Toxicology 410 (2018) 1–9

Contents lists available at ScienceDirect

Toxicology

journal homepage: www.elsevier.com/locate/toxicol

# Transcriptional profiling of irritant contact dermatitis (ICD) in a mouse model identifies specific patterns of gene expression and immune-regulation

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ARTICLE INFO

*Keywords:*
Skin
Irritant contact dermatitis (ICD)
Interleukin-6
RNAseq

ABSTRACT

*Background:* Irritant contact dermatitis (ICD) is a cutaneous inflammatory response to a variety of triggers that requires no sensitization and accounts for up to 80% of occupational dermatitis cases. IL-6 has been alternately associated with both allergic and irritant dermatitis and is closely linked to skin wound healing, therefore making it an ideal candidate to investigate in the mechanism of ICD.

*Results:* Despite being a well-known pro-inflammatory cytokine, IL-6 deficient (IL-6KO) mice show much more severe ICD than controls. Transcriptome analysis was employed to examine irritant-exposed and control skin samples from C57BL/6 and IL-6KO mice. Over 1900 transcripts were found differentially modulated between C57 (1184 total) and IL-6KO (802 total) mice with the magnitude of expression significantly disparate. Overall gene ontology revealed metabolic and cellular enriched functional processes but numerous pro-inflammatory and immune associated genes (*Cxcl2, Cxcl3, Cxcl5, Acod, Hamp, c-Lectins*, for example), keratin associated genes (Krt6b and various *Krtaps*), and members of the *Sprr and Lce* family, which promote skin barrier integrity and keratinocyte functions, were also differentially modulated.

*Conclusions:* The altered expression of these genes may provide a potential mechanism to explain the increased ICD severity in IL-6-deficient mice. Overall, this study offers new insight into the pathogenesis of ICD, indicates new mediators/biomarkers that may influence the variability of responses to irritants and provides potential targets for therapeutic development.

## 1. Introduction

Due to the large surface area directly exposed to the work environment, the skin is particularly susceptible to occupational injury. Skin-related complaints are prevalent in occupational illness, with atopic dermatitis being one of the most common inflammatory skin diseases (Coman et al., 2015). Over 3000 potential hazardous substances have been identified in the workplace, and exposure may result in allergic contact dermatitis (ACD) or irritant contact dermatitis (ICD) (Esaki et al., 2015). The exact number of ICD vs. ACD cases in work related dermatitis varies in the literature; however, it has been speculated that ICD accounts for 80% and the remaining 20% represent ACD (Belsito, 2005).

The major difference between ACD and ICD is often described as whether the disease is of immunological origin (allergic) where T cells are the primary source of inflammatory cytokines, or non-immunological origin (irritant) where physical damage is thought to be the initiating event. Irritant contact dermatitis is the result of activated innate immunity in response to a direct cytotoxic effect of a chemical/physical agent, and unlike ACD, requires no prior sensitization. ICD was previously thought of as a simplistic, non-specific reaction of the skin to an irritant. However, there is increasing evidence that indicates ICD is in fact a complex interplay of events that involves skin barrier disruption, cellular changes, and the release of numerous pro and anti-inflammatory mediators (Lee et al., 2013b; Effendy et al., 2000). Numerous factors (intrinsic or extrinsic) can influence the irritancy potential of a particular agent. Intrinsic factors comprise an individual's genetic predisposition, age, and sex, and body region, where extrinsic factors include the inherent nature of the irritant, exposure duration, concentration, and any other mechanical factors (Chew and Maibach,

*Abbreviations:* ICD, irritant contact dermatitis; ACD, allergic contact dermatitis; IL-6, interleukin-6; IL-6KO, interleukin-6 knock out; BKC, benzoalkoniumchloride; C57, C57 normal skin; C57-BKC, C57 BKC-treated; KO, IL-6 deficient normal skin; KO-BKC, IL-6 deficient BKC-treated

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https://doi.org/10.1016/j.tox.2018.08.014
Received 11 April 2018; Received in revised form 7 August 2018; Accepted 27 August 2018
Available online 29 August 2018
0300-483X/ 

2003; Slodownik et al., 2008).

The potential influence of irritants on gene expression associated with skin and inflammation has not been clearly defined for ICD. Of the cytokines implicated in inflammation, interleukin-6 (IL-6) has been alternately associated with both allergic and irritant dermatitis. In addition to its immune-modulatory activities, IL-6 is involved in the growth and differentiation of numerous cell types, including those of dermal and epidermal origin (Sehgal, 1990), and is closely linked to skin wound healing (Gallucci et al., 2000; Lin et al., 2003). IL-6 treatment also appears to modulate stratum corneum regeneration and skin barrier function (Wang et al., 2004) to maintain skin homeostasis

In the present study, our goal was to comprehensively characterize the immune and cellular transcriptional response in normal and irritant-treated skin samples using next generation sequencing (RNA-Seq). Additionally, since IL-6 has been shown to have a direct role in skin inflammation and healing (Gallucci et al., 2000; Lee et al., 2013a; Lin et al., 2003) the modulatory role of IL-6 in the process of ICD was also investigated by the use of an IL-6 deficient mouse model. We anticipated a role of genes associated with skin barrier integrity, innate immunity, inflammation, keratinocyte activity, as well as skin development and indeed found a number of genes modulated with an association to these processes. As previously mentioned, most studies to date have only investigated a small number of genes with focus only on allergic dermatitis. Therefore, assessment of variability in skin responses to the well-known detergent and preservative irritant benzalkonium chloride (Willis et al., 1986; Wentworth et al., 2016), utilizing the well-characterized C57BL/6 and IL-6 deficient mouse models should provide further insight into the pathomechanism underlying ICD.

## 2. Methods and materials

### 2.1. Mice

IL-6KO ($IL6^{tm1Kopf}$) and WT (C57BL/6) male mice of 8–12-week-old, were acquired from the Jackson Laboratory (Bar Harbor, ME). Mice were group-housed in polycarbonate cages containing hardwood chip bedding at room temperature (21 ± 3 °C) on a 12-h light/dark cycle. Animals were allowed to acclimate to the animal facility for at least 1 week prior to BKC exposure. Throughout the studies, animals received humane care according to the criteria outlined in the *Guide for the Care and Use of Laboratory Animals* prepared by the National Academy of Sciences and published by the National Institutes of Health (2011).

