

Pathomechanisms of Contact Sensitization

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Abstract Contact sensitization is the initial process involved in the development of an allergic reaction to xenobiotic environmental substances. Here, we briefly describe the differences between irritant and allergic contact dermatitis. Then, we highlight the essential steps involved in the development of an ACD reaction, i.e., the protein binding of haptens, genetic factors influencing the penetration of sensitizers into the skin, the different mechanisms driving the initial development of an inflammatory cytokine micromilieu enabling the full maturation of dendritic cells, the role of pre- and pro-haptens, antigen presentation and T cell activation via MHC and CD1 molecules, dendritic cell (DC) migration, and potential LC contribution as well as the different T cell subsets involved in ACD. In addition, we discuss the latest publications regarding factors that might influence the sensitizing potential such as repeated sensitizer application, penetration enhancers, humidity of the skin, microbiota, Tregs, and phthalates. Last but not least, we briefly touch upon novel targets for drug development that might serve as treatment options for ACD.

Keywords Contact dermatitis · Contact hypersensitivity · Hapten · Pathomechanism · Potency · Sensitization

Abbreviations

ACD	Allergic contact dermatitis
ATP	Adenosine triphosphate
CD	Contact dermatitis
CHS	Contact hypersensitivity
DAMP	Damage-associated molecular pattern
DNCB	2,4-Dinitro-1-chlorobenzene
DNFB	2,4-Dinitro-1-fluorobenzene
DINP	Di-iso-nonyl phthalate
FITC	Fluorescein isothiocyanate
HBD	Human beta defensin
HMGB1	High-mobility group box 1
HSA	Human serum albumin
ICD	Irritant contact dermatitis
LN	Lymph node
MCI	Methylchloroisothiazolinone
OXA	Oxazolone
SDS	Sodium dodecylsulfate
PPD	Para phenylenediamine
QSAR	Quantitative structure-activity relationship
TCR	T cell receptor
TLR	Toll-like receptor
T _{RM}	(Skin) resident memory T cell
T _{CM}	(Lymph node) resident central memory T cell

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Introduction

Contact dermatitis (CD) is a sterile inflammatory disease of the skin, caused by a contact with environmental xenobiotic chemicals resulting in erythema and eczema formation. Its pathogenesis relies on a chain of complex processes involving the immune system and underlies many levels of orchestrated fine control [1]. At least two distinct forms of contact

dermatitis can be distinguished—irritant (ICD) and allergic contact dermatitis (ACD).

Irritant contact dermatitis (ICD) activates innate but not adaptive immune responses. It is both clinically and histopathologically a promiscuous disease that is acute or chronic in nature, depending on the causative chemical and the circumstances of exposure [2]. While strong irritative chemicals such as acids, bases, and detergents immediately lead to the clinical symptoms, weak irritants may need repeated skin exposure before an ICD occurs. In ICD, innate immune responses are primarily triggered via the intrinsic toxicity of the causative chemical or by physical/mechanical irritation of the skin. The most prevalent example of physical irritation is dry skin occurring due to air condition-induced dry humidity [3]. Irritation and damage of epidermal skin cells in ICD then most likely lead to the release of stress-associated danger signals such as ROS and ATP and of damage-associated molecular patterns (DAMPs). ROS, ATP, and DAMPs such as extracellular matrix components, high-mobility group box 1 (HMGB1), and others then activate innate immune cells resulting in the release of chemokines and cytokines. This generally produces a local pro-inflammatory microclimate in the skin. However, with irritants, there is no formation of a T cell epitope and thus no induction of an adaptive immune response. Therefore, ICD leads to a rapid response, usually within minutes/hours after contact with the irritant, and is usually confined to the area of contact with the irritant.

