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Immunology of metal allergies

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Summary

Allergic contact hypersensitivity to metal allergens is a common health concern worldwide, greatly impacting affected individuals with regard to both quality of life and their ability to work. With an estimated 15–20 % of the Western population hypersensitive to at least one metal allergen, sensitization rates for metallic haptens by far outnumber those reported for other common triggers of allergic contact dermatitis such as fragrances and rubber. Unfortunately, the prevalence of metal-induced hypersensitivity remains high despite extensive legislative efforts to ban/reduce the content of allergy-causing metals in recreational and occupational products.

Recently, much progress has been made regarding the perception mechanisms underlying the inflammatory responses to this unique group of contact allergens. This review summarizes recent advances in our understanding of this enigmatic disease. Particular emphasis is put on the mechanisms of innate immune activation and T cell activation by common metal allergens such as nickel, cobalt, palladium, and chromate.

Introduction

Metal ions are among the most common triggers of allergic contact dermatitis (ACD). In fact, the current contact allergen “hit list” – derived from the patch test cohort of hospitals associated with the German Contact Dermatitis Research Group (DKG) and the Information Network of Departments of Dermatology Consortium (IVDK) – ranks three metal allergens among the top 10 for the period between 2010 and 2012. Nickel (II) sulfate ranks at number 1 with a sensitization rate of roughly 15 %; cobalt chloride, at number 4 with approximately 5 %; potassium dichromate, at number 9 with roughly 3 % [1]. Moreover, a recent international study reported a median prevalence of 7.8 % with respect to palladium allergy [2]. This figure may even represent an underestimation, as an adequate test substance for reliable patch testing is lacking [3], resulting in the absence of palladium in the standard series of test allergens.

In this review, we will summarize recent advances in our understanding of immunologic mechanisms underlying

ACD induction by this unique group of contact allergens. Particular focus will be put on metal-induced innate immune activation.

Basic mechanisms of allergic contact dermatitis

ACD is a T cell-driven human inflammatory skin disease induced by small non-proteinaceous substances (haptens) [4]. Immunologically, ACD is a delayed-type hypersensitivity (DTH, type IV) disorder, characteristically showing visible signs of hypersensitivity 48–72 h after hapten exposure. Unlike classical DTH, however, data generated with a murine contact hypersensitivity model (CHS, the murine equivalent to ACD) suggest that ACD is vitally dependent on cytotoxic T cells (Tc) [5]. Allergic contact dermatitis can be divided into two phases: 1) an asymptomatic sensitization phase characterized by subclinical innate immune activation and 2) a clinically symptomatic elicitation phase representing the

adaptive phase of ACD. The latter eventually gives rise to pruritic lesions characteristic of contact dermatitis [4, 6]. The two key events during sensitization involve a) hapten-processing and/or binding of haptens to self-peptides at the surface of antigen-presenting dendritic cells (DCs) and b) generation of a costimulatory innate immune signal. This signal results in adequate activation of hapten-loaded DCs and their migration to dermal lymph nodes, where they prime hapten-specific T-cells, which subsequently enter the bloodstream [5]. In murine CHS, dermal DCs rather than epidermal Langerhans cells fulfill the task of transporting antigens to dermal lymph nodes where they are presented to T lymphocytes, eventually leading to sensitization [5]. By contrast, Langerhans cells have recently been shown to play a pivotal role in inducing tolerance in mice [7]. Upon re-exposure to an antigen (elicitation phase), DCs encounter effector T cells in dermal perivascular areas, where by the latter are activated and promoted to proliferate in situ [8]. Thus, a complex inflammatory milieu is created, clinically manifesting as contact dermatitis [6, 9].

