

Nickel allergies: paying the Toll for innate immunity

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Abstract Allergic contact hypersensitivity responses to the transition metal nickel (Ni^{2+}) affect millions of people worldwide despite extensive legislative efforts to ban the use of Ni^{2+} in products coming into direct contact with the skin. Like other contact allergens, Ni^{2+} triggers a T lymphocyte-driven delayed-type hypersensitivity reaction that is characterized by leukocyte infiltration at sites of allergen exposure. The last years have revealed that besides a hapten-specific T cell response, Ni^{2+} can also directly trigger an innate immune response in resident skin cells that is necessary for mounting an allergic hypersensitivity reaction to Ni^{2+} . Recently, the receptor for the bacterial membrane component lipopolysaccharide, Toll-like receptor 4, has been identified as the crucial mediator of the innate immune response to Ni^{2+} , demonstrating that Ni^{2+} employs signaling components of the bacterial defense system to elicit its allergic reactions. Here, we provide an overview of Ni^{2+} -induced signaling events that have been implicated in contributing to the hypersensitivity response to this transition metal. We briefly review the causes and genetic predisposition fostering allergic responses to Ni^{2+} and discuss potential therapeutic and prophylactic strategies and chances evolving from the novel insights into the molecular basis of this disease.

Keywords Nickel · Allergic contact dermatitis · Innate immunity · Toll-like receptor · Inflammation

Introduction

Allergic contact hypersensitivity to metal ions has first been described in 1930 by the dermatologist Stephan Rothman who published a case report on a 42-year-old patient suffering from a mysterious allergic response to coins [1]. The man was an employee from a local street railway company, mainly occupied with the counting of silver, copper, and nickel coins and had been admitted to his clinic because of a severe itching rash. Rothman suspected a causal relationship and exposed the back skin of the patient to cleaned specimens of coins containing the different metals. He was able to show that coins composed of nickel (Ni^{2+}) induced a strong local reddening and swelling at the site of exposure. After confirmation that a strongly diluted metal salt solution could elicit the same hypersensitivity response, he deduced that the metal ion itself was responsible for the reaction and stated “obviously only those metal amounts are effective that are solubilized by the contact to the skin.” This perfectly describes what is state of the art more than 80 years later: It is the metal ions solubilized by sweat and other body fluids that serve as sensitizing allergen. Rothman concluded his study by mentioning that treatment of his patient had been unsuccessful until the patient had changed his job, thus illustrating the complication of severe contact hypersensitivity now and then: It may eventually lead to occupational incapability although nowadays the possibility of topical corticosteroid treatment somewhat ameliorates the situation.

While the risk of occupational disability arguably is an issue for a small group of professionals such as metal workers, cashiers, or hairdressers [2, 3], the major problem associated with Ni^{2+} -induced contact hypersensitivity is its wide spreading in modern industrial products. For instance, Ni^{2+} is not only released from coins [4], but is also a

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common component of earrings, watches, belt buckles, bras, mobile phones, dental and orthopedic implants, and cardiovascular stents [5–10]. Moreover, modern lifestyle, in particular the recent fashion of piercing as well as a largely uncritical consumer attitude towards the wearing of cheap decoration from overseas, has led to a renaissance of incidences of Ni^{2+} -induced contact dermatitis. In Europe, currently around 65 million individuals are estimated to be sensitized to Ni^{2+} [11]. Due to their exposure habits, females show higher sensitization rates than males [12, 13]. Legislative interventions limiting the use of Ni^{2+} in products of daily life such as the Danish nickel regulation [14] and the EU directive 94/27/EG (also known as “nickel directive”) appear to result in decreasing sensitization rates, but prevalences of contact eczema to Ni^{2+} are still considerably high [11, 15, 16].

Here we review the current advances on origin, progression, and the molecular mechanisms of Ni^{2+} -induced contact hypersensitivity and discuss novel therapeutic and preventive perspectives, which emerge from an improved understanding of the pathophysiology of metal-induced contact hypersensitivity.

Principles of contact hypersensitivity

The terms “allergic contact dermatitis” (ACD) referring to the clinical manifestation of contact allergy in humans and “contact hypersensitivity” (CHS) used in the context of experimental mouse models describe an eczematous skin reaction (dermatitis) in response to an allergen, i.e., to a foreign substance that generates a hypersensitivity response by triggering the immune system. In immunological terms, contact allergies represent a subform of a delayed-type hypersensitivity response (type IV immune reaction), which is characterized by activation of antigen-specific T lymphocytes resulting in clinically manifest skin inflammation evolving 48 to 72 h after re-exposure to the antigen. Contact allergens typically are haptens (half antigens), which can only act when bound by endogenous proteins. ACD can be subdivided into two separate phases: An inapparent sensitization phase, which is initiated upon first contact with the hapten leading to the generation of allergen-specific T cells, and an elicitation phase that results in clinically apparent local skin inflammation upon re-exposure to the allergen [17–19]. The sensitization process gets started when resident antigen-presenting cells (APC) such as epidermal Langerhans cells or dermal dendritic cells perceive the hapten, become activated, and migrate to the dermal lymph node [17, 18]. There, they prime naive T lymphocytes, leading to the generation of hapten-specific Th1/Tc1 and Tc17/Th17 effector T cells [18, 20], which subsequently enter the bloodstream. Upon

re-exposure, the elicitation phase commences: T cells enter the skin at sites of contact and together with infiltrating neutrophils, macrophages, and resident skin cells, they elicit the typical skin inflammation characterized by erythema, tissue edema, spongiosis, and scaling.