In contrast, *allergic contact dermatitis* (ACD) induces widespread to systemic hypersensitivity reactions. Systemic hypersensitivity reactions often result from the (cross-) reactivity of topically applied substances with implant materials. For example, the release of titanium from metal osteosynthesis implants can lead to a fever as well as papulo-nodular infiltration and mild erythema in skin regions with black tattoos containing metallic pigments [4]. Removal of the implants leads to complete disappearance of symptoms while patch test application of aqueous extract from screw material as well as TiO₂ results not only in the skin but also systemic flare up reactions [4]. Another case of systemic contact dermatitis was recently reported by Cobb et al., where once again erythema formation on the tattooed skin was resolved after implant removal [5]. As more and more people get implants as well as tattoos, these reactions are likely to increase.

ACD reactions are caused by contact with one of the > 4350 different potential chemical sensitizers [6]. The prevalence for ACD in the European countries is high, affecting ~ 20% of the general population [7]. As observed by Landsteiner and Jacobs ~ 80 years ago, ACD is an immune response to self-proteins/-peptides, which have been modified by exogenous chemicals in a way that renders them immunogenic [8]. The step after this foremost chemical event involves the activation of both the innate and the adaptive arms of the immune system and can thus be divided into two distinct

phases: the initial sensitization phase, where the skin comes into contact with the sensitizing chemical for the first time, and the elicitation phase occurring after a second encounter with the causative agent at the same or a distant spot. Successful sensitization may require repeated contacts with the sensitizing agent.

Sensitizers can be metal ions [9] or low molecular weight chemicals. Interestingly, two recent studies addressed the question if a molecular weight (MW) cutoff of < 500 Da, as previously reported to be a pre-requisite for a chemical to be a sensitizer, really holds true or not [10, 11]. Both studies refuted this assumption as the authors realized that also substances with a higher MW (up to 2285.61 Da) were classified as potent sensitizers [10, 11]. This might be especially important for the development and interpretation of skin sensitization quantitative structure-activity relationship (QSAR) models working with the assumption that sensitizers need to have a MW of < 500 Da. Similarly, the notion that highly hydrophilic substances, i.e., $\text{LogK}_{\text{ow}} \leq 1$, are expected not to penetrate effectively to induce sensitization has been questioned recently and found not to be valid [12].

Mechanisms Involved in Sensitization

One important aspect of sensitization is the ability of sensitizers to penetrate the *stratum corneum* and into the deeper layers of the epidermis [13, 14] as shown for example for 2,4-dinitro-1-fluorobenzene (DNFB) [15]. Here, the influence of *genetic pre-dispositions* such as filament-aggregating protein (filaggrin) mutations leading to skin barrier defects might play an important role not only in skin diseases such as atopic eczema [16] but also in the development of ACD, though the definite impact is still under debate [17–19]. Another genetic pre-disposition associated with sensitization in ACD is a mutation in the Claudin-1 gene, playing a major role in the formation of tight junctions and thus in skin barrier formation [18]. CLDN1 polymorphisms rs9290927, rs893051, and rs17501010 are associated, respectively, with nickel contact sensitization in individuals without ear piercings, contact sensitization to fragrances, and with both organic compounds and nickel contact dermatitis [18]. An exhaustive review on the role of tight junctions in skin inflammation was recently published by Bäsler and Brandner [20]. Further evidence that *skin barrier defects* are associated with ACD comes from a study showing that ACD patients present an impaired skin barrier function even in non-lesional skin regions showing delayed barrier recovery due to insufficient generation of bulk ceramides [21].

Among the sensitizing metal ions, nickel, gold, palladium, and cobalt are the most prevalent patch test-positive metals in a recent survey from Lithuania and at lower prevalence levels in the EU [22]. Despite the EU Nickel Directive, nickel still remains a problem [23] affecting 8–18% of the general

population [24]. Apart from metal workers, professions especially in close contact with fragrances, preservatives [25], or dyes such as hairdressers, who are exposed to the sensitizing chemicals throughout the whole process of hair dying including cutting freshly dyed hair [26], are at risk.

