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Role of oxidative stress in chemical allergens induced skin cells activation

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ABSTRACT

Allergic contact dermatitis (ACD) is an important occupational and environmental disease caused by topical exposure to chemical allergens. It describes the adverse effects that may result when exposure to a chemical elicits a T cell-mediated inflammatory skin disease. The ability of contact sensitizers to induce the oxidative stress pathway in keratinocytes and dendritic cells has been confirmed by several authors. Reactive oxygen species (ROS) can serve as essential second messengers mediating cellular responses resulting in immune cells activation. Oxidative stress may be the starter point, as it leads to the activation of transcription factors and signaling pathways, including NF- κ B and p38 MAPK, which leads to the release of cytokines and chemokines. ROS are also involved in the activation of the NLRP3/NALP3 inflammasome, which is required to direct the proteolytic maturation of inflammatory cytokines such as IL-1 β and IL-18, which are all integral to the process of dendritic cells mobilization, migration and functional maturation. Moreover, emerging evidence correlates ROS to changes in the constitution of the extracellular microenvironment found to facilitate ACD. The purpose of this review is to provide both conceptual and technical frameworks on the role of oxidative stress in chemical allergy.

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1. Introduction

Irritant and allergic contact dermatitis are undesired side effects in the development of drugs and cosmetics as well as after contact with environmental or industrial chemicals. A recent epidemiological study of the general population suggests that the prevalence of contact allergy to at least one sensitizing chemical is ~15–20% (Peiser et al., 2012; Thyssen et al., 2007), making contact allergy a common and important environmental and occupational health hazard.

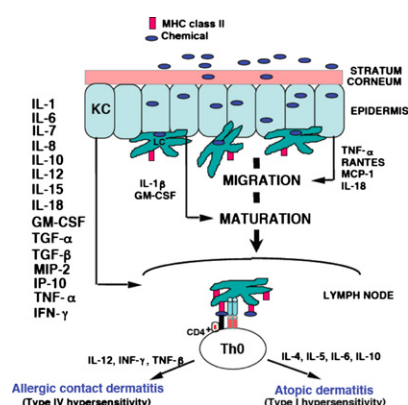
Whereas irritant contact dermatitis is a skin inflammation induced by primary contact with chemicals, and it is not mediated by lymphocytes, allergic contact dermatitis represents a delayed type hypersensitivity reaction caused mainly by reactive T helper 1 and interferon (IFN)- γ producing CD8⁺ T cells (Tc1), which requires previous sensitisation by the same chemicals (Cavani et al., 2007; Basketter et al., 2008; Nosbaum et al., 2009). Contact hypersensitivity can occur as a result of exposure to a wide variety of chemicals and drugs (Nethercott and Holness, 1989), cosmetics

(De Groot, 1988; Remaut and Thune, 1992; Paulsen and Andersen, 1993), and various metals including nickel (Picardo et al., 1990) and chromium (Nethercott and Holness, 1989). More than 3000 chemicals have been shown to cause skin sensitisation. In Fig. 1 a schematic representation of the key passages involved in the induction of chemical-induced skin sensitisation is depicted. In order for a chemical to induce skin sensitisation, it must satisfy certain requirements (key passages). Following absorption through the stratum corneum and gain access to the viable epidermis (usually chemical allergens have low molecular weight, <1000 Dalton, and appropriate lipophilicity, i.e. LogP ~2), as chemicals themselves are too small to cause an immunogenic reaction, they must bind to extracellular and cellular skin proteins to form a complete antigen. The development of allergic contact dermatitis then requires the activation of innate immune cells, including keratinocytes (KC) required for maturation and migration of dendritic cells (DC), and DC, required for the activation of T cells. For organic chemical allergens it is known that they fail to fully activate human or mouse DC in vitro, it is the skin microenvironment that provides the danger signals or DAMPs (damage associated molecular patterns), including ROS, uric acid, hyaluronic acid fragments and ATP, required for activation of pattern recognition receptors (PRRs) and full activation of DC, leading to the up-regulation of the co-stimulatory molecules CD40, CD80 and CD86. The acquisition of specific immune response will then take place at the level of draining lymph

Abbreviations: DC, dendritic cells; IL, interleukin; KC, keratinocytes; ROS, reactive oxygen species.