While for most contact allergens these two processes are still unclear in molecular terms, some basic principles of ACD have been found. So, irrespective of the individual hapten studied, the concept emerges that contact allergens generally require two signals to successfully trigger ACD [17, 21, 22]: the aforementioned hapten-specific T cell response and a more unspecific proinflammatory signal (throughout this review referred to as “innate immune signal”), which is effective in both phases. The latter is required for initiation of the adaptive immune response via activation of APCs as well as for activation of resident skin cells such as endothelial cells and infiltrating leukocytes that assist the adaptive T cell response during the elicitation phase and provide a critical proinflammatory microenvironment necessary for the development of a hypersensitivity response. While some haptens such as Ni^{2+} can autonomously provide this innate immune signal by directly activating proinflammatory pathways (see below), it is presently a matter of debate whether this holds true for all contact allergens. In particular, weak allergens such as certain perfume ingredients may perhaps not be able to generate the required threshold of innate immune activation alone but depend on unrelated stimuli such as, e.g., a microlesion or a coincidental viral/bacterial infection to exert their full allergic potential [22]. Indirect evidence for this hypothesis is provided by the observation that sensitization to an allergen is greatly facilitated when simultaneously an irritative substance (i.e., an agent with the capacity to activate the innate immune response) is applied [23, 24], which amplifies the innate immune signal. In some experimental systems, it has been proven impossible to elicit a CHS response unless a simultaneous adjuvant stimulus was provided in both sensitization and elicitation phases. A prominent example among the metal-induced contact allergies is the hitherto used mouse models of Ni^{2+} -induced CHS. These animal models strictly require the use of adjuvants as Ni^{2+} , in contrast to the human situation, is incapable to mount an innate immune response in mice [25]. While one model is based on stimulation with the bacterial cell wall component lipopolysaccharide (LPS) [26], which triggers activation of the innate immune system via binding to the pattern recognition receptor Toll-like receptor 4 (TLR4), a second model relies on combined treatment of Ni^{2+} with either complete Freund’s adjuvants, irritants such as sodium dodecyl sulfate (SDS) and PMA, or IL12, respectively [27]. These treatments activate stress-regulated signaling pathways [28] or the inflammasome [29], a multiprotein

complex, resulting in the release of the proinflammatory cytokine IL1 beta (IL-1 β) thereby providing the missing innate immune signal for Ni²⁺-induced CHS in mice. Importantly, activation of the innate immune system appears to be required for both the sensitization phase as well as for the elicitation phase as in the first mouse model, LPS co-treatment lowers the necessary amount of Ni²⁺ required for CHS in both phases [30]. Similar observations have been made with other contact allergens [23, 31]. For instance, the lowest amounts of oxazolone, which on their own fail to induce a CHS response in sensitized mice, can elicit CHS when a second irrelevant hapten is co-administered during the elicitation phase [31]. Thus, the intensity of CHS induction by a contact allergen apparently is directly correlated to its capacity to trigger an innate immune response. Notably, some irritant contact allergens can also elicit this response by indirectly activating TLR signaling via production of damage-associated molecular patterns (reviewed in [22]). These are endogenous TLR ligands such as soluble hyaluronan, which can be released by oxidative degradation of high molecular weight hyaluronan present in the extracellular matrix [32]. This degradation product of hyaluronan is highly proinflammatory and acts as a potent activator of TLR2 and TLR4 [33]. Its release has been proposed to mediate the CHS responses to 2,4,6-trinitrochlorobenzene (TNCB), which requires TLR2 and TLR4, since TNCB-mediated CHS is inhibited by a peptide inhibitor for hyaluronan [34].

In summary, the induction of hypersensitivity by contact allergens requires an innate immune signal, but apparently, divergent mechanisms are in charge for its generation.

Proinflammatory pathways triggered by Ni²⁺

From all of the more than 3,000 known contact allergens [35], Ni²⁺ thus far is the only one for which a direct mechanism of innate immune activation has conclusively been demonstrated and unraveled at the molecular level. Historically, the first evidence for a direct activation of proinflammatory pathways by Ni²⁺ originates from the observation that treatment of primary human endothelial cells (ECs) with Ni²⁺ triggers rapid expression of the surface adhesion molecules VCAM1, ICAM1, and E-selectin [36, 37], all of which are required for leukocyte adhesion. Likewise, Ni²⁺ is able to cause a rapid endothelial induction of the leukocyte-attracting adhesion molecule E-selectin within 24 h after ACD elicitation in sensitized individuals (Fig. 1a), a timepoint well before Ni²⁺-reactive T cells and other leukocytes infiltrate the skin. In situ hybridization furthermore demonstrated a clearly detectable mRNA expression of the monocyte-attracting chemokine MCP1/CCL2 as early as 6 h after