Due to their low molecular weight, the sensitizing chemicals called haptens are non-immunogenic but become recognizable by the immune system due their ability to bind to (epidermal) proteins [27, 28]—a process called *haptization*. For the black dye *para*-phenylenediamine (PPD), an irreversible binding to cysteine 34 in human serum albumin (HSA) was observed [29], while mass spectrometry methods revealed that the extreme sensitizers 2,4-dinitro-1-chlorobenzene (DNCB) and methylchloroisothiazolinone (MCI) modified a greater number of nucleophilic HSA sites than the moderate sensitizer cinnamaldehyde [30]. Interestingly, the weak/non-sensitizer 6-methyl coumarin was restricted to only a single nucleophilic residue within HSA. Moreover, Aleksic et al. showed selective covalent HSA binding by the sensitizers DNCB and phenyl salicylate and confirmed that non-sensitizers 2,4-dichloro-1-nitrobenzene (DNTB), butyl paraben, and benzaldehyde as well as the irritants benzalkonium chloride and sodium dodecylsulfate (SDS) did not covalently modify HSA under any conditions tested [31]. If the modification of a greater or lesser number of protein sites correlates with sensitizer, potency still remains to be shown.

Several (fragrance) substances can act as *pre*- and *pro*-haptens; i.e., substances that are able to become more potent than the parent substance in terms of sensitization by activation outside (via chemical/physical activators such as air oxidation or photo-activation) or inside (via biotic, enzymatic activation) the skin as recently reviewed by Karlberg et al. [32]. Some substances such as geraniol can even act as both *pre*- and *pro*-hapten [33]. This shift in potency from a non- or low sensitizing chemical to a potent sensitizer is an important risk factor and needs to be carefully addressed in patch tests and in vitro-based assays for the identification of contact sensitizers. A novel toolbox of methods for the quantification of skin-sensitizing hydroperoxides potentially formed from linalool and limonene in perfumes was recently provided by Natsch et al. [34].

As for the ICD, the initial generation of a *pro-inflammatory cytokine milieu* is an essential pre-requisite for efficient sensitization in ACD [35, 36] as shown by conversion of the tolerogen/weak sensitizer DNTB into an immunogen by the addition of interleukin (IL)-12 [37]. Without the full activation of immature dendritic cells via “danger” signals and the respective *pro-inflammatory milieu*, no activation and priming of naïve T cells and thus no adaptive immune response occur [38]. This induction of an inflammatory milieu can be generated in several ways, involving either direct or indirect activation of toll-like receptor (TLR) signaling pathways as well as

NF κ B and MAPK signaling. Interestingly, though tumor necrosis factor alpha (TNF- α) is known to play a crucial role during the sensitization process [39], no general consensus on the involvement of TNF- α single-nucleotide variants exists [40, 41], though TNFA-308 G/A polymorphism was linked to PPD sensitization [42]. Contact of the skin with *non-metal sensitizers* results in the release of ROS [43, 44]. The importance of ROS production becomes obvious in models where ROS detoxification is reduced, e.g., due to a lack in Nrf2 expression. In such models, contact hypersensitivity (CHS) responses to weak sensitizers such as cinnamic aldehyde are increased resembling reactions to strong sensitizers [45]. In combination with an upregulated hyaluronidase activity, ROS lead to the degradation of high molecular weight hyaluronic acid (HA) into *pro-inflammatory* low molecular weight HA fragments. These fragments act as *DAMPs* by activating TLRs 2 and 4 [43, 46–48]. Therefore, organic sensitizer contact results in an indirect activation of TLR signaling and subsequent cytokine release. Another molecule that is present in high concentrations in the epithelial layer of barrier tissues is cholesterol sulfate. Using a knock-out approach, it was shown that recognition of cholesterol by the C-type lectin receptor of the innate immune system, Mincle (Clec4e), plays an important role in the generation of a *pro-inflammatory cytokine milieu* and thus in the pathogenesis of ACD [49]. Mincle expression is upregulated after both tissue wounding as well as after DNFB application [49], further strengthening the hypothesis that tissue stress and damage are crucially involved in ACD. In addition, contact sensitizers induce the release of ATP into the extracellular space, leading to an activation of the P₂X₇ receptor. Subsequently, the *NLRP3 inflammasome* is activated, resulting in caspase-1 activation and the splicing of pro-IL1 β and pro-IL18 into their active forms, further contributing to the sensitization and elicitation process [50]. Moreover, the activation of *neutrophils*, *mast cells*, and *NK cells* by contact sensitizers directly contributes to the inflammation of the skin [51–54].

