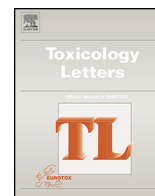




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Hyaluronan regulates chemical allergen-induced IL-18 production in human keratinocytes

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HIGHLIGHTS

- IL-18 has a key role in allergic contact dermatitis progression.
- Contact allergens enhance hyaluronan (HA) degradation in keratinocytes.
- Modulation of HA content reduces IL-18 production.
- Oxidative stress is involved in HA degradation and IL-18 production.
- HA fragments are mediators in contact sensitisation process.

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ABSTRACT

Interleukin-18 (IL-18) has been shown to play a key proximal role in the induction of allergic contact dermatitis. Low molecular weight hyaluronan (LMWHA), an endogenous molecule and a member of the so-called damage associated molecular patterns (DAMPs), has been suggested to elicit immune-stimulatory effects. The purpose of this study was to examine the role of hyaluronan (HA) degradation in IL-18 production in human keratinocytes following stimulation with the contact sensitizers 2,4-dinitrochlorobenzene (DNCB) and PPD. IL-18 production in the human keratinocyte cell line NCTC2544 was measured by ELISA, whereas changes in HA metabolism were determined by Real-time PCR and immunofluorescence. Both contact allergens were able to enhance hyaluronidase (HYAL) 1 and 2 expression inducing HA degradation. Modulation of HA production, by HYAL or aristolochic acid pre-treatment, resulted in a significant reduction of contact allergen-induced IL-18 production. Oxidative stress appears to be the initial step in KC activation, as all the sequels of events can be blocked using antioxidants. This is the first indication that LMWHA can act as a DAMP in keratinocytes. In conclusion LMWHA fragments are important mediators in the process of contact sensitisation leading to IL-18 dependent responses.

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1. Introduction

Irritant and allergic contact dermatitis¹ (CD) are undesired side effects after contact with environmental or industrial chemicals as

well as in the development of drugs and cosmetics. Due to a high prevalence of contact allergy in the general population, making it a common and important health hazard (Peiser et al., 2012), new approaches in mapping sensitisation pathways have been developed (Hartung and Immunotoxicology, 2013). Epidermal keratinocytes (KC) sense haptens, which in turn initiate a program of enhancement or de novo expression of inflammatory molecules, which ultimately represent the starting point of primary inflammation. IL-18, formerly known as IFN- γ -inducing factor (IGIF), which belongs to the IL-1 cytokine family, provides an early signal for development of Th1 lymphocyte responses (Dinarello et al., 1998). IL-18 has been shown to play a key proximal role in the

Abbreviations: IL, interleukin; KC, keratinocytes; HA, hyaluronan; LMWHA, low molecular weight hyaluronan; HYAL, hyaluronidase; ROS, reactive oxygen species; HMWHA, high molecular weight hyaluronan; DAMP, damage associated molecular pattern.

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induction of allergic contact dermatitis and to favor Th-1 type immune response by enhancing the secretion of pro-inflammatory mediators such as TNF- α , IL-8, and IFN- γ (Okamura et al., 1995; Cumberbatch et al., 2001; Antonopoulos et al., 2008). IL-18 also enhances the production of granulocyte macrophage-colony stimulating factor and IL-12, potentiates anti-CD3-induced T cell proliferation, and increases Fas-mediated killing of NK cells (Micallef et al., 1996; Tsutsui et al., 1996). Haptens, acting as danger signals, stress the cells, stimulate the assembly of the inflammatory and NF- κ B activation, resulting in IL-18 neosynthesis and release (Corsini et al., 2009). Therefore, IL-18 production is a representative biomarker of the initial phase “local trauma-proinflammatory cytokine production” necessary for maturation and migration of dendritic cells, and T cells activation (Corsini et al., 2009; Corsini et al., 2013a; Galbiati et al., 2011; Galbiati et al., 2014a).

Extracellular matrices (ECM) represent a complex network of macromolecules, including collagens, elastin, laminins, and glycosaminoglycans (GAGs), constituting the cell microenvironment. ECM provides the bulk, shape and strength of tissues *in vivo* (reviewed in 13). In addition to its structural role the ECM is critically important for cell growth, survival, differentiation and key to various disease processes including inflammation and cancer (Hynes, 2009; Sorokin, 2010; Afratis et al., 2013; Nikitovic et al., 2012). Importantly, it has been shown that the early/chronic inflammation stages of CD are associated with temporal changes in

the expression, deposition, and degradation of inducible extracellular matrix components including HA (Esser et al., 2012) as well as fibronectin, tenascin-C, fibulin-1, and 2 and various matrix metalloproteinase (MMP) (Kusubata et al., 1999). Moreover, a participation of increased oxidative stress and generation of radical oxygen species (ROS) have also been correlated to CD-related alterations in ECM constitution (Esser et al., 2012; Corsini et al., 2013b). Indeed, ROS induced ECM¹ remodelling in general seems to perpetrate a crucial role during the progression of pathological conditions (Nikitovic et al., 2013a; Nikitovic et al., 2014).