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KEY PASSAGES

1. Absorption and local trauma — proinflammatory cytokine production by keratinocytes (KC), and release of danger signals and damage associated molecular patterns, including ROS, uric acid, hyaluronic acid fragments, ATP, HMGB1, etc.
2. Protein binding and formation of a complete antigen
3. Antigen processing
4. Langerhans cells (LC)/dermal dendritic cells (DC) maturation and migration
5. Antigen presentation to Th cells and generation of memory and effector T cells (immunogenicity)

Fig. 1. Key passages in the induction of skin sensitisation.

nodes, where DC migrate via the afferent lymph vessels and stimulate the activation of hapten-specific responsive T-cells and the generation of Tc1 effector cells. Upon renewed contact with the same chemical, memory T cells are recruited to the site of contact, where interactions between T cells and antigen presenting cells can take place directly in the skin, thus initializing the inflammatory reaction (elicitation phase).

It is important to stress that hypersensitivity reactions are the result of normally beneficial immune responses acting inappropriately toward harmless chemicals, which are 'smelled' as pathogens in some individuals (Corsini and Kimber, 2005). In other words, chemical allergens mimic infection by triggering innate immune responses via pattern recognition receptors and endogenous danger signals, inducing 'sterile inflammation' (Martin, 2012).

1.1. Role of Ros and oxidative stress in contact allergy

Evidence indicates that allergic and inflammatory skin diseases are mediated by oxidative stress (Okayama, 2005; Byamba et al., 2010). Proteomic and genomic analyses of human keratinocytes as well as of dendritic cells revealed large numbers of clearly sensitizer-specific markers, with several interesting overlaps. Particularly, markers relating to the Nrf2-mediated oxidative response and oxidative stress in general were identified repeatedly, very much in line with the prominent position that oxidative stress holds in chemical sensitisation (Ryan et al., 2004; Ade et al., 2008; Hooyberghs et al., 2008; Johansson et al., 2012; Van der Veen et al., 2013).

Electrophilicity is one of the most common features of skin contact sensitizers and it is necessary for protein haptentation. The Keap1 [Kelch-like ECH-associated protein 1]/Nrf2-signaling pathway is dedicated to the detection of electrophilic stress in cells leading to the upregulation of genes involved in protection or neutralization of chemical reactive species. Several Authors have proposed that monitoring of this pathway may provide new biomarkers (e.g., Nrf2, hmox1) for the detection of the sensitisation potential of chemicals both in KC and DC (Natsch and Emter, 2007; Ade et al., 2008). A significant inhibition of the expression of oxidative stress associated genes and CD86 expression was observed by preincubated with N-acetyl cysteine, a glutathione precursor used to reinforce the redox potential of cells, further supporting the role of oxidative/electrophilic stress in chemical allergen-induced DC activation. Mizuashi et al. (2005) showed in human monocyte-derived dendritic cells that chemical sensitizers induced oxidative stress measuring the glutathione GSH/GSSG ratio, as a redox mar-

ker. These authors also showed that reduction of the glutathione GSH/GSSG ratio was accompanied by CD86 upregulation and p38 mitogen-activated protein kinase (p38 MAPK) activation, suggesting that the electrophilic properties of chemical sensitizers may be perceived by DCs as a danger signal leading to DC maturation (Sasaki and Aiba, 2007).

ROS have long been considered as harmful by-products of endogenous oxygen metabolism or cellular responses to hazardous agents. ROS have been implicated in the pathogenesis of several disorders, including cancer, aging, atherosclerosis, chronic inflammation, etc. (Brieger et al., 2012; Kleikers et al., 2012; Sosa et al., 2012). It is now known that ROS at relatively low concentrations also serve as essential second messengers mediating cellular responses to many physiological stimuli (Lenaz, 2012). It is now understood that ROS are critical mediators of intracellular signaling. ROS are products of normal cellular metabolism and under physiological conditions, participate in maintenance of cellular 'redox' homeostasis. Overproduction of ROS, results in oxidative stress. Within the cell, ROS are mainly generated by mitochondrial electron transport systems I and III, nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, xanthine oxidase, nitric oxide synthase, cyclooxygenase, peroxisomes, cytochrome P-450 and ribonucleotide reductase systems. The NADPH oxidase, first identified in phagocytes and now found in virtually every tissue, is an example of a system that generates ROS not as a byproduct, but rather as the primary function of the enzyme system. A large number of scientific studies have highlighted the importance of ROS as a second messenger in numerous cellular processes, including cell proliferation, gene expression, adhesion, differentiation, senescence, and apoptosis. The thiol/disulfide status of cysteine residues of cellular proteins is counterregulated by ROS and by endogenous disulfide reductase systems, and this redox regulation tightly controls transcriptional activities of transcription factors, including AP-1 and NF- κ B, as well as catalytic potentials of protein kinases and phosphatases and proteins more directly involved in oxidative stress detection (Keap-1 [Kelch-like ECH-associated protein 1]/Nrf2, hypoxia inducible factor-1, thioredoxin) (Cosentino-Gomes et al., 2012).