In contrast, *metal sensitizers* such as nickel, cobalt, and palladium induce the initial *pro-inflammatory milieu* by *direct binding* to human (but not mouse) TLR4 [55–57]. This observation provided an explanation for the long-known fact that CHS responses to nickel only worked if either LPS or an irritant was co-administered for the sensitization of mice. For *chromium*, a third mechanism was proposed; here, no direct binding to TLR4 occurs but the generation of mitochondrial ROS by chromium compounds leads to the activation of the NLRP3 inflammasome independent of ATP signaling [58].

A more general effect of sensitizers leading to an increase in cytokine levels might be the influence of *antimicrobial peptides* such as beta-defensins. Recently, an increase in beta-defensin gene expression in rats after DNFB skin treatment was observed [59], and a weak but significant upregulation of human beta-defensin (HBD) 2 gene expression levels

in skin samples from ACD patients compared to a strong upregulation in psoriatic skin was reported [60]. Interestingly, pre-incubation of primary human macrophages or THP-1 cells with HBD 2 or 3 leads to an increased pro-inflammatory cytokine release after re-stimulation with TLR ligands via a mechanism leading to an enhanced ATP release in response to defensin incubation and a subsequent ATP-gated channel P_2X_7 -mediated increase in cytokine/chemokine production [61]. In addition, Ferris et al. observed hBD3 to be inducing dendritic cell (DC) activation, migration, and polarization and reported that this effect was depending on NF κ B activation, further indicating that hBDs play an important role in the provision of a pro-inflammatory cytokine milieu and the activation of DCs in the skin [62].

Importantly, binding of a contact sensitizer to a protein abrogates the sensitizing ability of the chemical [63], further indicating that the reactivity and intrinsic irritant effect of the chemical are essential for the sensitization phase. Interestingly, the addition of an irritant such as SDS leads to a *synergistic effect* in nickel ACD [64], enhances nickel-specific CHS responses [65], and facilitates the immunogenicity of DNTB in the CHS model [66]. This might be an effect of the increase in cell death, mediated by irritants, and the release of ATP into the extracellular space, which also augments CHS responses [50, 67].

In humans, the *sensitization phase* takes 10–15 and 5–7 days in the murine CHS model. As a T cell-mediated delayed type (type IV) hypersensitivity reaction, T cell epitopes need to be presented to T cells by professional antigen-presenting cells on major histocompatibility complexes (MHC) or CD1 molecules [68, 69]. In contrast to the presentation of peptides via MHC molecules, CD1 molecules present lipid antigens to T cells. So far, the role of CD1a, which is highly abundant on human Langerhans cells in the skin, in inflammatory skin diseases remained unclear due to the lack of CD1a expression in murine cells. Recently, DNCB-pulsed CD1+ human APCs were shown to activate CD1a- and CD1d-restricted autoreactive T cells specific for cell-endogenous lipids [70]. In this study, some (DNCB, cinnamaldehyde, resorcinol, and isoeugenol) but not all (1,4-benzoquinone, oxazolone, and PPD) tested haptens potentiated CD1-restricted T cell activation via the release of cytokines in a CD1- and TCR-dependent manner [70], thus shedding further light on the importance of the CD1 family on effective sensitization in ACD. In addition, evidence that CD1a expression on Langerhans cells is vital for efficient induction of urushiol-mediated CHS responses driven by CD4+ T_H17 cells leading to IL-17 and IL-22 expressions was provided using a novel transgenic mouse model [71]. However, while the observed cytokine profile in this mouse model matched the one observed in poison ivy-allergic human patients, the results are in contrast to previous results obtained in WT mice, where urushiol induced only mild CHS responses that were

dependent on IFN- γ and TNF- α expressions [72]. This underlines the need for more exhaustive studies with a broader range of chemicals in reliable mouse models to investigate the role of CD1 responses in ACD.