HA is a free unbranched GAG composed of repeating disaccharides of N-acetyl-glucosamine and glucuronic acid units, synthesized in a unique manner by a family of hyaluronan synthases (HAS 1–3), degraded by hyaluronidases (HYAL 1 and 2) and exerts its biological effects by binding to families of cellular receptors, the hyaladherins (Antonio and Iozzo, 2001; Nikitovic et al., 2013b). HA is ubiquitously distributed in the ECM (Noble, 2002), and it is primarily produced by dermal fibroblasts and epidermal keratinocytes and to a smaller amount by other cell types including smooth muscle cells (Nusgens, 2010). A strict dependence on size is evident on HA biological roles as high molecular weight HA (HMWHA) ($<1 \times 10^6$ kDa) is anti-angiogenic, anti-inflammatory and immunosuppressive (Stern et al., 2006; Kim et al., 2008). On the other hand low molecular weight HA (LMWHA) breakdown products, occurring in the range from 1.2 to 500 kDa, induce pro-inflammatory innate immune responses

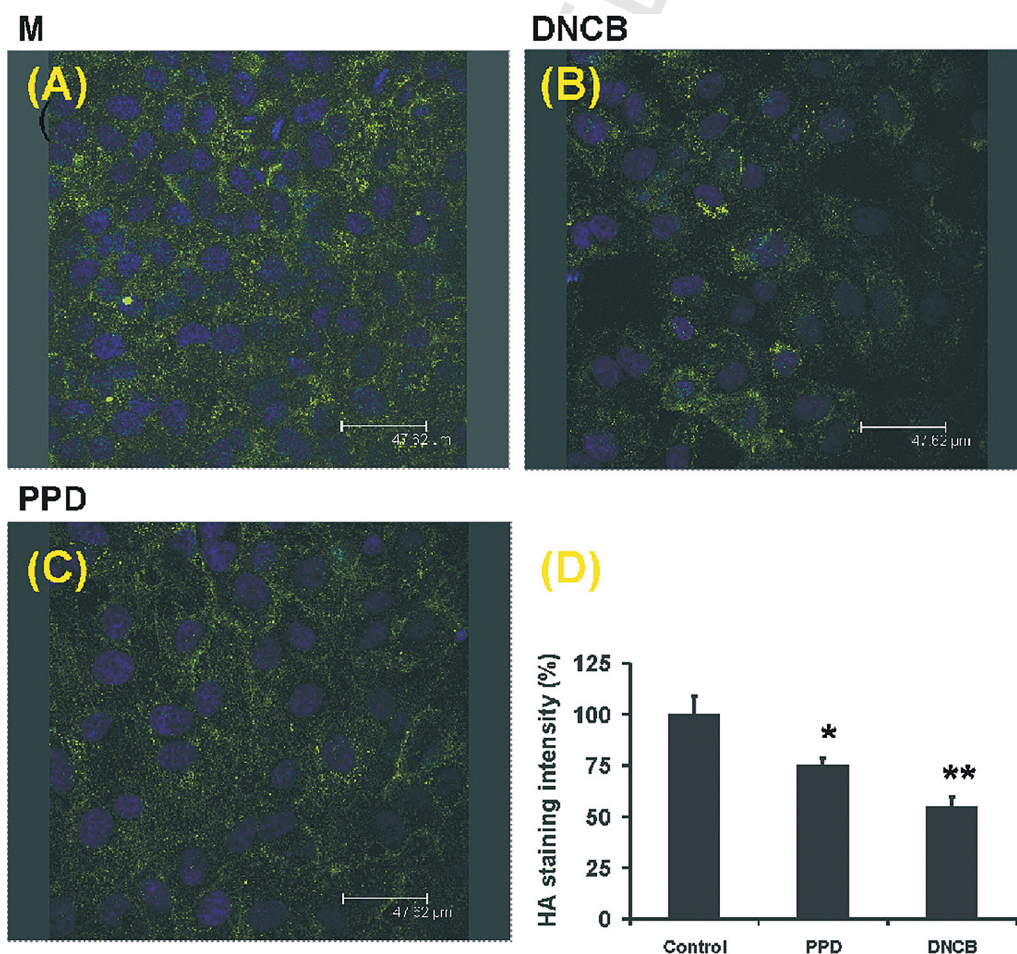


Fig. 1. Contact allergens affect HA expression in the human keratinocyte cell line NCTC2544. Cells were treated with DMSO as vehicle control (A), DNCB (2 μ g/ml) or PPD (30 μ g/ml) (C) for 24 h, whereupon biotinylated hyaluronan binding protein (HABP) was added. Cells were counterstained with DAPI. The stain intensity was monitored using a digital image processor connected to a microscope. The results represent the average of three independent experiments in triplicate. Means $\pm$ S.E. are plotted, with * $p < 0.05$ and ** $p < 0.01$ vs control.

(Noble, 2002), presumably via TLR2 and/or TLR4 in immune cells like dendritic cells (DCs) or macrophages (Termeer et al., 2002a) or via CD44 in other cells (Bourguignon et al., 2011). LMWHA fragments are generated during inflammation or tissue damage (Jiang et al., 2005), either enzymatically by HYAL 1 and 2 or non-enzymatically by ROS (Agren et al., 1997; Galbiati et al., 2014b; Monzon et al., 2014). HA breakdown in CD seems to be initially directly mediated by ROS, given the rapid induction of ROS by contact sensitizers (Galbiati et al., 2014b). Early chemical fragmentation (Agren et al., 1997) is followed by enzymatic cleavage, since ROS also regulate p38 MAPK dependent up-regulation of hyaluronidase, as recently described for lung inflammation (Monzon et al., 2014). In continuation, LMWHA fragments can regulate the activation of crucial immunomodulatory pathways (Scheibner et al., 2006) expression of proinflammatory genes (Collins et al., 2011) as well as cytokines, such as IL-8, IL-10, and IL-12 (Boodoo et al., 2006; Hodge-Dufour et al., 1997; Hodge-Dufour et al., 1998). Importantly, these molecules, including TNF- α , IL-18, and IFN- γ (Okamura et al., 1995; Cumberbatch et al., 2001; Antonopoulos et al., 2008), have been demonstrated *in vivo* to be directly responsible for driving the effector phases of chemical skin sensitisation (Martin et al., 2011). The participation of HA in the induction of skin inflammation by contact sensitizers like 2,4,6-trinitrochlorobenzene (TNCB), was demonstrated by significantly reduced contact sensitisation in germ-free mice pre-treated with an inhibitor of HA function (Pep-1) (Martin et al., 2008). This process is crucial for CD since sensitisation was completely prevented by pre-treatment of the skin with

antioxidants or the hyaluronidase inhibitor, aristolochic acid (Esser et al., 2012). The correlation of HA-metabolism with IL-18 production in keratinocytes stimulated by contact sensitizers has not previously been studied. Our results demonstrate that HA fragments are important mediators in the process of contact sensitisation leading to keratinocyte IL-18 dependent responses.