Within the immune system, ROS, besides acting as direct bacterial killing mechanisms, also stimulate the immune response through activation of signaling pathways, upregulation of surface co-stimulatory molecules, protein carbonylation, and cytokines secretion (Larbi et al., 2007; Sareila et al., 2011; Bertolotti et al., 2012). Amongst the mediators speculated to affect initial allergen sensitisation and the development of pathogenic allergic responses

to innocuous antigens are exogenously or endogenously generated ROS and reactive nitrogen species. It is known that ROS also plays a critical role in the regulation of NLRP3 inflammasome activation, which is required for the processing of interleukin-1 β and IL-18, and subsequent DC migration and functional maturation. Mitochondrial ROS drive NLRP3 inflammasome activation, leading to caspase-1 activation that, in turn, cleaves and activates the pro-inflammatory cytokines IL-1 β , IL-18 and IL-33 (Tait and Green, 2012). Engagement of certain Toll like receptors (TLR 1, 2 and 4) leads to mitochondrial translocation of the signaling adaptor TRAF6. At the mitochondria, TRAF6 interacts with ECSIT, a protein implicated in the assembly of complex I, leading to its ubiquitylation, which results in increased ROS production. Finally, among the protein carbonylated by contact allergens mitochondrial adenosine triphosphate (ATP) synthase was identified (Je et al., 2008), further supporting the central role of mitochondria in the pathophysiology of contact allergy.

To further support the role of oxidative stress in immune activation, antioxidants can prevent bidirectional DC-T cells activation processes during antigen presentation, and suppress the expression of adaptive immune response in animals (Matsue et al., 2003).

To summarize a key role of oxidative/electrophilic stress in the initiation of the sensitisation phase of contact allergy has been demonstrated. The redox imbalance provoked by sensitizers generates stress-related signal. In Fig. 2 a schematic view of contact allergens-induced oxidative/electrophilic stress is depicted. It has also been proposed that one of the biological roles of contact allergy is a defense reaction against chemicals having the potential to alter the redox balance (Sasaki and Aiba, 2007).

1.2. The development of skin sensitisation: chemical reactivity

One of the key molecular events in chemical-induced skin sensitisation is protein haptentation (the chemical modification of self-skin proteins). Chemical allergens are reactive exogenous compounds able to chemically modify skin proteins rendering them immunogenic and thus able to trigger a specific T-cell mediated immune response. Sensitizing chemicals have been divided according to their suspected reaction mechanism (Divkovic et al., 2005; Aptula et al., 2005). Many compounds are not allergenic themselves but are activated before skin contact (prehaptens), i.e. via air oxidation, or in the skin, i.e. metabolically (prohaptens), to form skin sensitizers (Karlberg et al., 2008).

The skin can best be described as a nucleophilic environment, rich in water and nucleophilic functional groups present in proteins, i.e. susceptible amino acids include tyrosine, tryptophan, histidine, lysine, methionine, and cysteine. The majority of contact sensitizers are electrophiles (Divkovic et al., 2005; Karlberg et al., 2008; Natsch et al., 2012). Five types of reactions have been recognized, including, S_N2 reactions, S_NAr reactions, Schiff base formations, Michael type additions, and acylation reactions. The possibility of a radical mechanism involved in antigen formation has also been proposed. Hydroperoxides, formed by air oxidation of many commonly used chemicals, have been shown to be strongly sensitizers. Most organic compounds, including turpentine, fragrance terpenes (i.e. limonene, linalool, geraniol), can undergo autoxidation, forming hydroperoxyde (ROOH). They are known to easily form radicals by cleavage of the labile O–O bond, the resulting radical may either bind directly with proteins or rear-

