} /CD45^{medium} expressing cells are resident leukocytes in the brain where an upregulation of CD45 expression has been shown to correlate with leukocyte activation (Hermiston, Xu et al. 2003; Denker, Ji et al. 2007). This seems to indicate that IL-6Rα function influences both resident and infiltrating leukocyte population dynamics in skin during ICD.

Skin gamma delta (γδ) T cells, also known as Dendritic epidermal T cells (DETC) are a subset of T cells that have been identified in murine skin (Girardi, Oppenheim et al. 2001; Havran, 2018), and produce a variety of cytokines and chemokines to modulate immune function in the skin (Boismenu, Hobbs et al. 1996). Following BKC and JP-8 exposure, IL6ra$^{\Delta ker}$ mouse skin had decreased numbers of γδ T cells relative to WT (Fig. 4). Interestingly, mice lacking γδ T cells display severe defects in wound healing suggesting that these cells are involved in skin regeneration and repair (MacLeod, Hemmers et al., 2013). Thus, the reduced levels of γδ T cells in the skin of IL6ra$^{\Delta ker}$ mice could lead to worse skin toxicity following irritant exposure. Murine skin γδ T cells also induce the production of Th2 cytokines like IL-4 and IL10 (Malik, Want et al. 2016). IL6ra$^{\Delta ker}$ mouse skin presented with significantly reduced expression of the Th2 cytokines, IL-4 and IL-10, relative to WT suggesting that the reduced percentage of γδ TCR cells may be associated with the reduced expression of these cytokines in IL6ra$^{\Delta ker}$ skin further influencing the severity of the inflammatory response.

Recently, several studies have looked at genetic susceptibility to occupational contact dermatitis (Kezic, Visser et al., 2009). Indeed, studies on variations in the genes involved in epidermal barrier function have shown that polymorphisms in these genes could influence susceptibility to contact dermatitis. For example, patients with a variant TNFA-308A allele have increased susceptibility to ICD following irritant exposure relative to patients with the wild-type TNF-α allele (DeJongh et al., 2008). Based on the findings that IL-6Rα function in epidermal keratinocytes confers a protective effect to lesional skin during ICD, it is speculated that polymorphisms in the IL-6Rα gene could influence individual susceptibility to ICD. Since different single nucleotide polymorphisms (SNPs) of the IL-6Rα gene exist, it will be interesting to evaluate the association between these SNPs and individual susceptibility to occupational contact dermatitis.

5. Conclusion

In summary, this model reveals that a deficiency of IL-6Rα in epidermal keratinocytes increases the risk of developing severe inflammatory response after exposure to both acute delayed (BKC) and cumulative (JP-8) irritants. Indeed, a functional IL-6Rα in epidermal keratinocytes acts to limit the expression of pro-inflammatory cytokines, reduce immune cell infiltration into lesion skin and promote the expression of anti-inflammatory cytokines during ICD. This study has demonstrated the involvement of IL-6Rα in epidermal keratinocytes in modulating leukocyte infiltration and inflammatory cytokine

Table 1
IL-6Rα function in epidermal keratinocytes modulates cytokine and chemokine expression in lesional skin after irritant exposure.