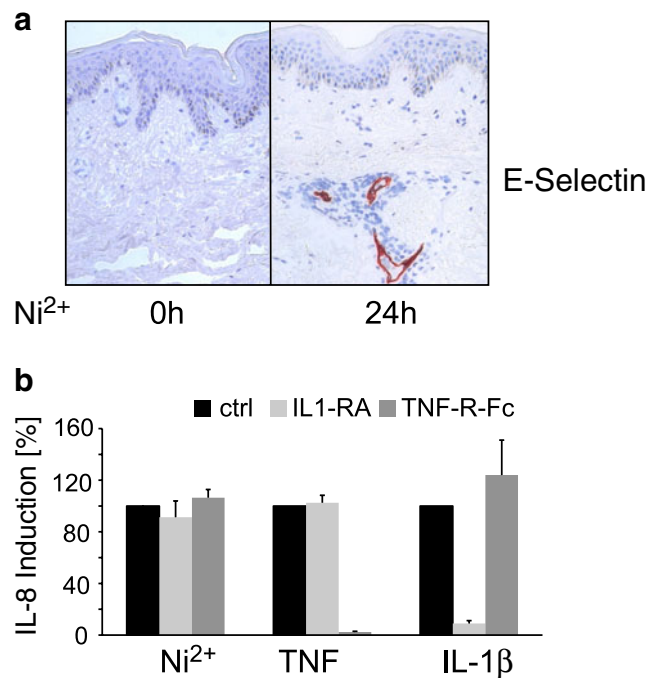


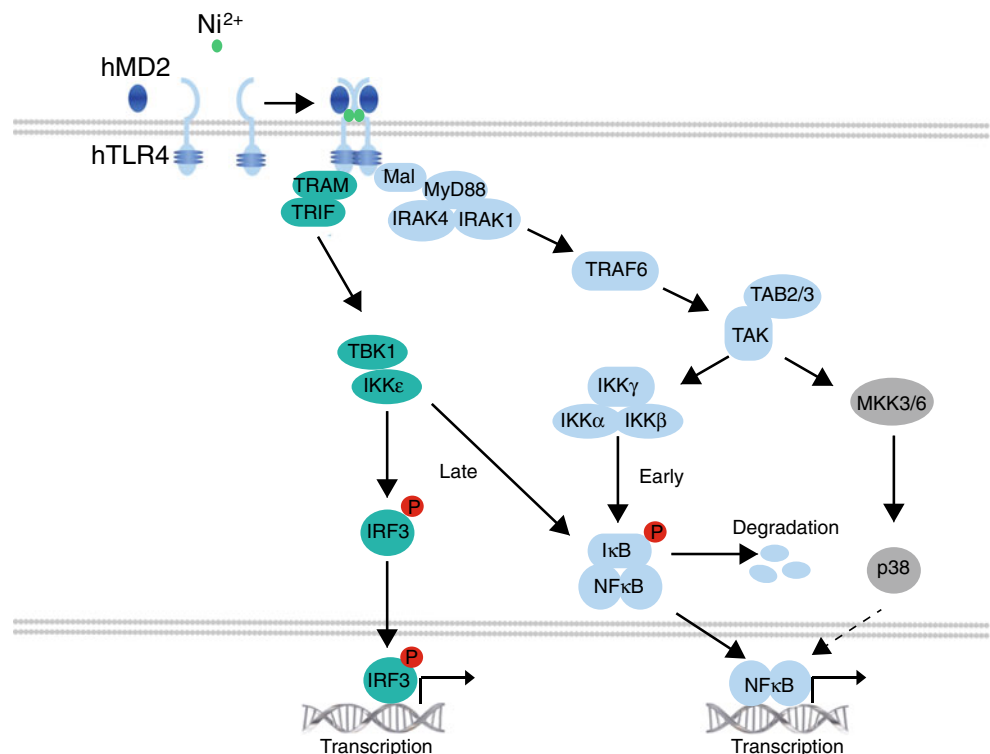
Fig. 1 Ni²⁺ elicits a rapid proinflammatory signal independent of TNF and IL-1 β induction. **a** Immunohistochemical staining for the endothelial proinflammatory adhesion molecule E-selectin, demonstrating endothelial activation within 24 h after epicutaneous application of Ni²⁺ to the skin of a sensitized individual. The perivascular leukocyte infiltrate reflects a very early stage of inflammation that requires the preceding upregulation of endothelial adhesion molecules such as E-selectin. **b** IL-8 ELISA of supernatants obtained after stimulation of human primary ECs for 8 h with Ni²⁺ (1.5 mM), TNF (2 ng/ml), or IL-1 β (50 U/ml) in the presence or absence of 400 ng/ml IL-1 receptor antagonist (IL-1-RA, Anakinra), 20 μ g/ml soluble TNF receptor 2 (TNFR2-Fc, Etanercept), or 20 μ g/ml IgG₂ control antibody (ctrl), showing independency of Ni²⁺-induced inflammatory gene expression from IL-1 or TNF induction, respectively. To facilitate comparison, IL-8 expression in the IgG₂-treated samples was arbitrarily set to 100%. Cells were pretreated with the indicated inhibitors 1 h prior to stimulation. Data represent means $\pm$ SEM of $n=3$ experiments