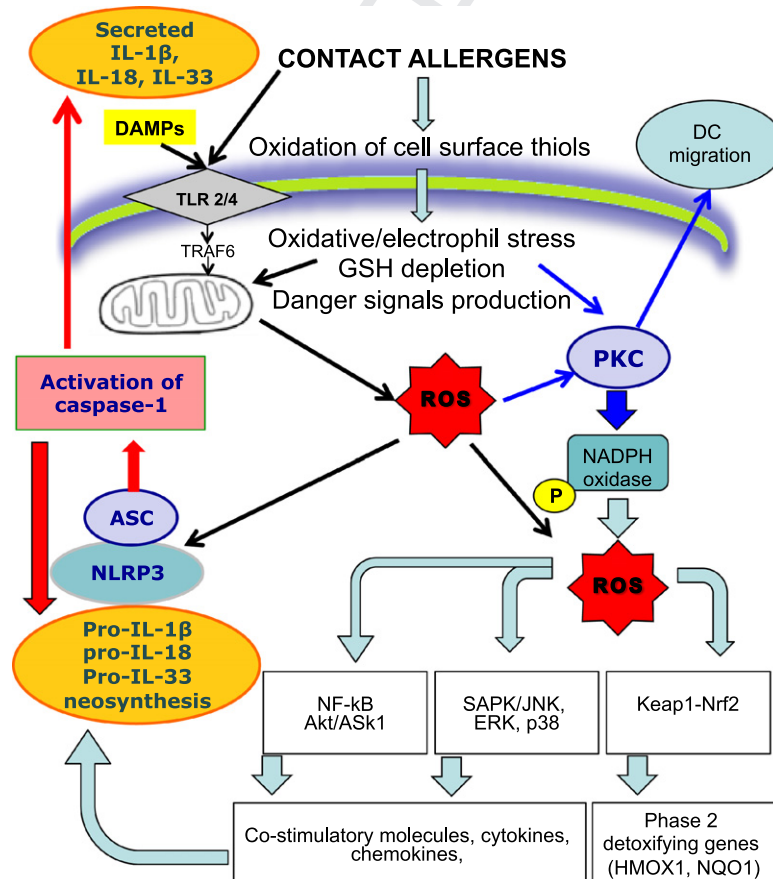


Fig. 2. Role of ROS and PKC in chemical allergen-induced DC/KC activation.

range to an epoxide to form a hapten–carrier complex (electrophilic haptens). A third type of contact allergens are metal ions, i.e. Ni, Cr, and Co, which form stable coordination complexes with proteins, then recognized as nonself.

Binding to the thiol group of cysteine is the one most often associated with sensitizers (Divkovic et al., 2005), which may lead to glutathione depletion and oxidative stress, tissue damage and increased inflammation.

On the basis of this mechanistic understanding, the relationship between reactivity of molecules toward proteins and their sensitisation potential has attracted increased interest and was used to develop experimental assay to predict the skin sensitisation potential of chemicals to replace in vivo animal tests (Gerberick et al., 2008).

1.3. The development of skin sensitisation: a role of KC

KC form an interface between the body and the environment, they are important guardians for the detection of danger signals and the consecutive initiation of an inflammatory response. KC play a role in all phases of allergic contact dermatitis, from the early initiation phase with the elaboration of inflammatory cytokines, that plays a role in Langerhans cells (LC) migration, and T-cell trafficking, through the height of the inflammatory phase with direct interactions with epidermotrophic T-cells, through the resolution phase of allergic contact dermatitis with the production of anti-inflammatory cytokines and tolerogenic antigen presentation to effector T-cells (Gober and Gaspari, 2008). KC sense haptens, and in turn initiate a program of enhances or de novo expression of inflammatory molecules representing the starting point of primary inflammation. KC mount immune responses through the secretion of a variety of inflammatory cytokines, soluble proteins and ROS. KC play a role in all phase of allergic dermatitis. They respond to allergen by producing a range of proinflammatory cytokines, which are important for dendritic cells maturation and migration to the draining lymph node, where the activation of the specific immune response take place (Vandebriel et al., 2005).

Recently, there have been several reports supporting the participation of ROS in the pathogenesis of contact dermatitis. Oxidative stress may be the starter point, as it leads to the activation of transcription factors and signaling pathways, including NF- κ B and p38 MAPK, which leads to the release of cytokines and chemokines (Gloire et al., 2005; Kim et al., 2010). Reactive oxygen species plays also an important role in the activation of the NLRP3/NALP3 inflammasome (Martinon et al., 2009; Bryant et al., 2009), which is required to direct the proteolytic maturation of inflammatory cytokines such as IL-1 β and IL-18 (Martinon et al., 2010). The ability of contact sensitizers to induce the oxidative stress pathway in keratinocytes (Natsch and Emter, 2008) has been recently confirmed by Vandebriel et al. (2010).