Recently, several novel factors influencing the *migration of DCs* and thereby the induction of CHS responses were reported, highlighting the importance of functional DC migration from the skin to the lymph nodes (LNs). The lipid mediator resolvin E1, which can be derived from ω 3 polyunsaturated fatty acids, mediates leukotriene B₄ (LTB₄)–leukotriene B₄ receptor 1 (BLT1) signaling blockade. Thereby, it inhibits DC actin polymerization, which in the end results in the downregulation of DC motility under both steady state—as shown in an elegant experiment using Kaede mice—and inflammatory conditions in the skin and exerts its anti-inflammatory effects in a DNFB CHS model [73]. This observation was supported by a report showing upregulated gene expressions of 5-lipoxygenase and leukotriene A₄ hydrolase, two critical enzymes for LTB₄ synthesis, BLT1, and elevated LTB₄ levels in skin lesions of oxazolone (OXA)-induced ACD [74].

Interestingly, in a repeated elicitation model of oxazolone ACD, selective deletion of E prostanoïd receptor 4 (EP4), one of the receptors for PGE₂, in T cells prevented hapten-induced IL-22 production in vivo and thereby limited atopic-like skin inflammation in the oxazolone-induced ACD model [75].

A new therapeutic target has recently been described by Ufer et al., who identified the neuronal plasticity molecule—activity-regulated cytoskeleton-associated protein/activity-regulated gene 3.1 (Arc/Arg3.1)—to be expressed exclusively in migratory dendritic cells in the skin [76]. In their fluorescein isothiocyanate (FITC) CHS model, knock out of Arc/Arg3.1 resulted in DC migration defects and abrogated mounting of T cell responses [76], further highlighting the role of DC migration in ACD.

Whether or not only dendritic cells need to take up the antigens, get fully activated in the skin expressing co-stimulatory molecules on their surface together with the release of pro-inflammatory cytokines, and thus are responsible for the initial priming of naïve T cells after egress from the skin to the draining local lymph node or if *Langerhans cells* (LC) also play a potentiating and not just inhibitory role is currently under debate.

While the role of DC activation and migration in ACD seems quite clear, understanding the role of LC in ACD pathology has been complicated by contradicting early results using different models for LC depletion. Mice constitutively lacking epidermal LCs due to the expression of the active human diphtheria toxin A subunit under the langerin promotor (huDTA mice) showed an increase in CHS responses, indicating that LC suppressed CHS via direct interaction between LC and CD4+ cells as well as via LC-derived IL-10 [77, 78]. In contrast, murine Langerin-diphtheria toxin receptor mice (muDTR) lacking all LCs as well as langerin+ dermal DCs

early after diphtheria toxin injection showed reduced but not completely abrogated CHS responses [79, 80], indicating that LCs act pro-inflammatory. In this model, the role of LCs seemed to be redundant [81] and the major cell type responsible for the reduction in CHS responses was dermal langerin-expressing DCs [80].

More recently, the development of huDTR mice, allowing the acute ablation of only epidermal LCs, indicated that LCs indeed are responsible for the suppression of CHS responses and that one mechanism might be the induction of anergy in sensitizer-specific effector T cells and the activation of CD4+ FoxP3+ regulatory T cells and IL-10 release [82, 83]. Another indication favoring the anti-inflammatory role of LCs came from a study using hu-DTA mice and *Candida albicans*-induced skin inflammation [83]. Here, in the absence of LCs, the inflammation was enhanced and Bobr et al. speculated that in this setting, LCs may function to limit CD49a+ NK cell priming as a mechanism to suppress inappropriate responses to benign stimuli.