ACD elicitation in skin sections of Ni²⁺-sensitized individuals [38]. In contrast to the early expression of monocyte-attracting cytokines, mRNA expression of primarily lymphocyte-attracting cytokines such as IP10, Mig, MDC, PARC, and TARC was only evident at later timepoints and correlated well with the infiltration of T cells into the dermis and epidermis at 72 h after allergen challenge [38]. Of note, the early induction of proinflammatory marker genes was independent of the induction of IL-1 or TNF alpha [37] (Fig. 1b) ruling out secondary effects by the induction of those cytokines. Molecular analysis of the intracellular signaling pathways responsible for the Ni²⁺-induced proinflammatory gene expression revealed that it can strongly activate the conserved IKK2/NF κ B signaling pathway to an extent comparable to inflammatory cytokines in primary human ECs [39].

Likewise, Ni^{2+} treatment of ECs results in rapid activation of stress-activated mitogen-activated protein kinase (MAPK) pathways such as p38 [40]. Since both the IKK2/ $\text{NF}\kappa\text{B}$ and the p38 MAPK pathways critically contribute to proinflammatory gene expression induced by either Ni^{2+} [40] or TNF [41, 42], we performed gene chip analysis of Ni^{2+} -stimulated human primary ECs [43] and compared those to the gene expression pattern induced by TNF [42, 43]. While we expectedly found a close overlap between TNF- and Ni^{2+} -induced proinflammatory gene expression, which was sensitive to blockade by expression of a dominant-negative IKK2 mutant and, partially, to chemical inhibition of p38, the direct comparison of all regulated transcripts for both stimuli revealed a much wider panel and a generally greater number of regulated genes for Ni^{2+} as compared to TNF [43]. This corroborated the previous finding that Ni^{2+} -induced proinflammatory gene expression cannot simply be accounted for by secondary induction of TNF [39] (compare Fig. 1b). Because of the rapid kinetics of Ni^{2+} -induced IKK2 and p38 activation [40], we early on suspected the existence of a cellular receptor for Ni^{2+} that mediates the induced innate immune response in human cells. However, for a long time, the upstream regulators for Ni^{2+} -induced activation of those two proinflammatory cascades remained unknown since expression of various dominant-negative mutants of potential upstream mediators for those pathways proved unsuccessful (Schmidt and Goebeler, unpublished data). The winning hint then came from the observation that expression of a dominant-negative mutant of IRAK1 kinase, which critically mediates signaling events downstream of the IL-1 receptor family and the TLR class of pattern recognition receptors [44], was able to abrogate Ni^{2+} -induced expression of $\text{NF}\kappa\text{B}$ target genes such as CCL2/MCP1 [25]. Having excluded a role for the IL-1 receptor family by blocking experiments with a recombinant form of the soluble IL1-receptor antagonist (IL-1Ra) anakinra (Fig. 1b), which tightly binds to the IL1-receptor 1 (IL-1 R1) thereby blocking activation by the most relevant family members IL-1 α and IL-1 β [45], we concentrated on the TLR family. Out of the ten TLR family members currently known [46, 47], only TLR3 and TLR4 were found to be expressed and functional in human umbilical vein ECs while expression and functionality of other candidate TLRs such as TLR2, which previously has been implicated to mediate CHS induction by other contact allergens [34], could not be detected under our culture conditions ([48] and unpublished results). Accordingly, we tested the effect of small interfering RNA (siRNA) for the most likely candidate, human TLR4 (hTLR4), on Ni^{2+} -induced expression of the $\text{NF}\kappa\text{B}$ target gene IL-8. siRNA-mediated depletion of hTLR4 abrogated Ni^{2+} -induced IL-8 expression in human primary ECs [25],