Haptenization of proteins by metal allergens

Surprisingly, little is known about haptenization of metal allergens. Available data is still based on a few early hallmark studies using nickel as exemplary metal allergen. In 1975, it was first realized that nickel directly interacts with the surface of human lymphocytes. However, binding was found to occur with both nickel-sensitive lymphocytes and insensitive controls [10], suggesting that lymphocyte interaction alone was insufficient for any reaction to occur. In 1991, Romagnoli et al. provided the first evidence that nickel could bind to a histidine residue in a major histocompatibility complex (MHC)-bound peptide. The authors postulated that metal allergens likely interacted with one or multiple metal-binding motifs displayed by self-peptides complexed with MHC molecules [11]. Twelve years later, this theory received even stronger support by the isolation of a human nickel-reactive T cell clone that bound nickel in combination with a specific MHC-loaded self-peptide generated in human and mouse B cells but not other antigen processing-deficient cells [12]. In the same year, Weltzien's group reported on another nickel-reactive human T cell clone. Here, reactivity was neither dependent on protein processing in antigen-presenting cells nor affected by the nature of MHC-associated peptides. Instead, it relied on peptide-independent crosslinking of the T cell receptor (TCR) and the MHC complex by nickel [13]. Recently, the peptide/MHC-binding model has attracted new attention by the identification of mimotope peptides able to replace nickel

in the natural TCR ligand [14]. However, it is not yet clear whether both described mechanisms equally contribute to the haptenization of nickel and other metals. Moreover, it is currently unclear if there are yet other mechanisms involved. These may include neoantigen formation by metal-induced peptide or MHC changes [15], potentially involving peptide stripping from the MHC complex as described for certain noble metals including palladium [16] or indirect modification of preexisting self-MHC-peptide complexes as shown for beryllium [17]. Remarkably, nickel-reactive human T cell clones have been found to cross-react with palladium but not cobalt when presented by identical MHC class II molecules [18], suggesting the existence of both overlapping and unique haptenization mechanisms.

Nature of the innate immune signal produced by metal allergens

Extensive research in recent years has provided experimental evidence that adequate innate immune activation is an essential requirement for the development of ACD [19]. Two major underlying mechanisms have been identified: functional mimicry of pathogen-associated molecular patterns (PAMPs) resulting in hapten-mediated activation of one or multiple innate immune receptors; alternatively, hapten reactivity to other cellular products, such as extracellular matrix components, triggering the release of damage-associated molecular patterns (DAMPs), which serve as endogenous agonists of innate immune receptors [4, 20]. Nickel and cobalt are prime examples of functional PAMP mimicry, as they can interact with distinct histidine residues at the dimerization interface of human Toll-like receptor 4 (TLR4) [21, 22], the receptor for bacterial lipopolysaccharide (LPS) (Figure 1). As a result, the receptor homodimerizes [22], which likely facilitates recruitment of the TLR4 co-receptor MD2 necessary for subsequent activation of intracellular proinflammatory signaling pathways such as the IKK2/NF- κ B pathway. Consequently, key proinflammatory cytokines – including tumor necrosis factor alpha (TNF α) and interleukin-8 – are induced [21–23] (Figure 1a, b). Thus, nickel and cobalt mimic the innate immune signal normally induced by LPS from gram-negative bacteria. This view is also supported by global gene expression analysis revealing proinflammatory nickel and cobalt-induced gene signatures similar to those induced by LPS and TNF [22, 24–26]. This concept apparently also applies to palladium, which has likewise been reported to trigger proinflammatory gene expression via TLR4 [27]. However, that study did not examine the exact perception mechanism in detail. Thus, it remains unclear if palladium-induced TLR4 activation requires the same histidine residues H431, H456,