### 2.2. BKC treatment

One day prior to exposure, mice were sedated with isoflurane 2% by inhalation and a ~9-$cm^2$ section of fur was clipped completely to bare skin, on the dorsal surface just posterior of the cranium. Treatments were initiated 24 h post-hair removal to ensure that minimal irritation and were devoid of any nicks that may have occurred from the hair removal process. If nicks were identified, animals were excluded. 2% aqueous benzoalkonium chloride (Sigma, St. Louis, MO) or PBS (negative control) was applied (50 µl) to the denuded skin of the animals daily for 7 consecutive days as this time point and concentration of BKC has been shown to be sufficient in inducing ICD (Lee et al., 2013a). Treatment groups (n = 3/strain and treatment) of each mouse genotype and the respective WT were placed in separate treatment chambers to minimize exposure and grooming form other mice. Twenty-four hours following the final exposure, skin samples were collected via a 4-mm full thickness punch biopsy. Harvested skin was immediately homogenized in TriReagent (Molecular Resource Center, Cincinnati, OH) with 10 µl of protective carrier (Molecular Research Center) and processed for RNA or embedded in Tissue-Tek O.C.T compound (VWR, Radnor, PA) and immediately frozen for histology. 5-µm skin cross-sections were hematoxylin and eosin (H&E) stained. Digital images of the skin histopathology (under 20× objective) were acquired utilizing a Leica 4000b microscope (Leica Microsystems, Buffalo Grove, IL).

### 2.3. Illumina RNAseq

RNAseq libraries were constructed using the Illumina TruSeq RNA LT v2 kit and established protocols. The library construction was done using total RNA isolated from mice (1 µg). RNA quality for each prep was analyzed prior to construction using the Agilent Bioanalyzer 2100 and RNA nano total RNA chips. Each library was indexed during library construction in order to multiplex for sequencing on the Illumina MiSeq platform. Samples were sequenced in batches of three libraries per 2 × 150 bp paired end sequencing run on the Illumina MiSeq. On average, a total of 40 million reads (6Gb) of sequencing data was collected per run. Standard RNASeq workflow parameters within CLC Genomics Workbench were used. The read alignment values were set at: mismatch cost 2, insertion cost 3, deletion cost 3, length fraction 0.8, similarity fraction 0.8, “auto-detect paired distance” was selected, strand specific : both, and maximum number of hits for a read was 10. Raw data for each sample was analyzed using CLC Genomics Workbench software (Qiagen (formerly CLCBio). Raw sequence reads were mapped to the *Mus musculus* genome for identification of genes expressed under each condition. Pairwise comparison of the expression results were performed using the total mapping results for C57-BKC vs. C57 and KO-BKC vs. KO. Differential gene lists were created using the “Differential Expression for RNA-Seq” tool in the CLC Genomics Workbench software with a 5-fold expression cutoff (and FDR p values of < 0.1) to identify genes that were up- or down-regulated under each condition. This stringent cutoff was used to allow for a more definitive and concise list of genes that were modulated in each comparison after *t*-test statistical measures were performed. Each experimental group had an n = 3.

## 3. Results

### 3.1. IL-6 deficient (IL-6KO) mice display epidermal defect, increased inflammatory cell infiltration and altered gene expression patterns in response to BKC treatment

Skin is an ideal model to study the inflammatory process. Disruption of the barrier and ultimate activation of the innate immune response and inflammation are characteristic and easily discernable features of ICD. IL-6 deficiency exacerbates inflammation by modulating several cytokine and chemokine mediators following treatment with various irritants (Lee et al., 2013a). Analysis of hematoxylin and eosin (H&E) stained skin sections showed both C57BL/6 (C57) and $IL6^{tm1Kopf}$ (IL-6KO) mouse skin had increased cellular infiltrate and displayed epidermal hyperplasia in response to BKC treatment (C57-BKC and KO-BKC, respectively) as compared to normal, untreated skin (Fig. 1A vs. B, C vs. D). Indeed, IL-6KO BKC-treated skin showed more severe thickening of the epidermis and a higher influx of inflammatory cells into the treated area than C57 (Fig. 1B vs. D) supporting earlier reports (Lee et al., 2013a). In addition to being thicker, the epidermis in IL-6KO mice also appeared to have less structural organization as compared to C57-BKC treated and normal skin, indicating more BKC-induced damage.

Although ICD is more prevalent than allergic skin reactions, the associated transcriptional response is not well characterized. Therefore, to further define the transcriptional changes associated with ICD, total RNA sequencing was performed on untreated C57BL/6 (C57) and IL-6 deficient (KO) skin samples as well as BKC-treated C57BL/6 (C57-BKC) and IL-6KO (KO-BKC) skin samples. Overall, 1986 genes were differentially modulated in response to BKC or between strains (Accession Number GSE95317) after our statistical cutoff was applied (FC > 5, p-value < 0.1). The highest number of genes modulated was within the untreated vs. BKC-treated C57 (C57 vs. C57-BKC) pairwise comparison