2. Materials and methods

2.1. Materials

Skin sensitizers 2,4-dinitrochlorobenzene (CAS No 97-00-7, DNCB) and p-phenylenediamine (CAS No 106-50-3, PPD), aristolochic acid, diphenylene iodonium (DPI), hyaluronidase (*Streptomyces hyalurolyticus*) were purchased from Sigma (USA). Mouse monoclonal (blocking) anti human CD44 antibody was from Lifespan Biosciences (USA), hyaluronan binding protein (HABP) was from Seikagaku (Japan) and the low molecular weight hyaluronan (10 kDa, LMWHA) from R&D Systems (UK). Non-blocking anti-CD44 (HCAM, sc-9960) antibody as well as all secondary antibodies (antigoat and anti-mouse) were purchased from Santa Cruz Biotechnology (USA). Cell culture reagents were obtained from GIBCO-Invitrogen (USA).

2.2. Cells

The human keratinocyte cell line NCTC2544 (Istituto Zooprofilattico di Brescia, Italy) was cultured in RPMI 1640 containing 2

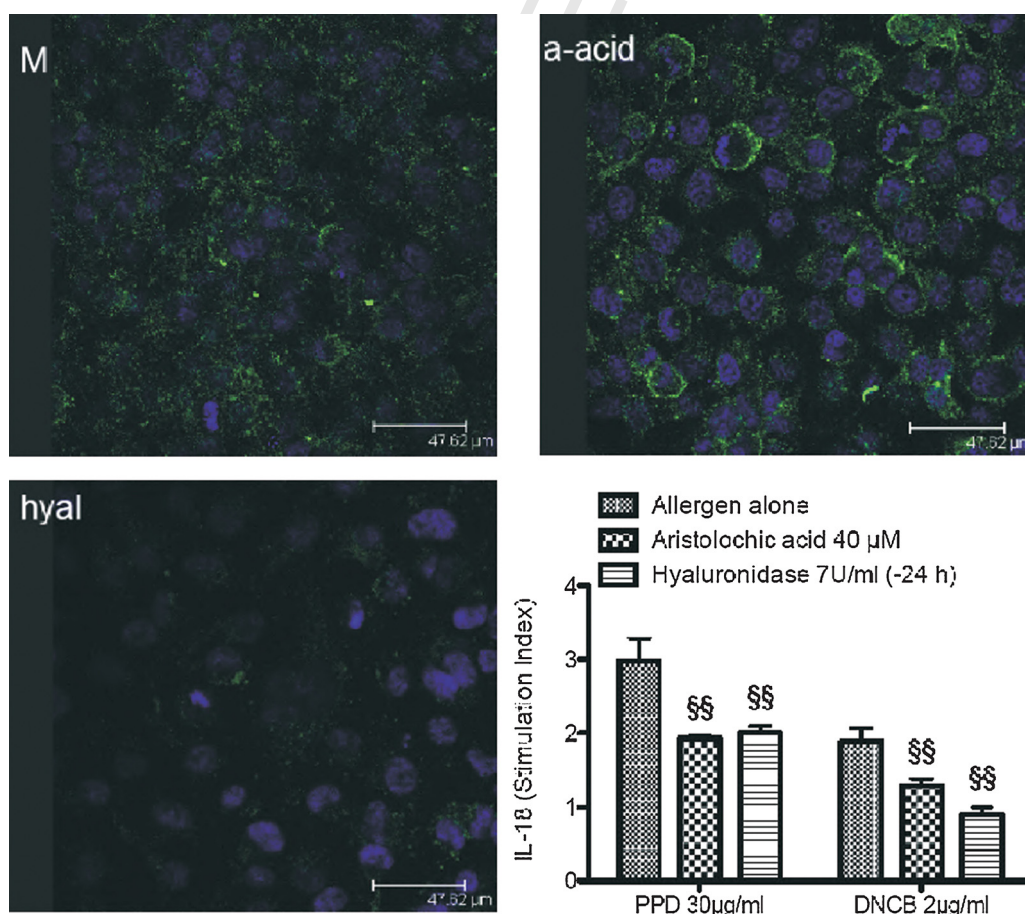


Fig. 2. Contact sensitizer-induced IL-18 production is dependent on HA expression. Cells were treated with aristolochic acid (a-acid, 40 μM) (B) or hyaluronidase (7 U/ml) (C) for 24 h and HA deposition visualized with HABP. (D) Following aristolochic acid or HYAL treatment cells were treated for 24 h with PPD (30 μg/ml) and DNCB (2 μg/ml). IL-18 production was assessed by ELISA. Results are representative of three independent experiments. Means $\pm$ SE are plotted, with §§ p < 0.01 vs cells treated with allergens alone.

mM L-glutamine, 0.1 mg/ml streptomycin, 100 IU/ml penicillin, supplemented with 10% heated-inactivated fetal calf serum (media) at 37 °C in 5% CO₂. For all experiments, cells were seeded at the density of 2.5×10^5 cells/ml.