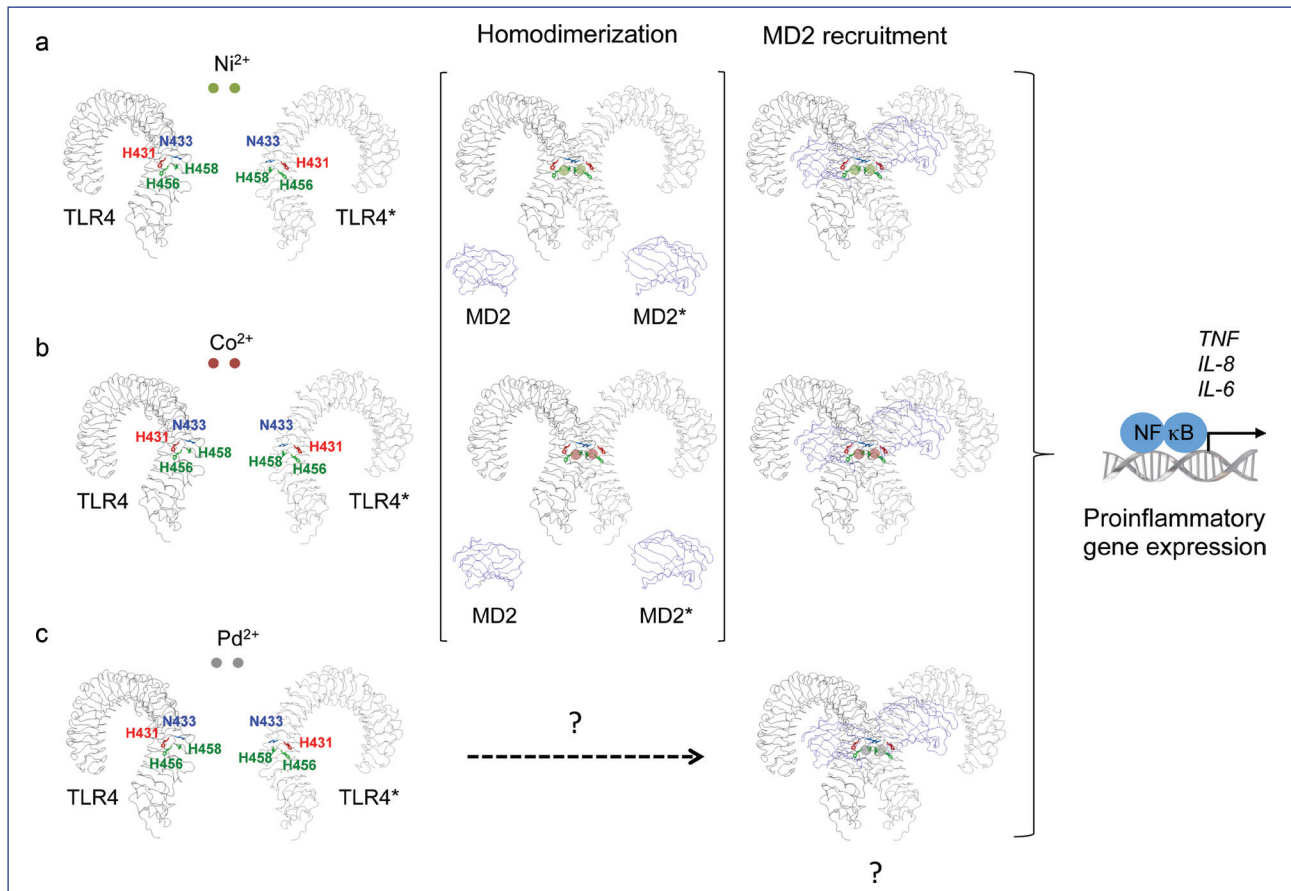


Figure 1 Model of metal-induced TLR4 activation. Nickel (Ni^{2+} , green spheres) (a) and likely also cobalt ions (Co^{2+} , brown spheres) (b) interact with two non-conserved histidines (H456 and H458, green residues) and one conserved histidine (H431, red residue) exposed at the dimerization interface of two opposing TLR4 molecules (black structure shown in wire representation). This triggers the formation of a tetrameric TLR4/MD2 complex that initiates proinflammatory gene expression, most importantly via MyD88-dependent activation of the IKK2/NF- κ B cascade. Unlike the natural TLR4 ligand LPS, nickel and cobalt can trigger TLR4 dimerization in absence of its coreceptor MD2 [22] (blue structure), but MD2 is strictly required for initiation of downstream proinflammatory signaling [21, 22]. Activation of the TLR4/MD2 complex by nickel and cobalt requires TLR4 dimerization as evidenced by the abortion of nickel/cobalt-induced NF- κ B activation by mutation of asparagine 433 (N433, blue residue) in human TLR4, disrupting TLR4 dimerization [22]. However, it is currently unclear whether TLR4 homodimerization is a prerequisite for MD2 recruitment or whether nickel and cobalt interact with preformed TLR4/MD2 heterodimers. In analogy to nickel and cobalt, palladium (Pd^{2+} , grey spheres) triggers TLR4/MD2-dependent NF- κ B activation [27], but it is unknown if this is also mediated by interaction with the above-mentioned metal-binding histidine residues and requires TLR4/MD2 heterodimerization (c).