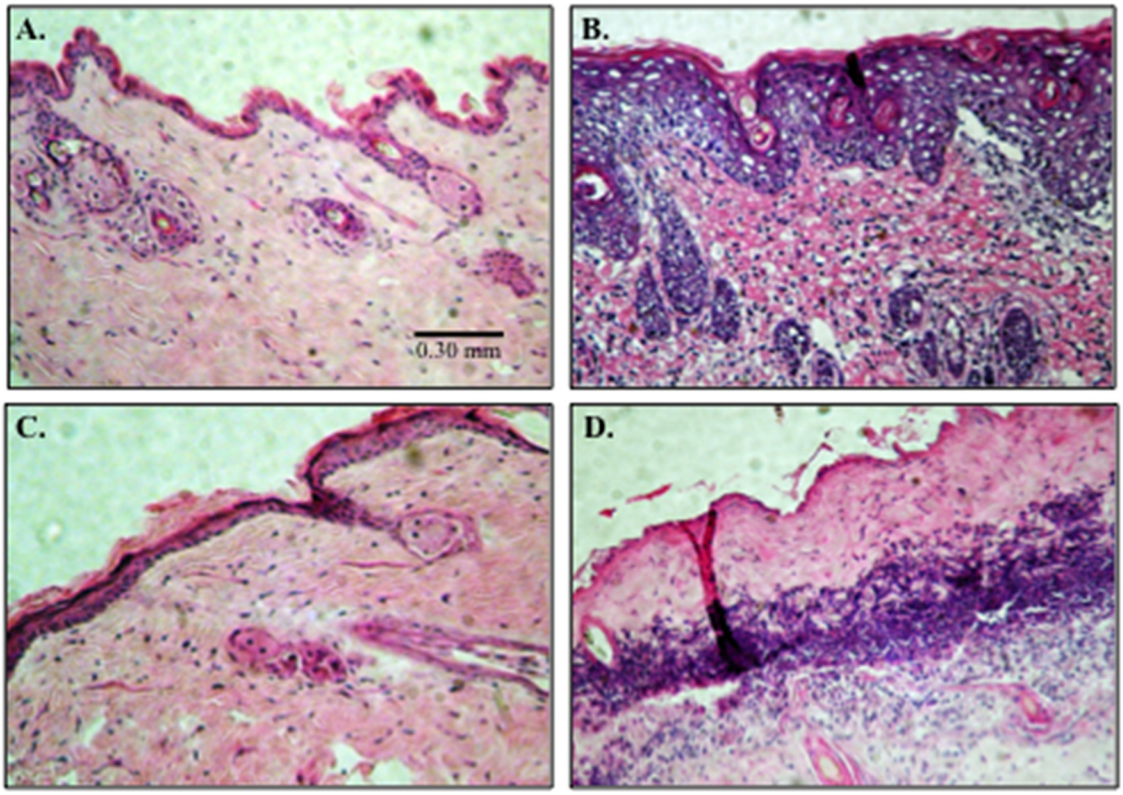


**Fig. 1. IL-6 deficient (IL-6KO) mice display epidermal defects and increased inflammatory cell infiltration in response to BKC treatment.** Punch biopsies (4 mm) were obtained from C57 or IL-6 deficient mice. 5 μm tissue sections were stained with haematoxylin and eosin dye. A) Untreated C57, B) BKC treated C57, C) Untreated IL-6KO, and D) BKC-treated IL-6KO. Data are representative of 15 animals per treatment group. (20x magnification, scale bar: 0.30 mm).

with over 1000 genes differentially expressed (Fig. 2A, gold circle). Table 1 identifies the most significantly up- and down-regulated genes in C57-BKC skin as compared to C57. *Krt6*, various members of the *Sprr* family and chemokines showed a significant up-regulation while *Upk3b*, a cell differentiation marker, the antimicrobial peptide *Hamp2*, and *Tgm7*, an enzyme involved in the construction of the multilayered epithelium were among those down-regulated in C57-BKC as compared to C57. Additionally, when comparing BKC-treated IL-6KO to normal, untreated skin (KO vs. KO-BKC), the total number of genes differentially expressed decreased to 802, with 393 uniquely modulated by the absence of IL-6 (Fig. 2A, blue circle). Genes that were significantly up-regulated include *Mrgpra2b*, a G-protein coupled receptor that mediates mast cell activation (Dwyer et al., 2016) *Reg1* and *Reg3g* potential modulators of keratinocyte proliferation (Parikh et al., 2012; Lai et al., 2012), the acute phase protein *Saa3* (O'Reilly et al., 2014), and various chemokines; while the majority of genes down-regulated were keratin-associated proteins (Table 1). To better understand the biological and functional significance of genes modulated within each comparison, the gene ontology terms associated were analyzed. The majority of genes differentially modulated were in involved in cellular (Ex. cell cycle, cell communication and movement) and metabolic processes (Fig. 2B). Supplemental Fig. 1 shows the overall gene expression changes within each sample and their hierarchical association.

### 3.2. IL-6 deficient and C57 BKC treated skin display altered transcriptional changes of immune associated genes

To evaluate the immune process following BKC exposure and the involvement of IL-6 in the resulting irritant dermatitis, genes that affect overall and innate immunity, as well as those focused on inflammation were evaluated using various functional annotation tools (DAVID, Panther, and CLC) under the same statistical measures used in Fig. 2. Clustering analysis in Fig. 3A shows all 49 immune associated genes that were significantly modulated in C57-BKC skin as compared to C57, as well as expression patterns and their association with other genes. Of those with a characterized function, 36 genes were up-regulated, while 13 genes were down-regulated in response to BKC treatment (Fig. 3A, Supplemental Table 1). KO-BKC skin compared to control skin resulted in 17 immune associated genes being up-regulated and 8 down-regulated (Fig. 3B and Supplemental Table 2).

### 3.3. BKC-treatment of C57 and IL-6KO mice induces structural and cellular changes in skin

In addition to immune dysregulation, ICD presents with many structural alterations of skin as demonstrated in Fig. 1. The classifying pathology associated with ICD is disruption of the epidermal barrier, and the main cellular component of the epidermis is keratinocytes. Therefore, genes that had an association with barrier function, skin development, as well as those specific for keratinocyte function were assessed (Figs. 4 and 5, Supplemental Tables 3–4). Interestingly, inflamed C57 skin (C57-BKC), only showed 16 genes (*Areg*, various *Sprrs, Inhba, Tgm3*, and *Sfn*, for example) up regulated with skin barrier and developmental functions (Fig. 4, Supplemental Table 3). A large number of *Sprr* and *Lce* genes, which strongly influence epidermal differentiation, were also up-regulated following BKC treatment, with *Sfrp4, Hoxa7* and *Lce1m*, which all promote epidermal differentiation, being the only keratinocyte-specific genes whose expression decreased in C57-BKC skin. Interestingly, KO-BKC skin only increased expression of a small number of skin-associated genes that again are associated with keratinocyte differentiation and barrier homeostasis (*Ltb, MMP9, Adra2a*, and *Ryr1*), with more being significantly down-regulated (Fig. 5, Supplemental Table 4).