Among the cytokines produced by KC, IL-18 is of particular interest as it is a potent inducer of IFN- γ by activated T cells (Okamura et al., 1995). This cytokine has been shown to play a key proximal role in the induction of allergic contact sensitisation and to favor Th-1 type immune response by enhancing the secretion of pro-inflammatory mediators such as TNF- α , IL-18 and IFN- γ (Okamura et al., 1995; Cumberbatch et al., 2001; Antonopoulos et al., 2008). Human keratinocytes constitutively express IL-18 mRNA and protein (Naik et al., 1999), and works published by Naik et al. (1999) and Van Och et al. (2005) showed the induction of IL-18 following exposure to contact sensitizers.

We have recently demonstrated in human keratinocytes that allergen-induced IL-18 production requires ROS, as IL-18 production could be completely prevented by the antioxidant PDTTC (Galbiati et al., 2011). We have now extended this observation with the identification of the cellular sources of allergen-generated ROS.

As mentioned above, there are several known sources of cellular ROS, including NADPH oxidase, the mitochondrial respiratory cycle and the xanthine oxidase, which catalyses the oxidation of xanthine to uric acid, generating superoxide, a major ROS (Pacher et al., 2006). As shown in Fig. 3, IL-18 production by the contact allergens para-phenylene diamine (PPD) and 2,4-dinitrochlorobenzene (DNCB) can be significantly modulated by both rotenone and allopurinol, indicating mitochondria and xanthine oxidase as source of ROS involved IL-18 production.

Mehrotra et al. (2005) were the first to demonstrate that both contact allergens and irritants can induce ROS and nitrogen species in the human keratinocyte cell line A431. More recently, Kim et al. (2011) demonstrated in HaCaT cells that both the allergen DNCB and the irritant benzalkonium chloride can generate ROS in a time- and dose-dependent manner, however, only DNCB induced a ROS-dependent protein carbonylation, suggesting that the characteristic of ROS generated in response to chemicals might differ. Changes in ROS levels were detected after treatment for 30 min. Interestingly, not all allergens tested induced ROS production as assessed by DCFH, nickel and hydroquinone failed, suggesting that cellular oxidative stress after contact with chemicals might depend on the specific nature of the chemicals themselves. Further studies are necessary to verify oxidative stress-dependent or oxidative stress-independent mechanisms in chemicals-induced KC activation and to establish its role in contact allergy.

1.4. The development of skin sensitisation: the role of DC

In the induction of contact sensitisation, DC represents the most important initiators and regulators of contact allergy. Within the skin, there are at least two distinct subsets of DC, LC, localized in the epidermis, and dermal DC (dDC). LC are the only DC subset to reside in the epidermis and, were once considered to be wholly

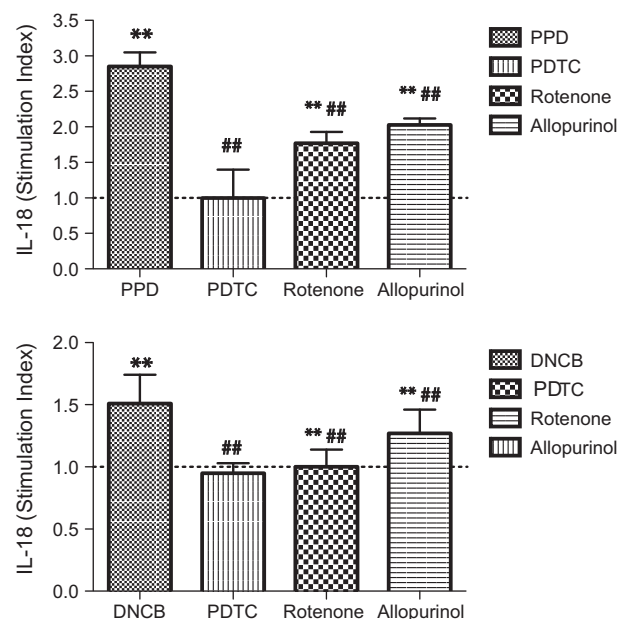


Fig. 3. Identification of the cellular source of ROS involved in allergen-induced IL-18 production. The human keratinocyte cell line NCTC 2544 was treated with PDTTC (100 μ M), rotenone (20 μ M) and allopurinol (100 μ M) for 1 h and after with PPD (30 μ g/ml) or DNCB (2 μ g/ml) or DMSO (0.2% final concentration) as vehicle control for 24 h. Results are expressed as IL-18 stimulation index (intracellular IL-18 in allergen treated cells / intracellular IL-18 in relative control cells), each value represents the mean $\pm$ SD, $n = 4$. Statistical analysis was performed with Tukey's multiple comparison test, with ** $p < 0.01$ versus relative controls and ## $p < 0.01$ vs cells treated with allergen alone.

responsible for presentation of chemical allergen to T-cells. Recently, some studies have challenged this belief, with the suggestion that also the other subsets may contribute to induction and regulation of skin sensitisation (Ainscough et al., 2012).