CYTOKINE	WT ACETONE	KO ACETONE	WT JP8	KO JP8	WT BKC	KO BKC
IFN-gamma	0.160517979 ± 0.02	0.000566182 ± 0.06	0.005788819 ± 0.00003	0.332853297 ± 0.016	0.293313 ± 0.04	0.243080791 ± 0.03
IL-2	2.437015074 ± 1.25	0.605436564 ± 0.22	0.675082472 ± 0.09	5.25192379 ± 0.43	1.05047 ± 0.38	2.010723843 ± 0.45
IL-27	21.35969718 ± 5.78	28.83089328 ± 7.82	34.66932561 ± 5.67	27.24492699 ± 6.89	20.85105 ± 7.45	8.9087 ± 3.54
IL-4	0.97483 ± 0.01	1.602705457 ± 0.10	**1.605275885 ± 0.27**	**0.838404048 ± 0.15**	**8.9893 ± 0.19**	**4.98383 ± 0.40**
IL-5	0.138808272 ± 0.02	0.505614548 ± 0.05	0.600654371 ± 0.06	0.290783773 ± 0.07	0.441267 ± 0.07	0.435398652 ± 0.05
IL-6	7.852340756 ± 2.16	3.445534825 ± 2.60	6.06891997 ± 0.24	13.79688248 ± 0.89	**63.71107 ± 10.30**	**130.1314267 ± 14.86**
IL-10	**41.80688892 ± 10.82**	**3.188440153 ± 2.83**	1.704800078 ± 0.63	24.8465489 ± 0.36	**11.58629 ± 2.11**	**3.282852883 ± 0.74**
IL-13	0.168051391 ± 0.03	0.005661821 ± 0.0003	0.005788819 ± 0.002	0.707118458 ± 0.004	1.116336 ± 0.15	1.285594032 ± 0.20
IL-3	0.019310202 ± 0.001	0.005661821 ± 0.0002	0.005788819 ± 0.0007	0.043311764 ± 0.008	0.000234 ± 0.00008	0.000291144 ± 0.00004
IL-31	0.444933346 ± 0.05	0.005661821 ± 0.0003	0.056618211 ± 0.009	0.003411538 ± 0.00004	0.002342 ± 0.00004	0.002911444 ± 0.0005
IL-1 beta	**0.190586374 ± 0.06**	**0.050262558 ± 0.056**	**0.057888189 ± 0.004**	**1.090788408 ± 0.24**	**7.938752 ± 1.31**	**19.82422559 ± 1.58**
IL-1 alpha	**11.56784134 ± 2.00**	**126.7091334 ± 3.67**	**182.2929471 ± 7.56**	**32.19558578 ± 24.99**	**31.19045 ± 3.18**	**70.1571501 ± 10.90**
IL-22	**1.231273732 ± 0.15**	**0.005661821 ± 0.0032**	0.005788819 ± 0.0003	2.186546973 ± 0.04	**6.011907 ± 0.68**	**22.88001456 ± 2.38**
TNF-alpha	3.103414442 ± 0.59	4.978292739 ± 1.76	5.135250074 ± 0.55	5.930268622 ± 0.69	20.31726 ± 3.47	17.78713373 ± 2.98
IL-18	13.85077428 ± 1.74	66.46842016 ± 19.24	66.03116337 ± 7.25	49.49495518 ± 21.80	35.12718 ± 2.54	37.02777923 ± 3.29
IL-17 alpha	0.004362092 ± 0.0005	0.005661821 ± 0.005	0.005788819 ± 0.0003	0.009847724 ± 0.004	0.368331 ± 0.04	0.43810349 ± 0.06
IFN-alpha	5.478095124 ± 0.57	0.000566182 ± 0.0005	0.005788819 ± 0.004	7.703692991 ± 0.69	0.000234 ± 0.0001	0.002911444 ± 0.00002
IL-9	17.40005169 ± 4.82	9.105069454 ± 8.13	11.17195091 ± 1.59	29.79459286 ± 5.26	29.02828 ± 5.57	29.60068519 ± 11.67
IL-23	1.957295384 ± 0.18	0.000566182 ± 0.00004	0.000578882 ± 0.00003	2.884865562 ± 0.86	3.310275 ± 0.71	2.106256959 ± 0.39
IL-12p70	0.112404884 ± 0.02	0.07281548 ± 0.002	0.012403337 ± 0.03	0.582963821 ± 0.05	0.5305 ± 0.11	0.755659915 ± 0.17
IL-15R	0.191932032 ± 0.06	1.420207921 ± 0.23	1.607977332 ± 0.22	0.521929371 ± 0.25	0.74649 ± 0.05	0.648166777 ± 0.06
IL-28	0.000436209 ± 0.00005	0.005661821 ± 0.002	0.005788819 ± 0.003	0.000492386 ± 0.0003	0.848682 ± 0.23	0.002911444 ± 0.06
LIF	1.779231128 ± 0.17	11.01479846 ± 0.45	11.63393333 ± 0.51	4.122185553 ± 0.94	35.61094 ± 4.85	75.06152986 ± 9.49