and H458 that mediate proinflammatory signaling by nickel and cobalt [21, 22] (Figure 1c). Notably, these histidines have recently been experimentally validated as genuine nickel-binding sites mediating a rather pH-stable interaction of nickel with an isolated human TLR4-derived peptide encompassing amino acids 439 to 460 [28].

Intriguingly, cross-species comparison of TLR4 proteins have revealed that – with respect to metal binding histidines – only H431 is fully conserved, whereas the histidine duplet

H456/H458 is exclusively present in primates [19]. Accordingly, nickel and cobalt fail to stimulate murine TLR4, but readily trigger TLR4-dependent proinflammatory activation upon introduction of histidines into murine TLR4 at the H456/H458-equivalent sites [21, 22]. The lack of proper metal binding residues in mouse TLR4 also provides a reasonable explanation for why early attempts at inducing CHS with these metal allergens were unsuccessful, unless LPS or other adjuvants had been coadministered [29, 30]. In the absence

of any adjuvants, nickel effectively induces CHS in mice only once human TLR4 has been introduced as transgene [21]. These data demonstrate that adequate innate immune stimulation is crucial for proper CHS induction, but also show that this signal can be provided by alternative means.

Interestingly, in the latter study, we also noticed that nickel triggered human TLR4-transgene-dependent release of interleukin-1 beta (IL-1 β) from bone marrow-derived mouse macrophages (BMDM) [21]. Interleukin-1 isoforms are essential for CHS induction by model haptens [31–35] and – along with TNF α – represent key cytokines regulating mobilization of DCs and T-cell priming [36, 37]. Unlike TNF α , however, which is immediately active and released upon production, biological activity and secretion of IL-1 β additionally requires activation of the inflammasome. The latter is a multi-component protease complex consisting of a nucleotide-binding oligomerization domain, leucine-rich repeat and pyrin domain containing protein (NLRP), ASC and the protease caspase-1, which senses cellular danger signals such as extracellular ATP and responds to their presence by cleaving inactive pro-IL-1 β into mature IL-1 β [38]. Macrophages show very low basal expression of pro-IL-1 β , but markedly increase its transcription upon LPS stimulation [39]. The mechanism requires the TLR/IL-1-receptor-associated adaptor protein MyD88 [40], which likewise contributes to nickel-induced proinflammatory gene expression [21]. Nickel may thus be expected to prime pro-IL-1 β transcription. However, the effective release of IL-1 β from human TLR4-transgenic murine BMDM upon nickel treatment suggests that it may additionally trigger inflammasome activation. Indeed, a recent study has provided experimental evidence for nickel-induced IL-1 β secretion via the NLRP3 inflammasome [41]. Remarkably, upon LPS priming, nickel is also capable of triggering IL-1 β release from wild-type murine BMDMs, suggesting that – unlike TLR4 stimulation – the inflammasome-activating capacity of nickel is conserved among species. Analogous to unrelated inflammasome activators [42], nickel-dependent inflammasome stimulation occurs through induction of mitochondrial reactive oxygen species (ROS) and requires caspase-1 and ASC [41].