In order to activate a specific immune response, DC must migrate through the dermis, towards the lymph nodes. Particularly important are the cytokines tumor necrosis factor- α , interleukin-1 β and IL-18, produced by KC and DC following DAMPs generation in the skin microenvironment, which are all integral to the process of DC mobilization, migration and functional maturation. It has been demonstrated in murine macrophages that LPS-induced NLRP3 protein expression was regulated through the NADPH oxidase/ROS/NF- κ B-dependent, JAK2/PI3-kinase/AKT/NF- κ B-dependent, and MAPK-dependent pathways, whereas ATP-induced caspase-1 activation was regulated through the NADPH oxidase/ROS-dependent pathway (Liao et al., 2012).

In DC, Mizuashi et al. (2005) showed that all the sensitizers tested (Ni, formaldehyde, DNCB, MnCl₂ and thimerosal), but none of the non sensitizers reduced the GSH/GSSG ratio, which was accompanied by p38 MAPK activation. The antioxidant N-acetyl-L-cysteine, which suppressed the reduction of the GSH/GSSG ratio, abrogated p38 MAPK activation and CD86 expression, a common marker of DC maturation induced by chemical sensitizers.

Chemical-induced oxidation of the cell surface thiols appears to be one of the triggers of DC maturation, resulting in intracellular redox imbalance (Suzuki et al., 2009; Kagatani et al., 2010). Interestingly, in human DC, ROS were produced by both contact allergens DNCB and thimerosal and the irritant benzalkonium chloride, and were both decreased by pre-treatment with N-acetyl-L-cysteine. However, only DNCB up-regulated CD86 and HLA-DR molecules and induced protein carbonylation, suggesting the DNCB-induced ROS are somehow different from those produced by irritant (Byamba et al., 2010). The same Authors demonstrated that DNCB induced ROS were reduced by diphenylene iodonium (DPI), while benzalkonium chloride-induced ROS were slightly decrease by N-monomethyl-L-arginine (a nitric oxide synthase inhibitor), suggesting a different cellular source of ROS. In particular, results suggest that irritant-induced ROS may consist of nitric oxides or peroxynitrite, while contact allergen-induced ROS may consist of hydrogen peroxide or superoxide, derived from the NOX2 pathway (Byamba et al., 2010). However, the involvement of mitochondria as source of ROS cannot be ruled out, as the inhibitor used DPI not only inhibits NADPH oxidase, but also potently inhibits mitochondrial ROS production (Li and Trush, 1998).

Proteins that are reversibly modulated by ROS are of high interest. In this context, protein kinases and phosphatases, which act coordinately in the regulation of signal transduction through the phosphorylation and dephosphorylation of target proteins, have been described to be key elements in ROS-mediated signaling events. The major mechanism by which these proteins may be modified by oxidation involves the presence of key redox-sensitive cysteine residues. The protein kinase C (PKC) family is composed of 10 serine/threonine protein kinases that are involved in a variety of pathways that regulate cell growth, differentiation, apoptosis, transformation and tumorigenicity. Most cells express more than one isoform, and each type of PKC mediates different cellular events. PKC isoforms are differentially distributed not only with respect to tissue but also in terms of subcellular localization, suggesting that each isoform could have specific functions. PKC isoforms differ in their structure, cofactor requirement and substrate specificity. The members of the PKC family have been divided into three major groups: the classical PKCs (cPKCs), including the α , β I, β II, and γ isoforms; the novel PKCs (nPKCs), including the θ , η , ϵ , δ isoforms; and the atypical PKCs (aPKCs), including the ζ and ι/λ isoforms. PKC isoforms have been shown to contain a unique structural feature that is susceptible to oxidative modification (Cosenti-