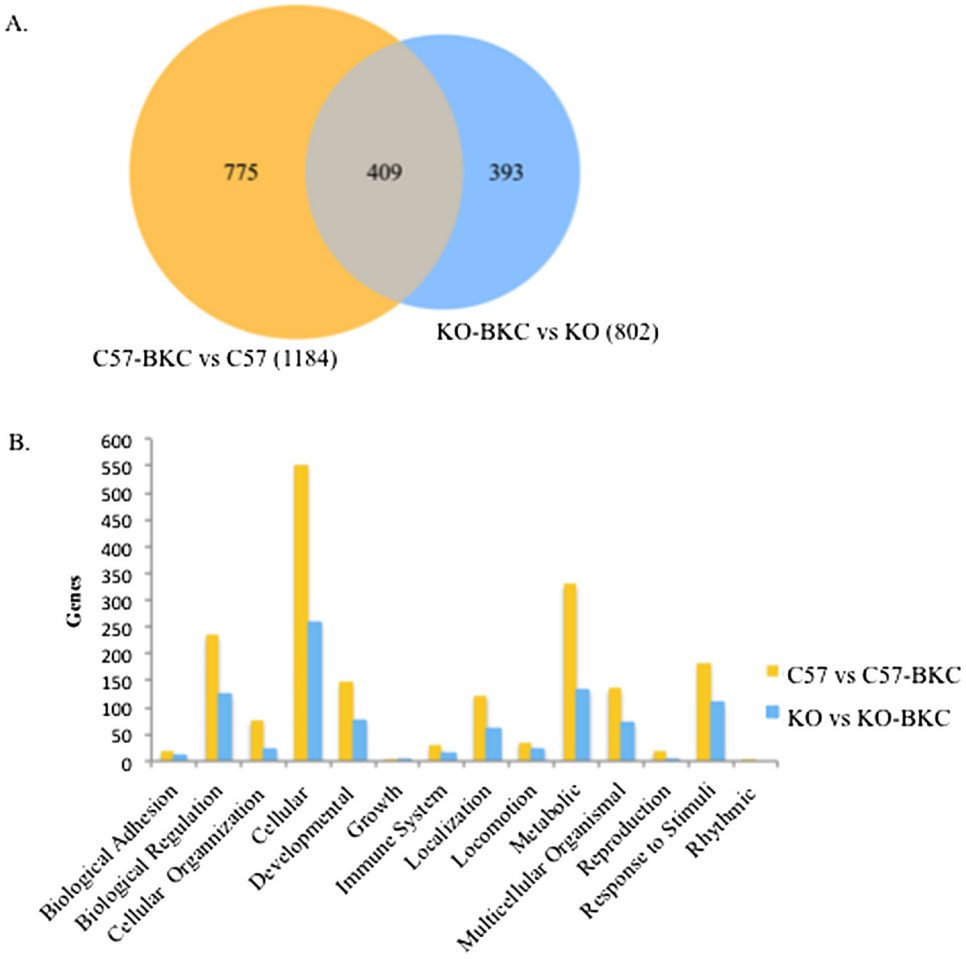


**Fig. 2. IL-6 deficient (IL-6KO) mice display altered gene expression patterns in response to BKC treatment.** Total RNA was isolated from untreated C57 (C57), BKC-treated C57 (C57-BKC), untreated IL-6KO (KO), and BKC-treated KO (KO-BKC) skin samples and subjected to transcriptome analysis by MiSeq. Differential gene lists were created with 5-fold expression cutoff and FDR p-values of $< 0.1$ ($n = 3$). A) Venn diagram showing total number of common and differentially regulated genes within each strain following BKC treatment. B) Overall gene ontology terms associated with the transcripts modulated in each group comparison as determine by the online database, Panther. (For interpretation of the references to colour in the text, the reader is referred to the web version of this article.)

## 4. Discussion

Transcriptome profiling of skin has gained acceptance as a sensitive tool to measure potential markers of pathology. However, the majority of dermatitis-associated genomic studies have been done using samples from atopic dermatitis patients (Bin and Leung, 2016; Suarez-Farinas et al., 2015; Sasaki et al., 2013) or ACD samples (Pierezan et al., 2016). It appears that no studies to date have examined the genome wide changes that occur during ICD, making this study novel in nature. The goal of this study was to identify overall transcriptional changes and specific gene signatures that could be attributed to skin inflammation, and more specifically the role of IL-6 in ICD. Therefore as an initial analysis, the global gene expression changes were described between untreated and irritant exposed skin from C57 and IL-6 deficient mice. Over 1900 differentially expressed genes were identified, but the overall magnitude of expression differences between mouse strains was most interesting. As shown in Table 1, the gene expression changes ranged from 4000 to −342 fold in BKC exposed C57 (as compared to untreated), but ranges were much more exaggerated when examining BKC exposed IL-6KO skin, where 234 to -40,000 fold differences were present compared to untreated C57. The significance of this phenomenon is unclear and would need additional investigation. However, this further emphasizes the pleiotropic nature of IL-6 and illustrates how the deletion of a single influential cytokine can profoundly influence the transcriptional response during skin inflammation. Interestingly, a study by Pedersen, M.B., et al. found that only 74 genes were differentially modulated in human skin during the elicitation of ACD with focus on the characterization of SMAD transcription factors and CCL19 in the elicitation phase which were not found in to be modulated in our ICD study (Pedersen et al., 2007). When immune associated genes were compared with the ACD study, only *Ltb* and *CCL2* were up-regulated in both studies and very few structurally associated genes overlapped as well indicating a different gene signature modulating ACD versus ICD.

### 4.1. Overall function

When studying gene ontology, the majority of genes were associated with either cellular or metabolic processes (Fig. 2B). This is not surprising as cellular processes can include cell communication, cell cycle, proliferation and signal transduction, all of which occur regularly in homeostatic and inflammatory conditions. Additionally, as nearly any chemical has the potential to be an irritant, skin metabolism plays a significant role in the chemical's irritancy potential and in the detoxification process of the chemical itself (Smith Pease et al., 2003). While describing the direct role of skin metabolism in ICD was not the main goal of this study, it was shown that a number of metabolically associated genes were modulated by BKC and IL-6, which may influence the chemicals irritant nature. Additionally, the analysis identified many potential targets for future studies to determine a more definitive link between various irritants, inflammation, and skin metabolism.