Much less information is available regarding inflammasome activation by other metal allergens. As to cobalt, both inhibition and activation of the inflammasome have been reported [43–45]. The capacity to induce inflammasome activation has been found to vary with particle size in cobalt-chromium-molybdenum (CoCrMo) alloy particles, of which cobalt and chromium form the active components [46]. In analogy to nickel, the latter study reported dependency of CoCrMo alloy particle-induced IL-1 β release on NLRP3, caspase 1, and ASC. However, instead of mitochondrial ROS production, inflammasome activation depended on

lysosomal damage [46]. Thus, with respect to related metal allergens, it is currently unclear whether inflammasome activation occurs equally effective and via the same mechanisms. Moreover, direct evidence is still pending whether inflammasome activation is equally important for metal-induced CHS as it is for other haptens [47–49]. Since hapten-induced pro-IL-1 β production, ROS-mediated NLRP3 inflammasome activation, and CHS sensitization have recently also been linked to activation of a novel ITAM/Syk/Card9 signaling axis present in DCs [50], it remains to be elucidated whether this signaling module may also contribute to metal-induced CHS responses. At any rate, IL-1 signaling appears to be relevant at some point, since cotreatment of nickel or palladium with LPS has failed to induce CHS in IL1 α/β double knockout mice [30].

Metal-induced adaptive immune response

Early experiments with model haptens in MHC I or II-deficient mice that naturally lack CD8 $^{+}$ Tc cells or CD4 $^{+}$ Th cell responses, or in wild-type mice depleted of CD4 $^{+}$ or CD8 $^{+}$ cells established CHS as primarily CD8 $^{+}$ Tc-driven response, which does not require CD4 $^{+}$ T cells, but is rather restricted by CD4 $^{+}$ T cells [51–53]. Analysis of cytokines released from lymph node-derived, hapten-stimulated T cells have identified the CHS-inducing CD8 $^{+}$ effector T cells as Tc1 subclass mainly producing type I cytokines such as interferon- γ . Negative regulatory CD4 $^{+}$ T cells, on the other hand, are of the Th2 type as evidenced by their predominant secretion of IL-4, IL-5, and IL-10 [54]. CHS and consequently ACD have thus classically been viewed as Tc1-induced diseases regulated by Th2 cells. However, depending on the hapten and experimental model (employed mouse strain, analyzed species), different subtypes may be prevalent. For instance, in some mouse models and haptens, CD4 $^{+}$ Th1 cells have also been shown to exert effector functions [6, 55]. In addition, Th17, Th22 [56] and most recently IL-9-producing Th9 cells, which inhibit Th1 function both directly and via production of IL-4 [57], have been added to the list of contributing cells. Interestingly, the latter study also showed nickel-dependent secretion of IL-9 from human peripheral blood mononuclear cells (PBMC) of nickel-sensitized individuals but not from PBMCs of healthy donors, suggesting that Th9 cells exert a regulatory role in nickel-induced ACD [57]. The question, whether different haptens might trigger distinct immune-polarizing responses in human ACD, has recently been addressed by Dhingra and colleagues [58]. They thoroughly profiled gene expression in skin samples of patients sensitized to different common haptens – including fragrances, rubber, nickel, and other metal allergens – in