no-Gomes et al., 2012). In particular, two pairs of zinc fingers are found within the regulatory domain (sites of diacylglycerol and phorbol ester binding). Each zinc finger is composed of six cysteine residues and two zinc atoms. The high levels of cysteine residues render the regulatory domain susceptible to redox regulation (Giorgi et al., 2010; Gopalakrishna and Jaken, 2000). Oxidative stress destroys the zinc finger conformation, and the autoinhibition is relieved, resulting in a PKC form that is catalytically active in the absence of Ca²⁺ or phospholipids. Currently, evidence supports the direct activation of different PKC isoforms by ROS generation (Gopalakrishna and Jaken, 2000; DelCarlo and Loeser, 2006). In the context of skin sensitisation, however, PKC is of particular interest, as it has been demonstrated almost 20 years ago by Halliday and Lucas (1993) to be required for LC migration, and that within the epidermis, the isoform β is exclusively expressed in LCs, and its downregulation impaired LC function with respect to contact hypersensitivity (Goodell et al., 1996). Furthermore, not only ROS can activate PKC, but PKC also has been shown to promote the production of endogenous ROS. The β isoform of PKC induces ROS generation through mitochondrial damage (Pinton et al., 2007). PKC β is responsible for the activation/phosphorylation of the mitochondrial p66shc protein, resulting in ROS generation (Migliaccio et al., 1999). In myeloid leukemia cells, ROS are not necessary for the translocation of PKC β II from the cytosol to the cell membrane. However, PKC β II was shown to be essential for ROS production induced by the PKC activator TPA (Datta et al., 2000). Also other PKC isoforms, including PKC α , PKC ϵ and PKC ζ , have been involved in mitochondrial ROS production in different experimental models (Wang et al., 2006; Agudo-López et al., 2011).

It is, however, outside the purpose of this article to review the role of PKC in the activation of the immune responses. Further studies are necessary to characterize the specificity by which ROS may activate different PKC isoforms, but the localization and the intensity of ROS generation could contribute to the explanation of the role of PKC in contact allergy.

1.5. ROS-dependent extracellular matrix changes facilitate ACD

Extracellular matrices (ECM) represent a complex network of proteins, including collagens, elastin, laminins, and glycosaminoglycans (GAGs), constituting the cell microenvironment. ECM provides the bulk, shape and strength of tissues in vivo (reviewed by Hynes, 2009). Moreover, ECM provides much more than just mechanical and structural support, as it is critically important for cell growth, survival, differentiation and key to various disease processes including inflammation and cancer (Hynes, 2009; Sorokin, 2009; Afratis et al., 2011; Nikitovic et al., 2011).

The interactions of both immune and tissue-resident cells with the ECM constitute an integral part of the immune response, as testified by an increasing number of reports (reviewed by Sorokin, 2009). Indeed, it is established that the ECM affects different aspects of the inflammation process, including leukocyte extravasation, penetration of the underlying endothelial cell basement membrane as well as subsequent migration through the loose interstitial matrix of the stroma to reach the site of inflammation (Karpos et al., 2010). On the other hand, in inflamed tissues, both ECM turnover and protease secretion by tissue-resident cells are affected by cytokines, including TGF- β (Leask and Abraham, 2004), IL-13 (Purwar et al., 2008) and TNF- α (Kelly et al., 2012) released by the activated infiltrating cells. Altered expression of ECM molecules can in turn influence immune cell activation, differentiation and survival, and thus ultimately affect the propagation of the inflammatory response and the development of chronicity (Sorokin, 2009).

In the case of ACD, available data suggest that early/chronic inflammation stages are associated with temporal changes in the

expression, deposition, and degradation of inducible extracellular matrix components including fibronectin, tenascin-C, fibulin-1 and 2 as well as various matrix metalloproteinase (MMP) expressions (Kusubata et al., 1999). Emerging evidence correlates oxidative stress to ACD-related alterations in ECM constitution (Esser et al., 2012). Specifically, changes in hyaluronan (HA) expression and fragmentation as well as MMP upregulation are closely related to increased oxidative stress in ACD.

HA is a free unbranched GAG composed of repeating disaccharides of N-acetyl-glucosamine and glucuronic acid units, with molecular weights ranging from 10^5 to 10^7 Da (Antonio and Iozzo, 2001). It is synthesized in a unique manner by a family of hyaluronan synthases, degraded by hyaluronidases and exerts a biological effect by binding to families of cellular receptors, the hyaladherins (Antonio and Iozzo, 2001; Kouvidi et al., 2011). The biological roles of HA are also dependent on its hydrophilic and hydrodynamic properties due to inherent negative charge, which allow it to retain water and play a structural role in tissue organization. HA is ubiquitously distributed in the ECM (Stern et al., 2006; 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). The versatile, in vivo, molecular weight of HA allows wide range of activities. Thus, 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, that are generated during inflammation or tissue damage, either enzymatically by a group of hyaluronidases or non-enzymatically by ROS (Jiang et al., 2011; Agren et al., 1997), induce pro-inflammatory innate immune responses (Noble, 2002), presumably via TLR2 and/or TLR4 in immune cells like DCs or macrophages (Termeer et al., 2002). Indeed, HA breakdown in ACD seems to be initially directly mediated by ROS, given the rapid induction of ROS by contact sensitizers. 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., 2010). In continuation, LMWHA fragments can induce the expression of pro-inflammatory genes (Collins et al., 2011), such as macrophage inflammatory protein (MIP)-1, MIP-1, keratinocyte chemoattractant, macrophage chemoattractant protein-1, and IFN-induced protein-10, as well as cytokines, such as IL-18, IL-12, and TNF- α (Scheibner et al., 2006; Boodoo et al., 2006; Hodge-Dufour et al., 1997; Horton 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). Likewise, inhibition of ROS-induced HA degradation was linked to a reduction of inflammation in other systems, as demonstrated in both bleomycin- and asbestos-induced pulmonary fibrosis (Eberlein et al., 2008; Gao et al., 2008).