Major executive cellular players in ACD are CD8+ T_c17 cells [84–86], though CD4+ cells (T_h1/T_h17) also seem to play a role [71, 72, 87–89]. An indication that not separate subsets but mixed T_h1/T_h2 responses are necessary for CHS development—at least in response to oxazolone stimulation—comes from a recent study using new T cell transcription factor-specific DNazymes targeting GATA3 or Tbet-positive T cells [90]. In addition to the conventional $\alpha\beta$ T cell subsets, dermal $\gamma\delta$ T cells also play an important role in primary CHS through their production of IL-17 [91].

Recently, it was shown for nickel ACD that the development of CD4+ T_h17 cells depends on TLR4, p38 mitogen-activated protein kinase, NF κ B and Jak-STAT activations, and on IL-23 production by DCs [92]. Interestingly, expression of the protein kinase CK2 (earlier known as casein kinase 2) also plays an important role in phenotypic maturation and cytokine production of DCs in response to nickel and DNCB and determines the CD4+ T cell activation and T_h1 T cell polarization [93].

Immunization with haptens has been shown to simultaneously generate antigen-specific skin resident memory T cells (T_{RM}) and lymph node central memory T cells (T_{CM}) in similar numbers from the same common naive T cell precursor [94]. Though both bear the same T cell receptor, T_{RM} mediate a rapid CHS response, while T_{CM} induce delayed and attenuated responses [94]. The T_{RM} population accumulates especially at the site of previous contact with the sensitizer, resulting in a rapid release of IFN- γ and IL-17 in turn leading to an increase in keratinocyte-produced IL-1 β [95].

Correlation of Contact Sensitizer Potency with ACD Reactions

A recent review by Lidén et al. compared the sensitizing potencies of 72 fragrance substances, 25 preservatives, and 107

hair dye substances as assessed via local lymph node assay data [96]. The conclusion was that the majority of sensitizing preservatives and hair dyes are to be categorized as strong or extreme sensitizers (EC3 value of $\leq 2\%$) and fragrances as mostly moderate sensitizers. However, they pointed out that factors other than potency need to be addressed more rigorously since the moderate fragrances are among the most frequent causes of ACD.

Interestingly, as shown for PPD-HSA, sensitizers induce expansion of T cells carrying selected TCRs consistent with antigen-driven clonal expansion [97]. However, while complementarity-determining region 3 (CDR3) sequences of the expanded TCRs could be grouped into several families of related amino acid sequences, the overall diversity of the expanded sample was not much less than that of a random sample of the same size [97]. Whether or not sensitizer potency correlates with T cell receptor frequency and T cell receptor repertoires for other sensitizers still remains to be shown [98].

Factors Influencing the Sensitizing Potential

As for ICD, the severity of ACD can be modulated via *repeated application* of the same substance at low (under single application non-sensitizing) concentrations as shown, e.g., for the sensitizer DNCB [99], although it seems to depend on the protocol used for the application and the assessment method of the effect whether or not an increase can be observed [100]. In addition, it has to be determined carefully whether or not a substance can accumulate in the skin and therefore low concentrations that would usually require repeated application to induce a response, resulting in a response comparable to a single, high-dose application as for example shown for PPD [101]. Moreover, combination of non- or low sensitizing concentrations of different weak fragrance sensitizers led to increased CHS reactions [102, 103], though whether or not this is a general mechanism needs to be addressed in more detail. For the weak sensitizers isoeugenol and cinnamic aldehyde, no synergistic effect on cell numbers and cytokine production in an adjusted local lymph node assay was observed [104].