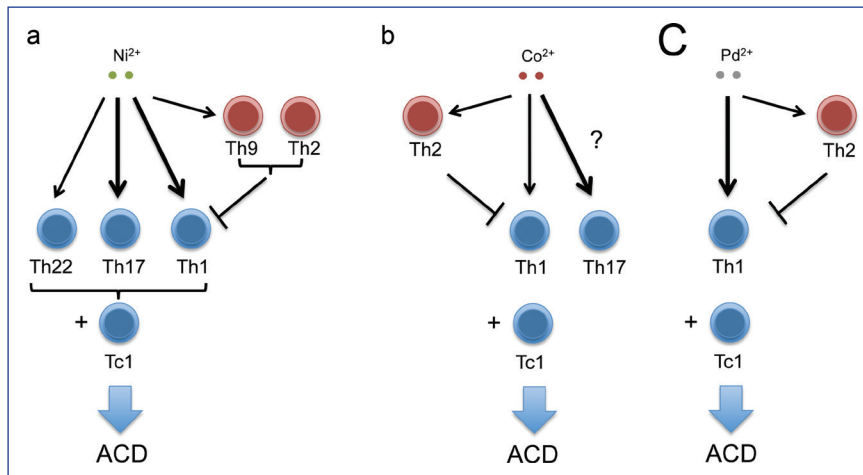


Figure 2 Overview of reported Th cell polarization patterns induced by nickel, cobalt, and palladium. Nickel-induced ACD is characterized by strong coinduction of Th1/Th17 cells with some Th22 contribution [58] (a). By contrast, cobalt-induced ACD has been implicated to exhibit strong Th17 bias [58] (b). Palladium has been reported to trigger a mixed Th1/Th2 response (c), which may also be true for other metal allergens including nickel and cobalt [60, 61]. Although metal-induced Th2 responses closely correlated with patch test reactivity in these studies, Th2 cells are likely to

fulfill a regulatory function. This is suggested by Th2-mediated IL-10 induction inhibiting Th1 cytokine expression without affecting Th2 cytokine production [63]. Besides Th2 cells, nickel has also recently been shown to induce IL-9-producing Th9 cells, which inhibit Th1 responses both directly and via Th2 cytokine induction [57].

comparison to petrolatum-occluded skin (as control). Employing gene arrays as well as qRT-PCR and immunohistochemistry, they found that nickel triggered potent innate immune activation together with induction of a strong Th1/Th17 response along with a Th22 component (Figure 2a). By contrast, the response to other metals, including cobalt and chromium, was predominantly characterized by induction of Th17 markers (Figure 2b). Fragrances and to a lesser extent rubber, on the other hand, showed a strong Th2 bias with some Th22 polarization but only a small Th1/Th17 contribution [58]. Yet, other studies comparing multiple metal allergens – including nickel, cobalt and palladium – have reported a close correlation between skin reactivity and the expression of Th2 cytokines in ACD, and found metal allergens to induce a mixed Th1/Th2 response [59–61] (Figure 2c). In a recently published novel mouse model of CHS to chromium, which required co-application of LPS during sensitization, there was a similar Th2-dominated response towards chromium [62]. However, in light of the above-mentioned CHS results with MHC II-deficient mice, the observed correlation of metal reactivity and Th2 polarization may also reflect a proportional counter response to Tc1/Th1 induction (Figure 2) as suggested by the coinduction of IL-10 by those cells shown to inhibit Th1 cytokine expression without affecting Th2 cytokine production [63]. Clearly, more work is required to resolve this issue and to define the exact role of different Th subsets in ACD-induction by individual metal haptens. Nevertheless, the observed correlation between the degree of hapten reactivity and the strength of the Th2 response holds prognostic promise with respect to in vitro

sensitization testing. Moreover, selective targeting of individual Th classes might be an interesting perspective for future treatment of ACD induced by metal allergens, particularly in case of chronification.

Conclusions

Much progress has been made regarding the mechanisms underlying metal-induced innate immune activation and T cell activation. The recent availability of a human TLR4-transgenic mouse model now enables us to study metal-induced CHS in the absence of additional adjuvants. Here, the objective is to decipher common and diverse mechanisms of metal-induced CHS in detail and to identify and test novel target structures for therapeutic intervention. The discovery of human TLR4 as common innate immune receptor for nickel, cobalt, and palladium as well as the identification of additional/alternative mechanisms of innate immune activation by metal allergens may allow for future development of safe and specific therapeutic strategies in ACD induced by this clinically highly relevant group of contact allergens. However, there is one important lesson to be learned from the example of species-dependent activation of TLR4 by metal allergens: native mouse models do not always adequately reflect the situation in humans. Therefore, each finding in mice should be thoroughly retested with either humanized mouse models or human cells. Moreover, the establishment of novel human skin-based test models is urgently needed to study the immunologic properties of the more than 4 000 currently known contact allergens [64].

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