It is also interesting to note that the majority of the most significantly modulated genes in BKC treated C57 skin are pro-inflammatory (Table 1). For example, *Cxcl2, Cxcl3*, and *Cxcl5* promote neutrophil infiltration, *Acod1* has been shown highly expressed in macrophages during inflammation (Michelucci et al., 2013), and members of the Sprr family, which have skin barrier functions and are highly associated with inflammation (Quigley et al., 2009). Furthermore, *Hamp2*, a well-known antimicrobial peptide (Plichta et al., 2014), and Skint7, which aids in wound healing (Keyes et al., 2016) were all significantly down-regulated (fold change of −158.39 and −59.46,

**Table 1**
**Top 10 up- and down-regulated genes in response to BKC treatment in C57 and IL-6KO skin**. Differences were considered significant when FDR was < 0.1 and Fold Change (FC) was > 5.0.

| Symbol | Description | Fold change | P-value | FDR p-value |
|---|---|---|---|---|
| **C57-BKC Upregulated** | | | | |
| Krt6b | keratin 6B | 4,536.77 | 0.00E+00 | 0 |
| Sprr2i | small proline-rich protein 2I | 3,401.75 | 4.43E−07 | 2.58E−05 |
| Tmprss11b | transmembrane protease, serine 11B | 3,174.76 | 4.92E−07 | 2.82E−05 |
| Sprr2a1 | small proline-rich protein 2 A1 | 2,839.49 | 6.23E−07 | 3.46E−05 |
| Cxcl2 | C-X-C motif chemokine ligand 2 | 2,657.66 | 0.00E+00 | 0.00E+00 |
| Acod1 | aconitate decarboxylase 1 | 2,516.18 | 1.04E−06 | 5.42E−05 |
| Cxcl5 | C-X-C motif chemokine ligand 3 | 2,196.85 | 2.23E−06 | 1.06E−04 |
| Sprr1b | small proline-rich protein 1B | 1,701.77 | 0.00E+00 | 0 |
| Sprr2b | small proline-rich protein 2B | 1,487.37 | 8.34E−06 | 3.32E−04 |
| Cxcl3 | C-X-C motif chemokine ligand 3 | 1,462.82 | 5.93E−06 | 2.46E−04 |
| **KO-BKC Upregulated** | | | | |
| 2300002M23Rik | emprin | 234.32 | 2.54E−03 | 0.05 |
| Mrgpra2b | MAS-related GPR, member A2B | 213.51 | 2.98E−03 | 0.05 |
| Reg1 | regenerating islet derived 1 | 199.57 | 5.21E−09 | 5.64E−07 |
| Gm14434 | | 159.74 | 4.97E−03 | 0.08 |
| Cxcl5 | C-X-C motif chemokine ligand 5 | 147.32 | 2.22E−16 | 6.73E−14 |
| Saa3 | serum amyloid A3 | 92.76 | 0 | 0 |
| Cxcl3 | C-X-C motif chemokine ligand 3 | 89.69 | 0 | 0 |
| Gm20431 | | 87.37 | 7.29E−06 | 3.49E−04 |
| Reg3g | regenerating family member 3 gamma | 75.38 | 3.47E−11 | 5.50E−09 |
| Prokr2 | prokineticin receptor 2 | 60.3 | 1.23E−05 | 5.52E−04 |
| **C57-BKC Downregulated** | | | | |
| Gm5849 | prediceted gene 5849 | −342.88 | 8.11E−04 | 0.02 |
| Upk3b | uroplakin 3b | −240.01 | 1.49E−03 | 0.03 |
| F830045P16Rik | RIKEN cDNA F830045P16 gene | −213.5 | 1.90E−03 | 0.03 |
| Gm26883 | | −184.98 | 4.41E−03 | 0.07 |
| Hamp2 | hepcidin antimicrobial peptide 2 | −158.39 | 4.49E−03 | 0.07 |
| Tgm7 | transglutaminase 7 | −149.67 | 3.42E−08 | 2.67E−06 |
| Gm26776 | | −145 | 5.85E−03 | 0.08 |
| Odf3l2 | outer dense fiber of sperm tail 3 like 2 | −137.22 | 5.07E−03 | 0.08 |
| Chrnb4 | cholinergic receptor nicotinic beta 4 subunit | −110.26 | 6.33E−03 | 0.09 |
| Skint7 | selection and upkeep of intraepithelial T cells 7 | −59.46 | 0 | 0 |
| **KOB-BKC Downregulated** | | | | |
| Krtap14 | keratin associated protein 14 | −40,684.71 | 1.62E−11 | 2.71E-09 |
| Gm10228 | | −13,868.91 | 9.76E−10 | 1.23E-07 |
| Krtap19-9b | keratin associated protein 19-9b | −12,098.88 | 1.85E−09 | 2.23E-07 |
| 4930553J12Rik | RIKEN cDNA 4930553J12 | −10,835.84 | 0.00E+00 | 0.00E+00 |
| Gm10229 | | −10,210.95 | 0 | 0 |
| Krtap19-5 | keratin associated protein 19-5 | −9,538.78 | 4.71E−09 | 5.16E−07 |
| Krtap6-2 | keratin associated protein 6-2 | −8,660.99 | 5.95E−09 | 6.34E−07 |
| Krtap19-2 | keratin associated protein 19-2 | −8,464.62 | 8.34E−09 | 8.56E-07 |
| Krtap16-3 | keratin associated protein 16-3 | −5,222.88 | 0.00E+00 | 0.00E+00 |
| Krtap6-1 | keratin associated protein 6-1 | −5,003.62 | 0.00E+00 | 0.00E+00 |

respectively) indicating possible compromised microbial protection and skin maintenance. Interestingly, BKC exposed IL-6KO skin presented a pro-inflammatory signature not all that dissimilar to C57 with the significantly increased expression of *Cxcl3, Cxcl5, Saa3* all of which induce inflammation. However, a majority of keratin-associated genes that were differentially expressed were down-regulated in irritated IL-6KO skin. These proteins play a critical role in skin integrity and could be associated with some of the structural differences obvious in IL-6KO skin as compared to C57 (Table 1). These results are similar to previous studies on the role of IL-6 in wound healing. In that, IL-6KO mice display significantly delayed wound healing as compared to the control C57 under the same wounding conditions (Gallucci et al., 2000; Lin et al., 2003). Additionally, the inflammatory cell infiltrate (ex. inflammatory/immune cells, chemokines, and cytokines) looks substantially different in IL-6KO mice as compared to C57 when exposed to the BKC indicating a different “repair” mechanism between the two strains (Calhoun et al., unpublished data) and a definitive role for IL-6 in the modulation of these two mechanisms.