M-CSF	0.132499453 ± 0.07	0.596432435 ± 0.05	0.550568387 ± 0.07	0.165054165 ± 0.08	0.301686 ± 0.04	0.50055519 ± 0.04
GM-CSF	0.043620916 ± 0.005	0.000578882 ± 0.0004	0.000566182 ± 0.0004	0.04923862 ± 0.01	0.932824 ± 0.14	0.801687289 ± 0.10
G-CSF	0.863803706 ± 0.10	3.91146599 ± 2.74	3.190381726 ± 0.90	5.293184982 ± 0.40	27.63619 ± 5.24	28.0767265 ± 5.69
CXCL1	1.012114788 ± 0.32	4.331610746 ± 4.15	4.18368809 ± 1.29	8.074185951 ± 0.78	73.18253 ± 14.96	70.11707442 ± 12.57
CXCL5	1.301909423 ± 0.18	1.843259041 ± 0.25	1.730053629 ± 0.08	1.904343468 ± 0.08	4.583139 ± 0.84	3.987626189 ± 0.81
CXCL2	**6.437280417 ± 3.75**	**45.86898441 ± 17.56**	48.84501411 ± 12.76	32.77859143 ± 8.60	**260.9162 ± 30.93**	**312.1665993 ± 40.66**
CXCL10	**3.740623204 ± 1.59**	**2.110674815 ± 1.99**	2.712509316 ± 0.48	8.216968318 ± 0.38	**9.145393 ± 1.41**	**26.02189329 ± 2.53**
CCL3	**1.231065576 ± 0.42**	**11.33248407 ± 5.87**	13.81253182 ± 2.98	9.6074242 ± 4.22	**54.80843 ± 5.06**	**82.97501705 ± 3.07**
CCL4	**0.638936619 ± 0.29**	**7.13279931 ± 2.53**	**7.803318094 ± 2.44**	**4.451031074 ± 2.28**	**46.83867 ± 5.70**	**81.94826384 ± 3.07**
CCL2	5.537508602 ± 1.35	41.31606776 ± 8.81	39.25375289 ± 6.72	32.64775281 ± 3.07	63.77459 ± 7.18	62.96559355 ± 7.49
CCL7	10.64544768 ± 1.63	27.58386681 ± 11.59	27.92751464 ± 5.91	35.98649279 ± 3.99	112.0832 ± 9.29	101.0117128 ± 13.61
CCL5	6.238693305 ± 1.33	14.30742023 ± 2.05	17.27686682 ± 2.02	11.97728945 ± 2.38	15.27458 ± 1.50	15.40099478 ± 1.48
CCL11	10.4207 ± 10.42	92.05021929 ± 30.29	89.3940307 ± 25.81	11.4758 ± 14.91	76.99657 ± 7.18	39.90745396 ± 4.68

Cytokine and chemokine expression for WT and IL-6Rα$^{\Delta ker}$ mice after 3-days of irritant exposure was determined by multiplex immunoassays (n = 15 per treatment per genotype). Data are shown as mean ± SD. Significant difference between genotypes (*$p < 0.05$) are highlighted in bold. WT = wild type, KO = IL-6Rα$^{\Delta ker}$. JP8 = Jet Propellant 8 fuel. BKC = benzalkonium chloride. Control = acetone.

expression and builds upon the previous knowledge on the protective effect of the IL-6/IL-6Rα axis during ICD. in vitro studies utilizing primary keratinocytes from IL-6KO and IL6ra$^{\Delta ker}$ mice will help better characterize the mechanism of this protective effect of the IL-6/IL-6Rα axis during ICD. This is the focus of ongoing research in this laboratory. The results presented herein calls for an evaluation of the association between IL-6Rα gene polymorphisms and susceptibility to ICD. These studies might find translation toward risk assessments in the human population.

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Conflict of interest

The authors declare no conflict of interest.

Author contribution

BF and RM conceived and designed experiments. BF and LC conducted experiments and analyzed data. KC developed flow cytometry protocol.BF wrote the manuscript. All authors reviewed and accepted text of manuscript.

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