2.3. Cytokine production

For the assessment of IL-18 production after exposure culture medium was discarded, monolayer gently washed twice with 1 ml of PBS, and cells lysed in 0.25 ml of 0.5% Triton X100 in PBS. Cell lysates were stored at –80 °C until measurement. Intracellular IL-18 content was assessed by specific sandwich ELISA commercially available (MBL, Nagoya, Japan). Results are expressed in pg/mg of total intracellular protein content or as Stimulation Index. The protein content of the cell lysate was measured using a commercially available kit (Bicinchoninic acid solution and Copper (II) sulfate solution from Sigma). Stimulation index (SI) was calculated as follows:

SI = IL-18 in chemical-treated cells/IL-18 in vehicle-treated cells

2.4. RNA isolation and real-time PCR

Total RNA was isolated using TRIzol™ (GIBCO, USA) according to the manufacturer's instructions. 500 nanograms of total RNA were used for cDNA synthesis using the PrimeScript RT reagent Kit (TAKARA, Japan) following manufacturer's instructions. Real-time PCR was performed using Mx3005P cycler (Stratagen, USA). The primers were mRNA specific to avoid misleading results from traces of DNA contamination. For real-time PCR reactions, the KAPA SYBR FAST Universal qPCR Kit (KAPA Biosystems, USA) in a total volume of 20 µL was used. The PCR conditions used for amplification were: 94 °C for 15 min, then 40 cycles at 94 °C for 20 s, 55 °C for 30 s, 72 °C for 30 s, followed by 72 °C for 10 min. Standard curves were run in each optimized assay, which produced a linear plot of threshold cycle (Ct) against log (dilution). The amount of each target was quantified based on the concentration of the standard curve and was presented as arbitrary units. GAPDH was used as housekeeping gene.

2.5. Immunofluorescence

Cells were seeded on round coverslips placed in 24-well plates and incubated for 24 h. At this point treatments were added, and the cells incubated for 24 h at 37 °C and 5% CO₂. Cells were fixed in 5% formaldehyde and 0.02 g sucrose in PBS for 10 min. After three washes with PBS containing 1% bovine serum, biotinylated hyaluronan binding protein (HABP; 1:10 dilution in PBS 1% bovine serum) was added for 1 h at RT. Coverslips not incubated with the primary antibody were utilized as negative control. The coverslips were washed again and incubated for 1 h, in the dark at RT, with FITC-Streptavidin (Zymed). DAPI nuclear staining was applied for 10 min. Coverslips were then placed onto slides using ProLong Gold antifade reagent (Molecular Probes, USA) as a mountant and visualised using a LEICA DM2500 microscope fitted with a DFC490 digital camera. The stain intensity was monitored using a digital image processor connected to a microscope, at five different positions of image taken. Stain intensity was quantified by image analysis (ImageJ 1.4.3.67 Launcher Symmetry Software) as previously described (Kouvidi et al., 2011).

2.6. Statistical analysis

All experiments were repeated at least 3 times, with representative results shown. Data are expressed as mean ± standard error (SE). The statistical significance was evaluated by

Student's *t*-test or one way ANOVA with Tukeys post-test, using GraphPad Prism (version 4.0) software. Effects were designated significant if *p* < 0.05.

3. Results

3.1. Contact allergens induce HA degradation in NCTC2544 keratinocyte cell line

In order to visualize both the content of HA in the ECM of KC and the possible effect of contact allergens, immunocytofluorescence using biotinylated HABP on fixed cells was performed. As shown in Fig. 1, KC pericellular matrix exhibits strong HA staining (Fig. 1A), confirming the ability of KC to synthesize HA. Treatment with PPD (30 µg/ml) and DNCB (2 µg/ml) for 24 h significantly decreased the HA staining intensity of the ECM surrounding KC (Fig. 1B and C), indicating that both allergens affect HA deposition. These data correlate well with results obtained in a mouse model of allergic