### 4.2. Immune modulation

Historically, ICD was thought to be a “non-immunological disorder” (Rietschel, 1997). However, that perception has changed and ICD is now generally accepted to involve endogenous and exogenous factors that activate the immune response (Slodownik et al., 2008). For instance, following irritant exposure, barrier disruption and keratinocyte damage induces release of pro-inflammatory cytokines that ultimately attract various innate immune cells. Certainly mouse strain affects immune responses, as is illustrated in a polygenic manner with C57 and Balb/c mice where each have been shown to have immune responses skewed towards Th1 or Th2 respectively (Watanabe et al., 2004). To access immune involvement during irritancy, genes were selected based solely on this function from the ~1900 total genes that were initially examined. In total, seventy-four genes were identified and characterized based on expression level. Hierarchical clustering revealed variable gene expression patterns with some association upon BKC exposure and others on IL-6 deficiency (Fig. 3). While some immune associated genes were similar between BKC exposed C57 and IL-6KO mice such as *Cxcl2, Cxcl3, Cxcl5, Acod1*, *C-lectins*, and *S100a9* all of which promote a state of inflammation (Supplemental Tables 1–2), irritated C57 skin had a greater number of genes uniquely modulated compared to IL-6KO skin. For example, *Chil3, Chil1* (HogenEsch et al., 2006), *Aqp3* (Qin et al., 2011), *IL-23a* (Wiekowski et al., 2001), *Ccl2* (Lai and Gallo, 2008), *Ccr5* (Kato et al., 2006), and *Ccl7* (Brunner et al., 2015) were significantly

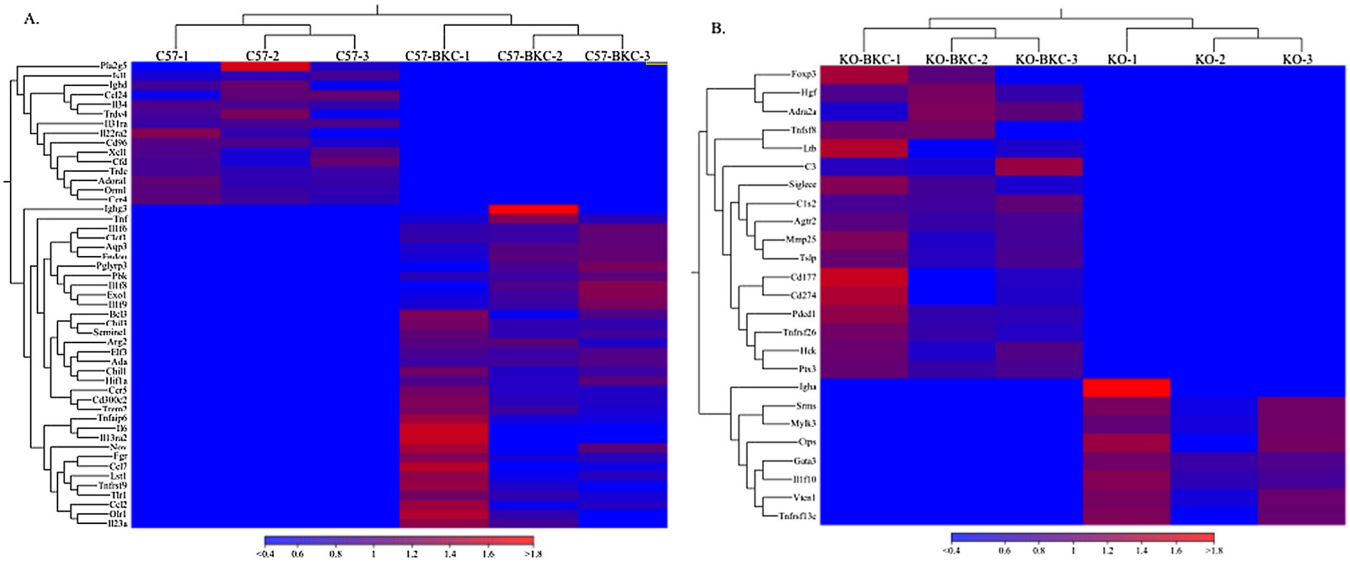


**Fig. 3. IL-6 deficient and C57 BKC treated skin display altered transcriptional changes of immune associated genes.** Total RNA was isolated from untreated C57 (C57), BKC-treated C57 (C57-BKC), IL-6KO (KO), and BKC-treated KO (KO-BKC) skin samples and subjected to transcriptome analysis by MiSeq. Differential gene lists were created with 5-fold expression cutoff and FDR p-values of < 0.1 (n = 3). Genes with an associated innate or overall immune function as well as an inflammatory associated function were selected for using various databases (DAVID, Panther and CLC). Expressional differences were shown via heat map in A) C57 vs. C57-BKC and B) KO vs. KO-BKC comparisons. The expression levels are visualized using a gradient color scheme, where the red is high expression levels and blue is low expression levels. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

up-regulated compared to control C57, which further contribute to a more Th1/pro-inflammatory immune response. Interestingly, this is similar to previous results showing that chitinase like proteins (Chil3L3 and Chil3L4) were found significantly up-regulated in the skin of a chronic proliferative dermatitis mouse model and this increased expression coincided with epidermal thickening and increased immune cell infiltration as in our model (HogenEsch et al., 2006). However, IL-6 deficiency resulted in the up-regulation of a completely different set of immune regulatory genes in irritated skin including *Pdcd1*, a co-inhibitory receptor that inhibits TCR mediated signaling and increased by continuous or repetitive TCR stimulation of T cells (Okazaki et al., 2013); *Foxp3*, a regulatory T cell (T-reg) transcription factor involved in immunological tolerance (Dudda et al., 2008); *Ltb*, a neutrophil chemoattractant; and *C1sb* and *C3*, produced by keratinocytes and all involved during skin inflammation (Grabbe and Schwarz, 1998). While the increased expression may initially indicate a role for T cell, neutrophils and complement in promoting damage, this may actually be a protective mechanism induced to counteract the increased inflammation that is seen in our IL-6KO ICD model. Indeed, it has been shown that C3 plays a novel anti-inflammatory role in T-cell mediated skin inflammation (Purwar et al., 2011). This differing transcriptional signature may influence which immune cells infiltrate damaged tissue and could ultimately be associated with the variable severity of ICD observed between strains.