which, for the first time, conclusively demonstrated an essential involvement of hTLR4 in Ni^{2+} -dependent proinflammatory gene expression. Further evidence for a crucial role of the hTLR4 receptor complex was provided by the observation that expression of hTLR4 in combination with its co-receptor MD2, but not expression of either of the single genes, was sufficient to confer Ni^{2+} responsiveness to HEK293 cells, which do not express endogenous TLRs [25]. As a result, we could show that human cells expressing hTLR4 and hMD2 including macrophages, fibroblasts, and dendritic cells were able to mount a proinflammatory response upon Ni^{2+} treatment ([25] and unpublished results). Strikingly, similar experiments using the respective mouse cells or cell lines showed resistance of the latter to proinflammatory activation by Ni^{2+} despite a functional expression of the murine TLR4/MD2 complex, as was confirmed by their responsiveness to the classical TLR4 ligand LPS [25]. Having excluded a species-dependent effect of MD2, expression of mouse/human chimeric TLR4 constructs as well as introduction of point-mutated hTLR4 variants into HEK293 cells stably expressing hMD2 finally revealed that two non-conserved histidine residues in hTLR4, H456, and H458 are critically required for Ni^{2+} -induced proinflammatory gene expression but are dispensable for LPS-induced responses [25]. Mouse TLR4 (mTLR4) lacks those two histidine residues, accounting for the failure of mice to elicit a CHS response to Ni^{2+} in the absence of co-stimulating adjuvants. Importantly, transgenic expression of hTLR4 but not of mTLR4 could restore the missing innate immune signal in mTLR4-deficient mice and was sufficient to confer susceptibility of those mice to Ni^{2+} -induced CHS [25]. Of note, expression of hTLR4 but not of a hTLR4 mutant bearing two inactivating alanine replacements at the amino acid positions H456 and H458 (hTLR4-H456A-H458A) not only restored Ni^{2+} -induced release of the known $\text{NF}\kappa\text{B}$ -dependent cytokine IL-6 in wild-type mouse macrophages but also triggered Ni^{2+} -dependent expression of the interferon-reponse factor 3 (IRF3)-restricted cytokines IFN α/β [25] that is mediated by a separate pathway downstream of TLR4 [46, 47]. Consistently, transgenic expression of hTLR4 but not of mTLR4 in mTLR4^{-/-} bone marrow-derived macrophages not only allowed expression of $\text{NF}\kappa\text{B}$ - and IRF3-dependent cytokines upon Ni^{2+} exposure but also phosphorylation of p38 and IRF3 [25]. Thus, besides enabling Ni^{2+} -dependent $\text{NF}\kappa\text{B}$ activation, hTLR4 expression is also sufficient to mediate activation of other TLR4-driven signaling events by Ni^{2+} in mice (Fig. 2), accounting for the efficient induction of CHS by Ni^{2+} in the hTLR4-transgenic mouse model. While one lesson from these studies is that hTLR4 is crucial for Ni^{2+} -induced proinflammatory activation, another conclusion is that contact allergens apparently

Fig. 2 Overview of TLR4-dependent signaling events triggered by Ni^{2+} . Ni^{2+} initiates proinflammatory signaling by presumably triggering dimerization of two hTLR4/hMD2 heterodimers resulting in MyD88-dependent rapid activation of the IKK2/NF κ B and MKK3/6/p38 pathways, which are both required for proinflammatory gene expression. Moreover, Ni^{2+} triggers activation of IRF3-dependent gene expression via recruitment of the adaptor proteins TRAM and TRIF, which additionally provides a late activation signal for NF κ B. Activation of all three pathways is abrogated by mutation of H456 and H458 in hTLR4, while those mutations do not affect LPS-induced hTLR4 activation



take advantage of host-specific pattern recognition structures, originally devised to fight infections, to elicit their allergic effects. This plausibly explains why co-treatment with LPS, which triggers mTLR4, can substitute the missing hTLR4 signal delivered by Ni^{2+} in mice and allows for CHS development in response to this hapten [26]. The observations furthermore underscore the crucial requirement of an efficient innate immune signal for the elicitation of CHS by contact allergens. On the other hand, the former implicates that the process of generating Ni^{2+} -specific T cell clones can even take place when the innate immune system is activated by alternative agonists. Indeed, Gamerding and colleagues showed that in the case of Ni^{2+} , the priming of T cells appears to result from a peptide-independent direct bridging between the hapten-specific T cell receptor (TCR) and the restricting histocompatibility human leukocyte antigen DR protein chain on the APC [49]. Thus, the priming of Ni^{2+} -specific T cells apparently is a direct consequence of hapten binding to protein structures within the TCR and MHC proteins and may occur as long as the respective APC receives an adequate activating stimulus that elicits its migration to the dermal lymph node. This concept could account for the finding that not only TLR4 agonists such as LPS [26] but also irritants such as SDS are capable to replace the missing innate immune response to Ni^{2+} in mice [27], since they may activate the innate immune system via the inflammasome and subsequent IL-1-receptor activation as has been shown previously [50].

Considering the obvious expendability of H456 and H458 in hTLR4 for LPS responses, it is currently unclear why humans have acquired these mutations. However, a sequence comparison of different species reveals that these replacements are present in all examined primates (Fig. 3, gray-shaded box), suggesting a common origin of these sequence variants for primates, which may indicate an as yet undetected evolutionary benefit for those species. Of note, the comparison predicts that no other domestic animals appear to be prone for Ni^{2+} -induced CHS albeit the common house cat (*Felis catus*) bears the equivalent of H458, and dogs (*Canis lupus familiaris*) exhibit a similarly charged arginine at this position and thus may exhibit some limited responsiveness (Fig. 3).

