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

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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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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