Patch testing of cobalt-sensitized patients with a range of aqueous cobalt concentrations alone or in combination with the *penetration enhancer* (and irritant) sodium dodecylsulfate (SDS) showed that surfactant treatment reduced the minimum eliciting concentration [105]. In the CHS model, nickel was shown to act as an adjuvant during cobalt sensitization [106], also indicating that the combination of different substances (and thereby most likely increasing the local skin irritation) leads to an augmented sensitization. Moreover, the addition of the irritants sodium lauryl sulfate (11 subjects) or anthralin (dithranol) (four subjects) to allergen application in patients showed that the irritants increased the ACD reaction [107], further strengthening the relevance of local inflammation on

the sensitizing potency. These findings can be explained by the fact that the essential pro-inflammatory signals required for the promotion of skin inflammation, DC activation, and migration in ACD do not necessarily have to be provided by the sensitizer that causes ACD. Heterologous inflammatory stimuli can derive from other contact allergens or irritants which are often combined in cosmetics and other products. Even infections may provide such heterologous stimuli [108, 109]. The result can be the amplification of pro-inflammatory stimuli or even the substitution of missing pro-inflammatory stimuli. Therefore, combinations of several contact sensitizers and of contact sensitizers and irritants may cause sensitizations that would not occur with a single sensitizer.

Another factor influencing the magnitude of CHS responses is the *humidity of the skin*, affecting the penetration of chemicals into the deeper epidermal layers. As shown by CHS experiments with different sensitizers under low, intermediate and high humidity levels, CHS responses to both water-insoluble (lipophilic) chemicals like TNCB and oxazolone as well as to water-soluble chemicals like TNBS were ameliorated under high humidity levels in Balb/c and C57BL/6 mice [110]. Interestingly, the sensitization phase was not affected by different humidity levels but only the elicitation response. The optimal time frame of high humidity needed to induce the amelioration via increased skin surface hydration was 6 h after elicitation [110]. However, in a recent study, no difference in skin hydration but just a difference in ceramide content was observed in ACD patients compared to healthy controls [21].

While the expressions of TLRs 2 and 4 are crucial for the development of CHS, the presence of *microbiota* seems to be dispensable in response to sensitizers like TNCB, oxazolone, and DNFB [43, 48, 111]. However, induction of oral tolerance to DNFB was impaired in mice lacking either normal intestinal microbiota or TLR4 as the migration of DNFB carrying retinaldehyde dehydrogenase (RALDH) expressing CD103+ CD11b+ DCs from the gut to mesenteric lymph nodes was abrogated, thereby inhibiting the neoconversion of FoxP3-expressing Treg cells [111]. Interestingly, TLR3 signaling is involved in determining the severity of acute and chronic contact hypersensitivity reactions to oxazolone and TNCB. Under repeated elicitation, the ear swelling response in *Tlr3* KO mice was reduced and the shift in the time course of the hypersensitivity response from a typical delayed-type response to an early-type response as observed in WT mice was delayed [112], indicating that other TLRs apart from the well-known TLR2 and TLR4 play a role in the severity of ACD reactions.

One important function downregulating the very early phases of sensitization is the degradation of extracellular ATP by *CD39+ regulatory T cells* (Treg cells) [113]. As recently shown by Mahnke et al., CD39+ Tregs are able to degrade ATP in the lymph nodes after sensitizer stimulation. This leads on the one hand to an enhanced accumulation of

CD8+ T cells in the LNs within 3 h after sensitization and on the other hand to an impaired egress of CD8+ T cells due to an inhibition of CD62L (a homing molecule mediating LN homing) shedding by the T cells in the LN that was attributed to an impaired ADAM17 activation by the lack of ATP. In addition, downregulation of ATP by Tregs also down-modulated the IFN- γ expression by CD8+ T cells [113]. *CD73* is the second ectonucleotidase involved in the degradation of ATP to adenosine. In a murine knock-out model, lack of CD73 expression results in an increase in DC motility and an increased migration of langerin+ DCs from the skin to the LNs compared to WT [114]. Interestingly, lack of CD73 also results in an increased CHS response to TNCB, implying that CD73-mediated adenosine production slows down the langerin+ DC migration from the skin to the LN and might be a crucial mechanism to prevent overboarding immune reactions against haptens [114]. Since ATP is an essential danger signal driving the activation of DC maturation and inflammasome activation and blocking ATP signaling is known to result in abrogated CHS responses; this provides an interesting way of Treg dampening the very early immune response.