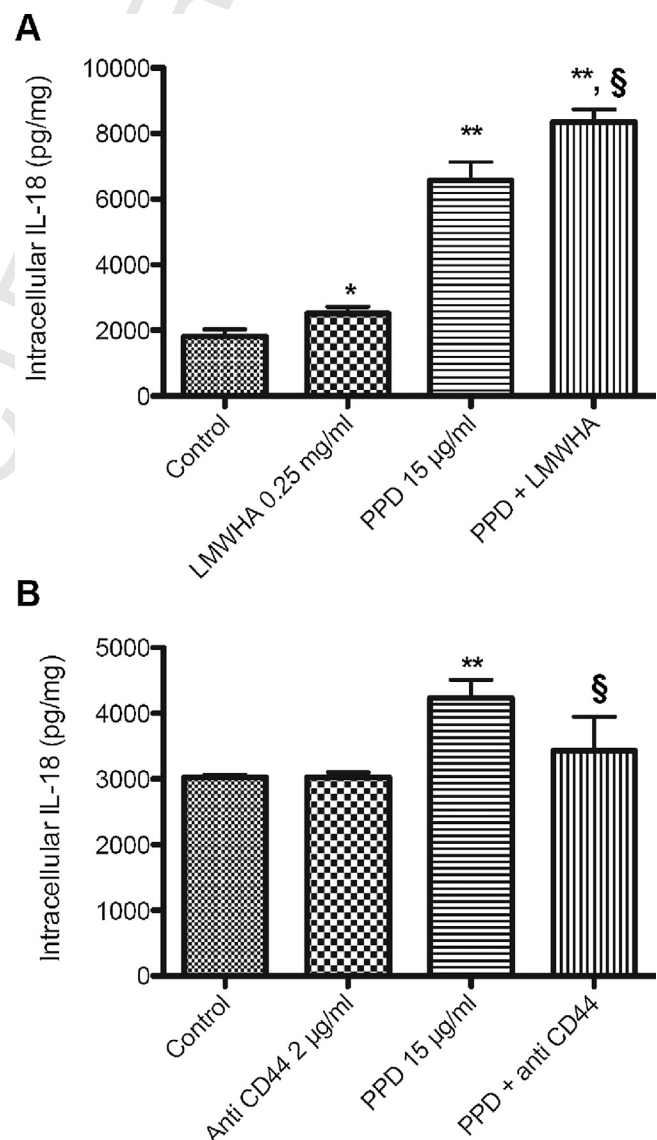


Fig. 3. Role of LMWHA and neutralization of CD44 in PPD-induced IL-18 production. KC cells were treated with LMWHA (250 µg/ml) or blocking anti human CD44 antibody (2 µg/ml) in the presence or absence of PPD (15 µg/ml) for 24 h. Results are representative of three independent experiments. Means ± SE are plotted, with **p* < 0.05 and ***p* < 0.01 vs control and §*p* < 0.05 vs cells treated with PPD.

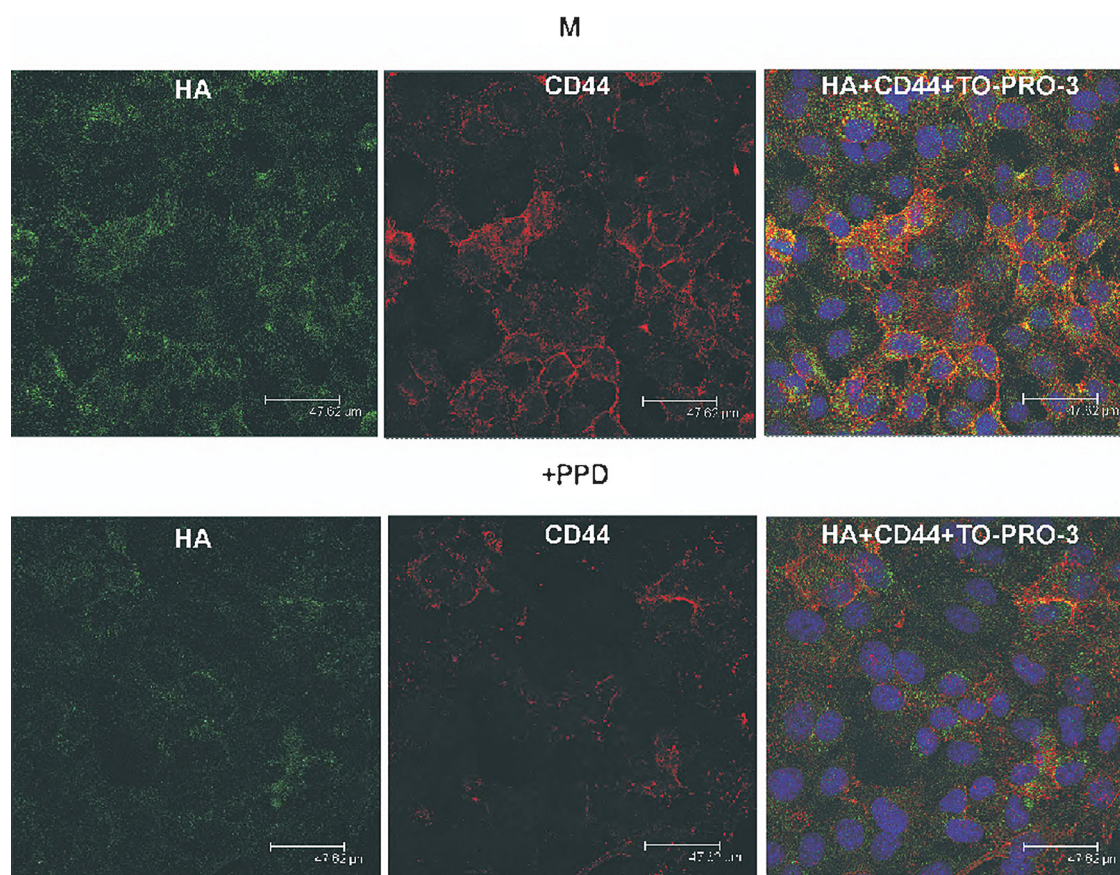


Fig. 4. Double staining of HA and CD44. Cells were treated with medium (M) or PPD (30 μg/ml) for 24 h and CD44 + HA staining was visualized with a non-blocking anti CD44 antibody and HABP, respectively. Nuclear staining was performed using TO-PRO-3. Slides were analyzed by confocal microscopy.

contact dermatitis (Esser et al., 2012), where exposure to the contact allergen TNBS resulted in HA degradation. Densitometric analysis of staining intensity (Fig. 1D) demonstrates that both PPD (30%, $p < 0.05$) and DNCB (45%, $p < 0.01$) significantly decrease HA expression in KC.