NF κ B-independent signaling pathways triggered by Ni^{2+}

As elaborated above, the prominent capacity of Ni^{2+} to elicit ACD in humans most likely results from a combination of its ability to elicit an innate immune signal and a T cell response [51]. While the former is dependent on TLR4-induced signaling pathways such as the IKK2/NF κ B cascade, there is at least indirect evidence that Ni^{2+} can also activate TLR4-independent responses. For instance, a microarray analysis of the Ni^{2+} -induced gene expression patterns in ECs revealed that Ni^{2+} can also potentially induce various target genes commonly regulated by the transcription factor hypoxia-

			H433	H456 H458	
TLR4 [Homo sapiens]	400	SLKYLDLSFNGVITMSSNFLGLEQLEHLDFQHSNLKQMSSEFSVFLSLRNLIYLDISHTHTRVAFNGIFNGLSSLEVLKMA			479
TLR4 [Gorilla gorilla]	400	SLKYLDLSFNGVITMSSNFLGLEQLEHLDFQHSNLKQMSSEFSVFLSLRNLIYLDISHTHTRVAFNGIFNGLSSLEVLKMA			479
TLR4 [Pan paniscus]	400	SLKYLDLSFNGVITMSSNFLGLEQLEHLDFQHSNLKQMSSEFSVFLSLRNLIYLDISHTHTRVAFNGIFNGLSSLEVLKMA			479
TLR4 [Pan troglodytes verus]	400	SLKYLDLSFNGVITMSSNFLGLEQLEHLDFQHSNLKQMSSEFSVFLSLRNLIYLDISHTHTRVAFNGIFNGLSSLEVLKMA			479
TLR4 [Pongo pygmaeus]	400	SLKYLDLSFNDVITMGSNFLGLEQLEHLDFQHSNLKQMSSEFSVFLSLRNLIYLDISHTHTRVAFNGIFNGLSSLEVLKMA			479
TLR4 [Saguinus oedipus]	313	SLRYLDLSFNDVITMGSNFLGLEQLEHLDFQHSNLKQMSSEFSVFLSLRNLIYLDISHTHTRVAFNGIFNGLSLKVLKMA			392
TLR4 [Hylobates lar]	400	SLKYLDLSFNDVITMSSNFLGLEQLEHLDFQHSNLKQMSSEFSVFLSLRNLIYLDISHTHTRVAFNGIFNGLSSLEVLKMA			479
TLR4 [Macaca fascicularis]	400	SLKYLDLSFNDVITMSSNFLGLEQLEHLDFQHSNLKQMSQFSVFLSLRNLIYLDISHTHTRVAFNGIFDGLLSLKVLMKMA			479
TLR4 [Macaca nemestrina]	400	SLKYLDLSFNDVITMSSNFLGLEQLEHLDFQHSNLKQMSQFSVFLSLRNLIYLDISHTHTRVAFNGIFDGLLSLKVLMKMA			479
TLR4 [Macaca mulatta]	400	SLKYLDLSFNDVITMSSNFLGLEQLEHLDFQHSNLKQMSQFSVFLSLRNLIYLDISHTHTRVAFNGIFDGLLSLKVLMKMA			479
TLR4 [Cercopithecus torquatus atys]	400	SLKYLDLSFNDVITMGSNFLGLEQLEHLDFQHSNLKQMSQFSVFLSLRNLIYLDISHTHTRVAFNGIFDGLLSLKVLMKMA			479
TLR4 [Felis catus]	400	RLKHLDFSNNIITISSNFLGLEQLEHLDFQHSNLKQMSQFSVFLSLRNLIYLDISHTHTRVAFNGIFDGLLSLKVLMKMA			479
TLR4 [Equus caballus]	302	RLKHLDFSNDVISMSSNFMGLEQLEHLDFQHSNLKQMSQFSVFLSLRNLIYLDISHTHTRVAFNGIFDGLLSLKVLMKMA			381
TLR4 [Canis lupus familiaris]	400	RLKHLDFSNEIITMSSNFLGLEQLEHLDFQHSNLKQMSQFSVFLSLRNLIYLDISHTHTRVAFNGIFDGLLSLKVLMKMA			479
TLR4 [Bos taurus]	400	NLKHLDLSFNDVITLGSNFMGLEQLEHLDFQHSNLKQMSQFSVFLSLRNLIYLDISHTHTRVAFNGIFDGLLSLKVLMKMA			479
TLR4 [Sus scrofa]	400	KLKHLDFSNEIITMSSNFMGLEQLEHLDFQHSNLKQMSQFSVFLSLRNLIYLDISHTHTRVAFNGIFDGLLSLKVLMKMA			479
TLR4 [Rattus norvegicus]	398	NLKYLDLSFNGVILMSANFMGLEQLEHLDFQHSNLKQMSQFSVFLSLRNLIYLDISHTHTRVAFNGIFDGLLSLKVLMKMA			477
TLR4 [Mus musculus]	398	SLRHLDFSNGAIITMSANFMGLEQLEHLDFQHSNLKQMSQFSVFLSLRNLIYLDISHTHTRVAFNGIFDGLLSLKVLMKMA			477