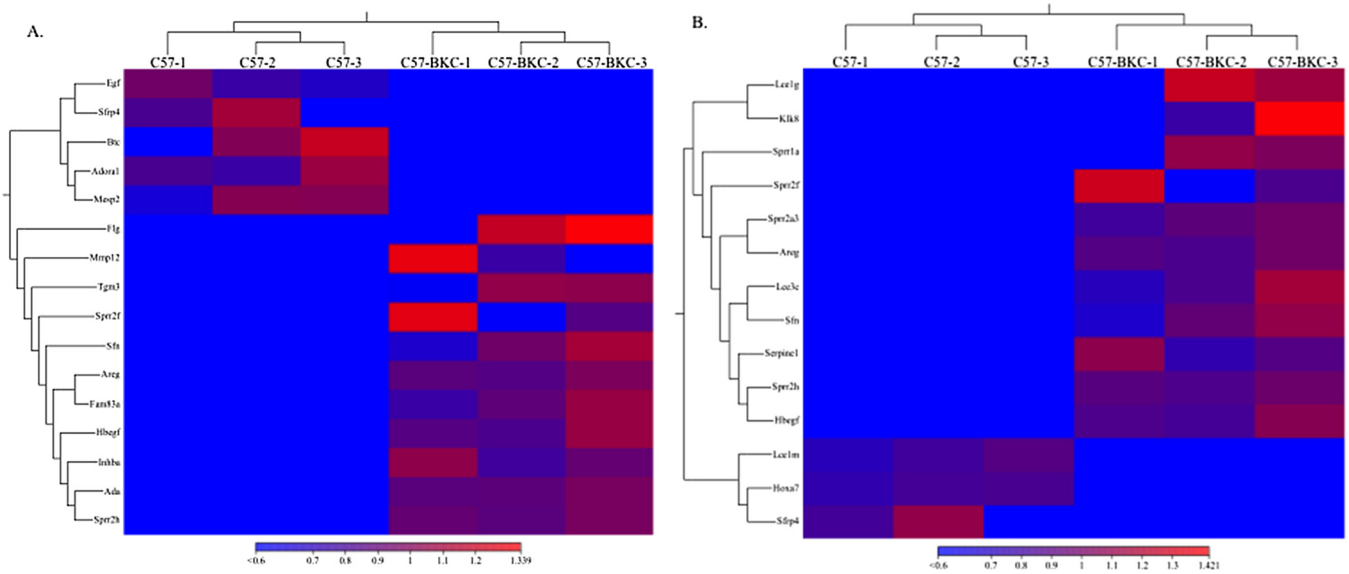


**Fig. 4. BKC-treatment of C57 mice skin induces structural and cellular changes.** Total RNA was isolated from untreated C57 (C57) and BKC-treated C57 (C57-BKC), skin samples and subjected to transcriptome analysis by MiSeq. Differential gene lists were created with 5-fold expression cutoff and FDR p-values of < 0.1 (n = 3) as well as an associated function with skin dynamics. Heat map of differentially expressed genes associated with either A) Overall skin maintenance or B) Keratinocyte specific associated genes. The expression levels are visualized using a gradient color scheme, where the red is high expression levels and blue is low expression levels. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

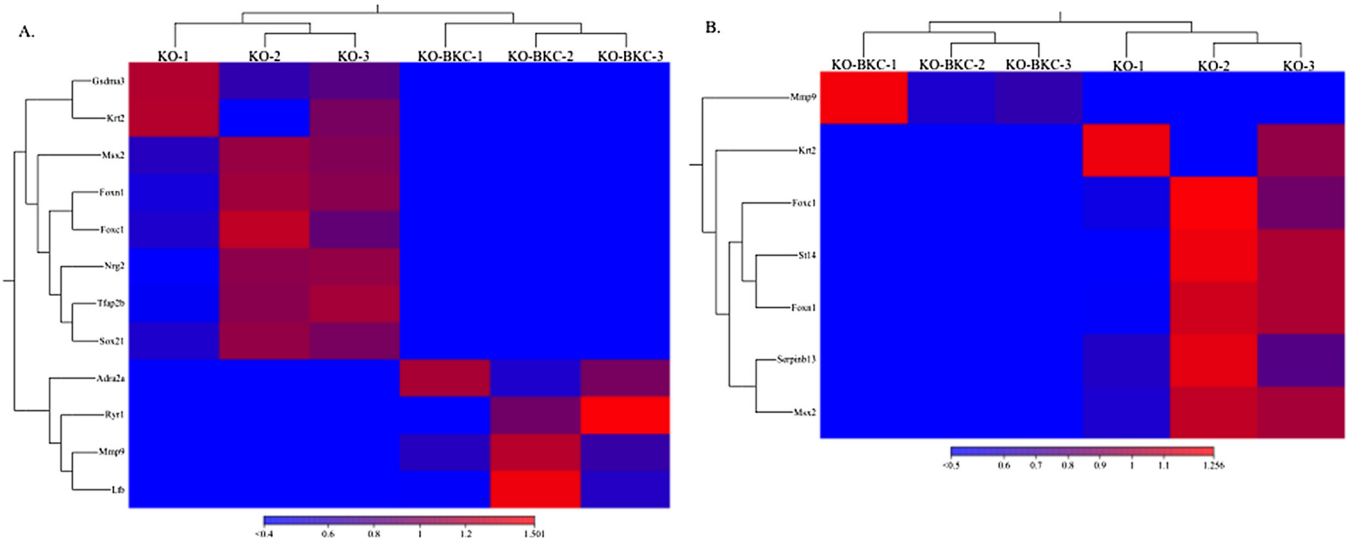


**Fig. 5. BKC-treated IL-6KO mice skin induces structural and cellular changes.** Total RNA was isolated from untreated IL-6KO (KO), and BKC-treated KO (KO-BKC) skin samples and subjected to transcriptome analysis by MiSeq. Differential gene lists were created with 5-fold expression cutoff and FDR p-values of < 0.1 (n = 3). Heat map of differentially expressed genes associated with either A) Overall skin maintenance or B) Keratinocyte specific associated genes. The expression levels are visualized using a gradient color scheme, where the red is high expression levels and blue is low expression levels. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