3.2. Contact sensitizer-induced IL-18 production is dependent on HA expression

As also previously reported, PPD and DNCB strongly induced IL-18 production ($p < 0.001$) in human KC (Fig. 2D). In order to examine the effect of HA-deposition on PPD and DNCB induced IL-18 production, endogenous HA expression was modulated by means of inhibition of HYAL by aristolochic acid treatment and HA depletion by hyaluronidase pre-treatment. Cells were cultured for 1 h in the presence of aristolochic acid (a-acid), a specific inhibitor of HYAL activity, and then PPD and DNCB were added for 24 h. Treatment with aristolochic acid resulted in increased deposition of HA at the pericellular matrix as visualized by HA-binding protein (HABP) and estimated as intensity of stain ($p < 0.01$) (Fig. 2B). Prevention of HA degradation significantly inhibited both PPD and DNCB induced IL-18 secretion ($p < 0.01$) (Fig. 2D). In order to deplete cells of HA, they were treated for 24 h with HYAL 1 (7 U/ml), medium removed and cells exposed for 24 h to PPD and DNCB. Treatment with HYAL induced a significant decrease in pericellular matrix HA deposition ($p < 0.001$) (Fig. 2C). Under these conditions, HA was previously found to be mostly degraded to 4-mers and 6-mers (Kouvidi et al., 2011). In the absence of HA, DNCB-induced IL-18 production was completely abrogated, while the effect of PPD

was significantly reduced (Fig. 2D), demonstrating that HA is required for contact allergen-induced IL-18 production in KC.

3.3. LMWHA can induce IL-18 and CD44 is involved in contact allergen-induced IL-18 production

To further corroborate the ability of HA degradation products to induce IL-18 production, cells were treated with commercially available LMW HA (<10 kDa). As shown in Fig. 3A, LMWHA 0.25 mg/ml was able to trigger IL-18 production ($p < 0.05$ vs control cells) and the co-treatment with PPD resulted in higher IL-18 levels ($p < 0.05$ vs cells treated with PPD alone). These data further support the pro-inflammatory effects of LMWHA, and its' possible role as DAMP in chemical allergen-induced KC activation.

The role of TLR4 and CD44 in LMWHA fragments-induced inflammatory responses has been shown to be cell specific (Jiang et al., 2005). In macrophages, cytokine production is mediated by TLR4 and it is independent from CD44 expression. In breast cancer cells, both receptors are involved in cytokine production (Bourguignon et al., 2011). We have previously demonstrated that blocking TLR4 in KC can reduce contact allergen-induced IL-18 production (Galbiati et al., 2014b). Using a CD44 blocking antibody, PPD-induced IL-18 production was almost completely abrogated (Fig. 3B), further supporting the involvement of HA in contact allergen-induced IL-18 production. To visualize CD44/HA binding in KC a specific non-blocking CD44 antibody and HABP were utilized and staining analysed by confocal microscopy (Fig. 4). Confocal microscopy demonstrated

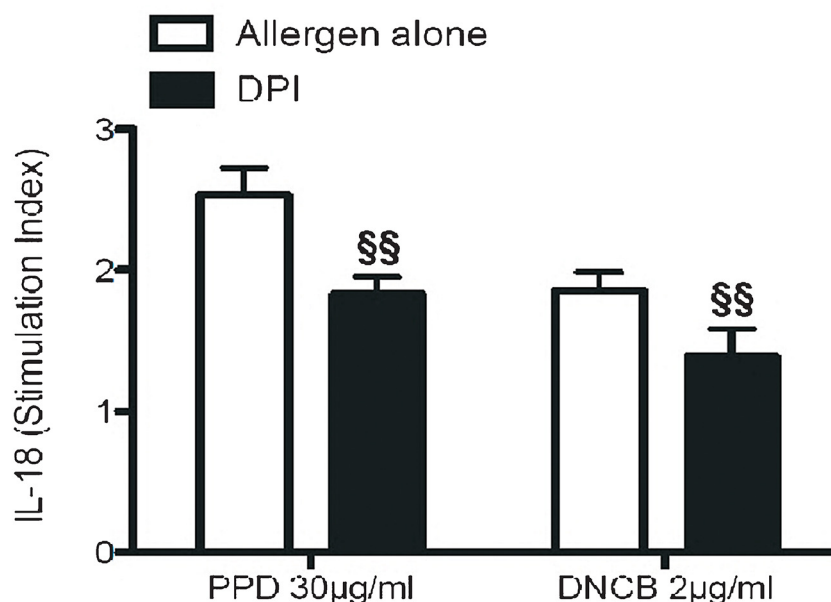
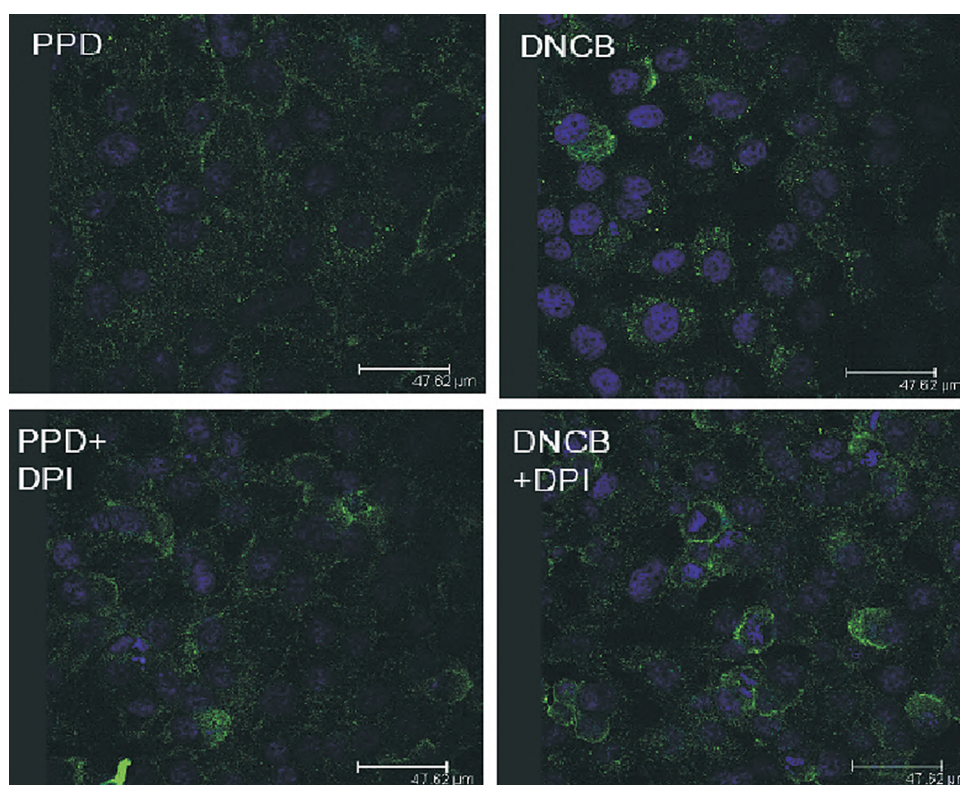