Importantly, HA degradation also occurs in human skin when contact sensitizers are applied ex vivo, indicating in this manner, a species spanning mechanism (Agren et al., 1997). Thus, contact sensitizers induce endogenous danger signaling by triggering ROS (Agren et al., 1997) and hyaluronidase mediated HA degradation (Monzon et al., 2010). This process is crucial for ACD since sensitisation was completely prevented by pre-treatment of the skin with antioxidants or the hyaluronidase inhibitor AA (Esser et al., 2012). Conclusively, HA-induced inflammatory mediators serve both in

the induction of sensitivity as well as in the enhancement of the ongoing inflammatory response.

MMPs are a family of zinc- and calcium-dependent endopeptidases involved in degradation and remodeling of the ECM, employed in many physiological and pathological processes. MMPs have been suggested as modulators of inflammation and innate immunity (Parks et al., 2004). Among the MMP family, some of them have been implicated in pathophysiology of ACD. Immune cells, including T cells, LC, and macrophages, produce MMPs such as stromelysin-1 (MMP-3) and gelatinase B (MMP-9). It was shown that MMP-3 is essential for the initiation of ACD by induction of T lymphocyte proliferation in response to specific antigen, and it was also demonstrated that MMP-9 is necessary for the resolution of skin inflammation (Pilcher et al., 1999). Importantly, the expression of MMP-3 and -9 was found to be persistently enhanced in ACD in vivo (Hartmann et al., 2006). It is noteworthy that these MMPs expression is up-regulated at the translation level with the advent of ROS generation (Hazane et al., 2005; Song et al., 2011), activating thus a vicious circle culminating in inflammatory response. Indeed, the antioxidant activity of superoxide dismutase was shown to inhibit TNF- α -induced MMP-9 expression via ROS-NF- κ B-dependent mechanisms in keratinocytes, and it was therefore suggested to be used as an immunomodulatory agent against inflammatory skin diseases related to oxidative stress (Song et al., 2011).

Other components of the ECM, like proteoglycans (Seidler et al., 2011), have been found to fine-tune tissue inflammation through a number of different mechanisms including those mediated by ROS (Gill et al., 2010). Whether these mechanisms are valid in the milieu of the skin inflammatory processes however, remains to be elucidated. The present data indicates that inhibitors of ECM degradation or antagonists of the pro-inflammatory function of ECM components related to oxidative stress appear to be worthwhile targets of further studies.

2. Conclusions

Even if more studies are necessary to elucidate the mechanisms that are involved in chemical allergens-induced oxidative stress, the signaling pathways activated and their role in contact allergy, data clearly indicated a pivotal role of ROS in chemical allergy.

It seems that following chemical allergens exposure, a general signaling mechanism is triggered in mitochondrial dysfunction. In this context, an increase in ROS production by mitochondria activates local PKCs, which in turn activate NADPH oxidase. Consequently, the redox state of the cell becomes imbalanced, with the activation of several pathways, including MAPK, such as SAPK/JNK, ERK1/2 and p38, or NF- κ B or Akt/ASK1 or Keap1/Nrf2 pathways, resulting in the production of costimulatory molecules, cytokines, chemokines, phase 1 detoxifying enzymes.

Conflict of Interest

Authors declare of not having any financial, personal, or association with any of individuals or organizations that have could inappropriately influence the submitted work.

3. Uncited references

Ade et al. (2009), Afratis et al. (2012), Corsini and Kimber (2007), Corsini et al. (2009), Corsini et al. (2012), Horton et al. (1997), Kim et al. (2012), Korpos et al. (2010), Johansson et al. (2011), Nikitovic et al. (2012) and Sorokin (2010).

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