Phthalates, which are widely used as plasticizers, may be able to influence inflammatory and allergic responses as reviewed by Dearman and Kimber [115]. Recently, oral application of di-iso-nonyl phthalate (DINP) was shown to enhance oxidative damage and NF κ B activation as well as TSLP expression in a CHS model in Balb/c mice with the weak sensitizer fluorescein isothiocyanate (FITC) [116, 117]. In addition, TRP cation channel, subfamily A, member 1 (TRPA1) expression and activation were upregulated via NF κ B signaling after long-time DINP exposure [116, 117]. Together, these changes resulted in an enhanced cytokine production and enhanced CHS responses, indicating that the oral uptake of phthalates may enhance the risk of sensitization and increase the severity of ACD.

Identification of ACD on a Molecular Level

As ACD, psoriasis (Pso), and atopic dermatitis (AD) are inflammatory skin diseases characterized by epidermal disruption, the similarities and differences of diseases make the diagnosis sometimes difficult. However, a recent approach using sera from healthy controls and different ACD, AD, and Pso patients for proteomic profiling provided 10 proteins exclusively upregulated in ACD patients [118].

In addition, matrix metalloprotease 2 (MMP2) was proposed as a novel marker for the detection of ACD by Wojciechowska et al. [119]. In their study, they observed no statistically significant difference between results obtained analyzing serum levels of MMP2 by ELISA and skin patch test results comparing nickel-allergic patients or healthy controls.

Novel Treatment Options for ACD

A novel approach for the treatment of ACD might arise from the observation that small (< 200 nm) negative and neutrally charged nanoparticles exhibited an immunosuppressive effect on DNFB and urushiol CHS [120] by impacting mast cell degranulation. Interestingly, positively charged NPs had no effect on CHS responses.

A new option for the treatment of ACD has been recently described by Balmert et al. using biodegradable TReg-Inducing (TRI) microparticles, engineered to release TGF- β 1, rapamycin, and IL-2. The prophylactic (2 days before sensitization) microinjection of these microparticles into the skin resulted in an expansion of antigen-specific T regulatory cells and suppressed CHS responses to DNFB. In addition, treatment of previously sensitized mice at the time of DNFB challenge led to a reversal of CHS symptoms to subsequent exposures [121].

Another approach was taken by Matsubara et al. showing that palladium CHS was reduced by oral administration of the antihistamine fexofenadine hydrochloride interfering with the development of antigen-presenting cells from the sensitization phase [122]. While unfortunately, controls like untreated mice and the double administration of the glucocorticoid prednisolone are lacking, the authors concluded that antihistamine treatment was even more effective in reduction of CHS responses than glucocorticoid treatment.

Conclusions

Given the central role of innate inflammatory responses, anti-inflammatory therapies hold promise for the treatment of ACD. In the mouse model, it was already shown that the neutralization of ROS by topical antioxidants, the prevention of P2X7R signaling by the antagonist KN-62, or the blockade of IL-1 receptor signaling by the IL-1R antagonist anakinra can successfully prevent sensitization and sometimes elicitation in sensitized mice. These promising pre-clinical results from the CHS model await translation into the human system.

Overall, basic research has provided many mechanistic insights into the pathomechanisms of CHS and ACD. These provide the basis—and further basic research is still crucially needed—for the development of mechanism-based therapies and of in vitro assays for contact allergen identification to replace animal testing.

Compliance with Ethics Guidelines

Conflict of Interest The authors declare no conflicts of interest relevant to this manuscript.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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