Fig. 3 Sequence comparison of the homologous TLR4 regions in different species encompassing the Ni²⁺-responsive sites H431, H456, and H458 of hTLR4. Reference protein sequences for TLR4 from the indicated species were compared using the freely available Constraint-

based Multiple Alignment Tool [70] provided by the U.S. National Library of Medicine, National Institutes of Health. The gray box indicates primates, and the red and green boxes indicate conservation in the positions homologous to H456, H458, and H431 in hTLR4

inducible factor 1 alpha (HIF-1 α) [43]. This transcription factor is usually highly unstable and becomes stabilized in response to hypoxia [52], resulting in the expression of various proangiogenic genes including vascular endothelial growth factor [52, 53]. Ni²⁺ treatment leads to a rapid and concentration-dependent stabilization of HIF-1 α thus mimicking a hypoxia response [43] (Fig. 4a). Interestingly,

Ni²⁺-dependent HIF-1 α stabilization is resistant to inhibition of the IKK2/NF κ B pathway by expression of a dominant-negative IKK2 [43], suggesting that it occurs independently of NF κ B and may hence occur independently of TLR4 activation. Indeed, unpublished results confirm that Ni²⁺-induced HIF-1 α stabilization is readily detectable in TLR4-deficient HEK293 cells (Schmidt and

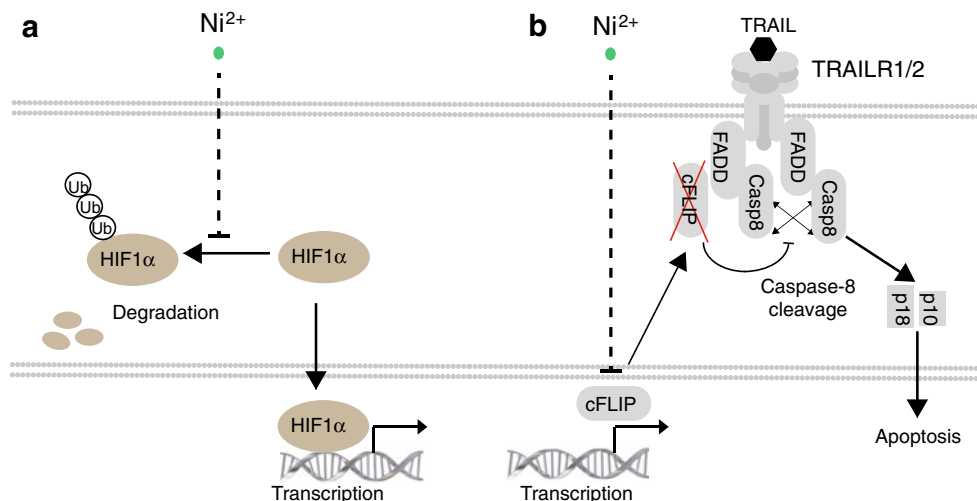


Fig. 4 Overview of TLR4-independent signaling events triggered by Ni²⁺. **a** Ni²⁺ induces stabilization of HIF-1 α in a hTLR4-independent manner, leading to HIF-1 α -dependent gene expression, which provides a proangiogenic response. **b** Ni²⁺ sensitizes primary human keratinocytes and ECs for TRAIL-induced apoptosis by downregulation of cFLIP mRNA expression via an as yet unidentified

mechanism. As a result, anti-apoptotic cFLIP protein is no longer available to counteract TRAIL-induced cleavage of the initiator Caspase-8 in the presence of TRAIL. The resulting initiation of apoptosis may be involved in the formation of epidermal spongiosis during ACD and amplifies the allergic response by destabilizing the barrier function of the skin

Goebeler, manuscript in preparation). While the significance of this observation for Ni²⁺-induced ACD is currently unclear, the fact that mice do not mount a CHS response to Ni²⁺ but still show induction of HIF1- α in the absence of hTLR4 suggests that HIF-1 α is unlikely to be causally related to Ni²⁺-induced ACD. However, activation of an angiogenic response may greatly facilitate the access of T cells into the exposed tissue and thus amplify the inflammatory response.

Another pathway that was demonstrated to be influenced by Ni²⁺ is the death receptor-mediated or extrinsic apoptosis pathway [54]. We could recently show that Ni²⁺ can transcriptionally repress expression of cFLIP, an anti-apoptotic cellular antagonist of the pro-apoptotic Caspase-8, in both primary human keratinocytes and ECs [55]. As a result, these primary cells, which show an unusually high resistance to death ligand-mediated apoptosis due to strong expression of endogenous cFLIP, are strongly sensitized to apoptosis by the death ligand TRAIL (Fig. 4b). While the functional consequence of this observation for Ni²⁺-induced ACD is currently unknown, there is evidence for an increased occurrence of death ligand-mediated keratinocyte apoptosis in the course of Ni²⁺-induced ACD in sensitized individuals [56]. While in this report increased keratinocyte apoptosis was attributed to FasL-dependent apoptosis, it is important to note that FasL and TRAIL use the same downstream signaling components to exert their pro-apoptotic effect, and apoptosis induction by both death ligands is refractory to cFLIP overexpression [57]. Hence, a transcriptional downregulation of cFLIP should principally sensitize cells to both death ligands. Of note, both primary ECs and keratinocytes express the receptors for both death ligands [55], demonstrating that they should generally be sensitive to modulations in cFLIP levels when death ligands are encountered. A known source of membrane-bound TRAIL and FasL is activated T cells [54, 58]. It is thus tempting to speculate that in the course of ACD repression of cFLIP might sensitize keratinocytes and ECs for T cell-mediated apoptosis via activation of death receptor-dependent apoptosis. This could perhaps explain the frequently observed phenomenon of spongiosis associated with Ni²⁺-induced ACD in humans, which previously has been proposed to result from death ligand-induced apoptosis of keratinocytes within the skin during elicitation of ACD [59]. Moreover, enhanced cell death of keratinocytes is predicted to impair the barrier function of the skin. Hence, Ni²⁺-mediated cFLIP downregulation might importantly tip the balance towards increased apoptosis of certain skin cell populations, which successively may augment the severity of the ACD response during the elicitation phase by increasing the efficient concentration of Ni²⁺ arriving in the epidermis.