### 4.3. Skin developmental and structural modulation

Irritants can be quite diverse in nature and interact with different structural components of the skin, eliciting a spectrum of reactions from local inflammation to widespread, systemic dysfunction. The presence of a competent epidermal barrier is a key determinant for the outcome of the interaction between most irritants and the skin. The stratum corneum is formed by terminal differentiation of keratinocytes into a cornified layer of cells that forms the main protective barrier of the skin. Members of the Sprr family utilize lysine- and glutamine-rich N- and C-terminal domains for transglutaminase cross-linking during the building-up of the cornified cell envelope. Interestingly, increased Sprr expression can accompany several pathological conditions associated with inflammation, where *Spr1* expression is shown to be elevated in psoriatic epidermis, as well as SLE, eczema, and ichthyosis (Tesfaigzi and Carlson, 1999). Sprr proteins are also thought to be involved in wound healing where they provide injured tissue with an efficient, finely tuned antioxidant barrier specifically adapted to the tissue involved and the damage inflicted. Sprr proteins protect keratinocytes from excessive ROS by direct quenching via their cysteine residues (Vermeij and Backendorf, 2010). Our results showed significantly increased expression (~18–100 fold higher than control) of *Sprr2f, Sprr2h, Sprr2a3, Sprr1a* in BKC-treated C57 skin as compared to control (Fig. 4), but not in irritated IL-6KO skin (Fig. 5). A similar decrease in *Sprr* proteins was shown by Nozaki et al. and Demetris et al. in an IL-6 deficient model and played a role in barrier function after bile duct ligation (Demetris et al., 2008; Nozaki et al., 2005) further highlighting the importance of *Sprr* activity in barrier function and lack there of in IL-6 deficient skin. Additionally, the gene for activin (*Inhba*) a member of the TGF-β superfamily that functions to regulate skin morphogenesis via keratinocyte proliferation and differentiation (Bamberger et al., 2005); the structural proteins epidermal-type transglutaminase 3 (*Tgm3*); *MMP12*, which not only aides in elastin degradation but also may be associated with macrophage migration in inflammatory skin conditions (Vaalamo et al., 1999); the stratum corneum protein *Lce*; and the dermal regulatory protein, stratifin (*Sfn*) are all up-regulated in C57 but not IL-6KO (Figs. 4 and 5, Supplemental Tables 3–4). The increased expression of these structural genes may work to promote a pro-healing, or protective phenotype in response to damage. Conversely, IL-6 deficiency was associated with a large number of skin-associated genes being significantly down-regulated as compared to C57 (Fig. 5). *Msx2* was the most significantly down-regulated gene in KO-BKC skin and is a member of the homeobox family of genes that play key roles in governing cellular competence and is a strong candidate regulator of skin wound healing (Yeh et al., 2009). It is differentially expressed between fetal and adult skin in which its expression coincides with the stage of fetal scarless skin repair (Stelnicki et al., 1997; Carlson et al., 1998). Decreased *Msx2* expression has been associated with accelerated wound closure, increased keratinocyte proliferation and re-epithelialization (Yeh et al., 2009). It is also interesting to note that *Krt2*, which is the second more abundantly expressed keratin in skin and associated with keratinocyte activation, proliferation and keratinization is decreased 26-fold compared to control treatment. Deletion of *Krt2* has shown acanthosis (epidermal thickening) and increased transepidermal water loss, inflammation, and hyperkeratosis of the ear, which is similar to our results (Fig. 1) (Fischer et al., 2014). Indeed, IL-6KO mice displayincreased epidermal thickening following irritant exposure (Fig. 1D and [Lee et al., 2013a#2785]), consistent with decreased expression of *Msx2* and *Krt2*. Therefore, it appears that the protective role of IL-6 in skin is not only associated with immune modulation, but it may also affect the basic architecture or remodeling of the skin during ICD.

### 4.4. Conclusions

The goal of this study was to identify differentially expressed genes organized by biologically functional themes (immune, inflammatory, and structurally associated) in an irritant contact dermatitis mouse model. Our results expand upon the current understanding of ICD by offering a snapshot of the concomitantly occurring transcriptional events that characterizes the mechanism of this dermal inflammatory process. Additionally, our results further confirm a role for IL-6 in ICD and highlight the varied transcriptional responses that occur with the deletion of this highly pleiotropic cytokine. Further investigation is necessary to determine the potential usefulness of the various biomarkers revealed by the current study relative to risk assessment associated with occupational dermatitis.

## Authors contributions

Lerin R. Luckett-Chastain designed the study, performed all animal

work, analyzed all data (in all treatment groups), and wrote the manuscript.

Jenny R. Gipson performed library preparation, sequencing and assisted with data analysis.

Allison F. Gillaspy and Randle M. Gallucci contributed to critically revising and providing final approval of the manuscript.

## Competing interests

The authors declare that they have no competing interests.

## Funding

This work was supported by the National Institutes of Health (NIH/NIGMS R01 GM67745-01A1); and the Center for Disease Control (CDC/NIOSH R01 OH010241-01).

## Acknowledgments

We thank the Laboratory for Molecular Biology and Cytometry Research at OUHSC for the use of the Core Facility which provided Illumina MiSeq sequencing and bioinformatics support.

## Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.tox.2018.08.014.

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