Fig. 5. Antioxidant prevents chemical allergen-induced HA degradation and IL-18 production. Cells were treated with PPD (30 μg/ml) and DNCB (2 μg/ml) without (A and B) and in the presence of DPI (2.5 μM) (C and D) for 24 h and HA deposition visualized utilizing HABP. Under same conditions, the production of IL-18 was monitored by ELISA (E). Results are representative of three independent experiments. Means ± SE are plotted, with §§*p* < 0.01 vs cells treated with allergen alone.

a high level of CD44/HA binding in human KC which is modulated by PPD treatment

3.4. Role of ROS in contact allergen-induced HA degradation and IL-18 production

ROS are suggested to participate in the modulation of HA expression perpetrated by contact sensitizers in an experimental model of ACD (Esser et al., 2012). We have previously

demonstrated a central role of oxidative stress in chemical allergen-induced IL-18 production in KC and their ability to rapidly induce ROS production (Galbiati et al., 2014b). In order to examine the involvement of ROS in chemical allergen-induced HA degradation, cells were sensitized with PPD or DNCB without (Fig. 4A and B) or in the presence of the antioxidant diphenylene iodonium (DPI), a NOX4/NADPH oxidase inhibitor (Fig. 5C and D). The blockage of the oxidative stress prevented PPD and DNCB induced HA degradation as quantified by stain intensity (Fig. 5C–

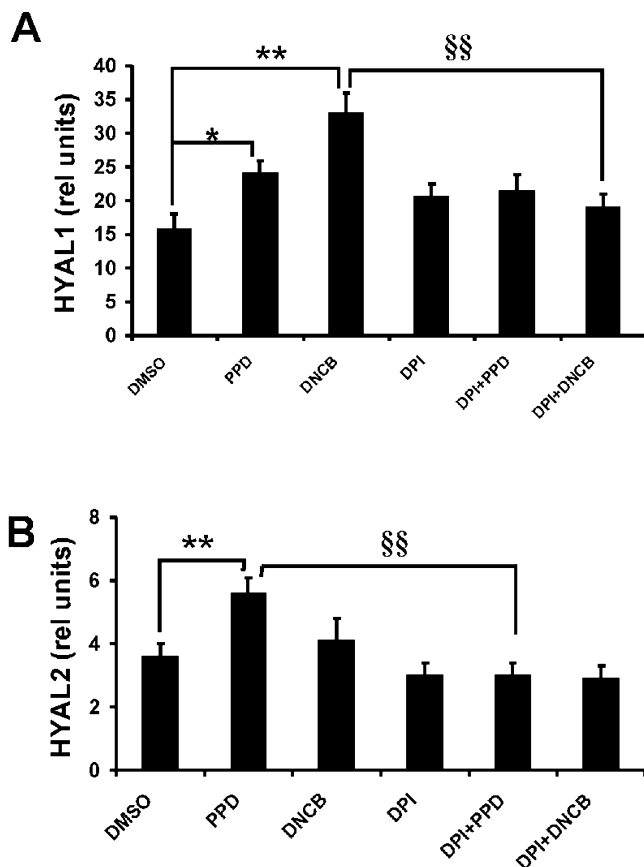


Fig. 6. Contact allergens induced HYAL 1 and 2 expression is abolished by antioxidant. Cells were treated with DMSO as vehicle control, PPD (30 μ g/ml), DNCB (2 μ g/ml) for 24 h or or pretreated with DPI (1 h) and treated with, PPD (30 μ g/ml), DNCB (2 μ g/ml) for 24 h. HYAL 1 (A) and HYAL 2 (B) mRNA expression were assessed by Real-time PCR. Results represent the average of three separate experiments in triplicate. Means $\pm$ S.E. are plotted, with * p < 0.05 and ** p < 0.01 vs control; with §§ p < 0.01 between treatments.

E), resulting in a significant reduction of IL-18 production (§§ p < 0.01 vs cells treated with allergen alone), further supporting the role of the oxidative stress as the initial step. In order to identify the mechanisms of HA catabolism we examined the PPD and DNCB effects on the expression of HA-degrading hyaluronidases. Real-time PCR analysis shows that KC express both HYAL 1 and 2 isoforms, (Fig. 6A and B). Both PPD (p < 0.05) and DNCB (p < 0.01) increased HYAL 1 mRNA expression as compared to the DMSO control (Fig. 6A). Likewise, a significant upregulation of HYAL 2 expression was evident upon treatment with PPD (p < 0.01). When the cells were pretreated with DPI (2.5 μ M) before treatment with contact allergens the induction in HYALS expressions was inhibited (Fig. 6A and B). Therefore, at least partly, the tested allergens through the up-regulation of HYAL isoforms can modulate the degradation of HA in human KC. Pretreatment with DPI demonstrates that the effect of PPD and DNCB on HYALS expression is ROS-dependent.