Albeit the exact mechanism of cFLIP mRNA repression by Ni²⁺ has not been unraveled yet, neither p38 MAPK nor

IKK2/NF κ B or HIF-1 α -dependent pathways appear to be involved since expression of dominant-negative mutants or RNAi-mediated depletion of central components of those pathways were incapable to block Ni²⁺-induced sensitization for TRAIL-induced apoptosis [55]. Thus, at least one other yet unknown signaling pathway is likely to exist that operates independently of TLR4 and HIF1- α and is responsible for the observed transcriptional repression of cFLIP by Ni²⁺.

Prophylactic and therapeutic implications from Ni²⁺-induced TLR4 activation

It is well known that certain risk factors such as piercings greatly increase the chance of developing ACD to Ni²⁺. Yet, only a minority of pierced individuals finally develop ACD. Genetic factors have been hypothesized to influence the risk of developing sensitization to Ni²⁺ [60]. First-degree relatives of probands suffering from Ni²⁺ allergy more often reported a history of ACD to Ni²⁺ than relatives of individuals lacking sensitization to this metal [61]. Twin studies revealed that the concordance rate regarding the prevalence of sensitization against Ni²⁺ was significantly higher in monozygotic twins than in dizygotic ones indicating that genetic factors may be of relevance [62]. Genome-wide association studies analyzing the genetic basis of ACD to Ni²⁺ are currently not available. However, polymorphisms and mutations of various genes have been assumed to be associated with ACD [63]. Interestingly, mutations in the filaggrin gene, a histidine-rich protein in the upper layer of the epidermis that is functionally related to epidermal barrier function [64, 65], support the development of Ni²⁺-induced ACD [66–68].

In light of the proven involvement of TLR4 signaling in Ni²⁺-induced CHS, it is conceivable that certain polymorphisms might exist in the hTLR4 gene that modulate its activity and/or the binding efficiency for Ni²⁺ thereby influencing the susceptibility of certain individuals for Ni²⁺-induced ACD. Currently no information is available on a potential correlation of hTLR4 polymorphisms with Ni²⁺ susceptibility. However, such association studies are highly desirable, since they might be extremely valuable for prophylactic risk assessment, particularly for those professional groups facing a higher risk of developing Ni²⁺-induced ACD.

Besides having implications for disease prevention, the newly discovered mechanism of Ni²⁺-induced innate immune activation may also yield potential implications for therapy. Apart from strict avoidance of exposure, the standard treatment of Ni²⁺-induced ACD is still the usage of topical corticosteroids. However, these drugs frequently show considerable adverse effects when used for a

prolonged period of time. In particular for the cases of occupationally acquired ACD to Ni^{2+} , alternative prophylactic and therapeutic strategies are highly desirable. The observation that the Ni^{2+} -responsive site of hTLR4 is different from its LPS binding site [25] immediately suggests that drugs may be developed that prevent TLR4 signaling by Ni^{2+} but leave the essential function of hTLR4 in bacterial defense untouched. However, structural modeling of the interaction site of hTLR4 with Ni^{2+} revealed an overlap with the dimerization interface of hTLR4 and suggests that Ni^{2+} -induced TLR4 signaling most likely results from the bridging of two hTLR4 monomers [25] (Fig. 2). The design of a specific inhibitor thus is complicated by the likelihood that an interfering peptide or small compound inhibitor may interfere with dimerization, which might likewise affect LPS responses and impair defense against gram-negative bacteria. A more promising strategy is to prevent the initial binding of Ni^{2+} to hTLR4. This could be achieved by application of soluble hTLR4 receptor protein fragments or peptides or, probably more economic, by Ni^{2+} -chelating agents. The recent observation that 70-nm-calcium carbonate and calcium phosphate nanoparticles epicutaneously applied to the skin may efficiently capture Ni^{2+} and reduce skin irritation refers to such a strategy [69].

In any case, the newly solved mechanism of Ni^{2+} -induced innate immune activation raises great hopes that novel, preventive, and therapeutic strategies can be designed, which curb this, in former times mysterious, disease.

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