4. Discussion

We found that chemical allergen-induced HA degradation is an important mechanism regulating KC functions during inflammatory responses. HA fragments, acting as DAMP, via CD44/TLR4, mediated IL-18 production following exposure to contact allergens. Oxidative stress appears to be the initial step in KC activation, as all the sequels of events can be blocked using antioxidants. Oxidative stress is followed by HA degradation, cellular damage

and release of other DAMPs, i.e. HMGB1, leading to inflammasome activation and IL-18 production.

During acute and chronic inflammation or tissue injury, reactive oxygen species (Tammi et al., 2002) and matrix metalloproteinases (Isnard et al., 2011) can significantly alter the HA turnover, generating local (Liang et al., 2011) and systemic (George and Stern, 2004) accumulation of LMWHA fragments. Endogenous LMWHA is a member of the so-called damage associated molecular patterns (DAMPs), which has been suggested to elicit immune-stimulatory effects by triggering CD44 (Campo et al., 2010), TLRs (Termeer et al., 2002b) or NOD like receptor (NLR) signaling. Indeed, LMWHA has been shown to be a potent stimulant of the inflammatory response able to induce cPLA_{2 α} , ERK 1/2, MAPK p38 and JNK phosphorylation, as well as activated COX2 expression and PGE₂ production in primary human monocytes, murine RAW264.7 and wild-type bone-marrow derived macrophages through a TLR4/MyD88 pathway (Sokolowska et al., 2014). LMWHA can induce the expression of several pro-inflammatory cytokines, including interleukin-8 (Campo et al., 2010).

In the present study, treatment of human KC with DNCB and PPI caused a significant degradation of HA at the KC pericellular matrix. The decrease in HA deposition was correlated to an upregulation of HYAL 1 and 2, enzymes responsible for HA cleavage.

Moreover, we demonstrated that the effects on HA-matrix were partly dependent on ROS generation as co-treatment with the antioxidant DPI prevented DNCB and PPI effects on HA expression. These results correlate well with an *in vivo* study performed in a mouse model of ACD demonstrating that contact sensitizers induce production of ROS and a concomitant breakdown of HA to pro-inflammatory LMWHA fragments in the skin (Esser et al., 2012). Moreover, a ROS scavenger, superoxide dismutase 3 (SOD-3) suppresses LMWHA mediated skin inflammation by inhibition of TLR-4 signaling pathway; more specifically SOD-3 inhibits ROS induced trafficking of TLR-4 to lipid rafts (Kwon et al., 2012).

The purpose of this study was to examine the potential correlation between LMWHA downstream signaling and IL-18 production by KC. Use of aristolochic acid, a potent inhibitor of HYAL action, resulted in a significant downregulation of IL-18 production by KC. Likewise, treatment of KC with hyaluronidase, which results in very small oligosaccharides, reduced the effect of contact allergens on IL-18 production, further supporting the role of HA degradation in KC activation. This is well in accord with previous studies demonstrating that, the effect of HA on the inflammatory response appears to be strictly related to its molecular size, i.e. larger HA has anti-inflammatory activity while smaller HA has pro-inflammatory activity (Jiang et al., 2005; Yamawaki et al., 2009; Pasonen-Seppänen et al., 2012). LMWHA increased TLR-4, MyD88, TRAF-6, iNOS, MMP-13 expression and pro-inflammatory cytokines IL-1 β and IL-8 expression, modulating both innate and acquired immune response of these cells (Campo et al., 2010). CD44 is the main cell surface receptor for HA, binds HA to the plasma membrane of many cell types and assists in HA degradation, including in KC (Pasonen-Seppänen et al., 2012; Kugelman et al., 1999). KC express mainly CD44 isoforms which contain variant exons v3-v10 (Kugelman et al., 1999). Interestingly, 6-mer HA oligosaccharides were previously found to stimulate pro-inflammatory responses in mouse synovial fibroblasts by modulating both CD44 and TLR4 downstream signaling. CD44 stimulation activates the protein kinase C (PKC) family which in turn activates the transcriptional nuclear factor kappa B (NF- κ B) responsible for the expression of the inflammation mediators such as tumor necrosis factor alpha (TNF- α), IL-18, IL-33 as well as matrix metalloproteinases (MMPs), that are important mediators in rheumatoid arthritis (Campo et al., 2010). The addition of HA-binding protein inhibited HA activity and was

able to reduce the effects of oligosaccharides and the consequent inflammatory response (Campo et al., 2012). Treatment of KC in this study with a specific anti-CD44 antibody ameliorated the effect of LMWHA on IL-18 production which correlates well with previous studies showing that CD44/LMWHA interactions elicit immunomodulatory responses (Bourguignon et al., 2011). Another potentially important point is that degradation and clearance of HA at inflammatory sites occur by a CD44-dependent uptake mechanism (Noble, 2002; Stern, 2003). Moreover in a previous study, treatment with HMWHA of genetically diabetic mice with diabetic nephropathy significantly reduces expression of inflammation mediators, including IL-18 (Campo et al., 2010) suggesting antagonistic effects of LMWHA and HMWHA on CD44-dependent inflammatory response. In conclusion, the present study demonstrates a direct involvement of HA metabolism on chemical allergens evoked IL-18 production in KC.

Transparency document

The Transparency document associated with this article can be found in the online version.

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