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Nickel sensitisation in mice: A critical appraisal

Pål Johansen^a, Ying Wäckerle-Men^a, Gabriela Senti^b, Thomas M. Kündig^{a,*}

^a Department of Dermatology, University Hospital Zurich, Gloriastrasse 31, 8091 Zurich, Switzerland

^b Center for Clinical Research, University Hospital Zurich, Raemistrasse 100, 8091 Zurich, Switzerland

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ABSTRACT

Background: The market release of new domestic and industrial chemical and metal products requires certain safety certification, including testing for skin sensitisation. Although various official guidelines have described how such testing is to be done, the validity of the available test models are in part dubious, for which reason regulatory agencies and research aim to further improve and generalise the models for testing of skin sensitisation.

Objective: We applied a recently published murine model of nickel allergy as to test its applicability in a regulatory setting and to study and better understand the events leading to type-IV hypersensitivity. Nickel was chosen as model hapten since it induces allergic contact dermatitis with high incidence in the general population.

Method: Typically, C57BL/6 mice were sensitised and challenged by intradermal applications of nickel, and cutaneous inflammation was analysed by the mouse ear-swelling test, by histology, and by lymphocyte reactivity in vitro.

Result: Surprisingly, the study suggested that the skin reactions observed were results of irritant reactions rather than of adaptive immune responses. Non-sensitised mice responded with cutaneous inflammation and in vitro lymphocyte reactivity which were comparable with nickel-sensitised mice. Furthermore, histological examinations as well as experiments in T-cell deficient mice demonstrated that lymphocytes were not involved and that nickel caused an irritant contact dermatitis rather a true allergic type-IV contact dermatitis.

Conclusion: The authors question the validity of the described murine model of nickel allergy.

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1. Introduction

Allergic contact dermatitis is a T-cell mediated type-IV hypersensitivity reaction induced through skin contact with an antigen or hapten. Such haptens are highly reactive, often lipophilic chemicals that become immunogenic after coupling to carrier proteins or cells, e.g. Langerhans cells and dermal dendritic cells. An important hapten is the metal nickel, which induces allergic contact dermatitis with high incidence in the general population, the incidence in the general female European population being more than 10% [1–4]. Nickel binds its carrier through an interaction with histidine-rich proteins, often on peptides present in the binding groove of MHC class I and II molecules, and thereby altering self-peptides such that they appear foreign to CD4 and CD8 T-cells [5]. Upon re-exposure to the same hapten, an eczematous reaction in the skin is elicited by

activated T-cells and as other inflammatory cells and mediators infiltrating the skin within 1–3 days.

Nickel and other haptens that induce allergic contact dermatitis are found in a variety of industrial and domestic products such as metals in jewellery, workshop tools, music instruments, cell phones, and coins, as well as in food, detergents, cosmetics and paints [3,4]. Hence, nickel is of interest both as a model for studying allergic contact dermatitis and for developing treatment options such as immunotherapy for patients with type-IV allergies; although scarce, epidemiological reports [6] and interventional clinical studies in the 1990s suggested that the induction of tolerance to nickel could be feasible [7–10].

The guinea pig model of nickel sensitisation was established during the 1960–1970s. The model was valid and reliable, but attempts to develop a murine model of nickel sensitisation continued. After all, murine models of sensitisation to other experimental compounds such as dinitrochlorobenzene and fluorescein isothiocyanate work well. More recently, it was reported that nickel sensitisation could be induced in C57BL/6 mice and that the sensitisation capacity of nickel increased with

* Corresponding author. Tel.: +41 44 255 1111; fax: +41 44 255 4418.

E-mail address: thomas.kuendig@usz.ch (T.M. Kündig).

higher oxidation states [11]; such oxidative conditions could be expected in inflamed skin [12]. In a subsequent therapeutic study, the same authors also described that oral application of nickel in sensitised mice induced anergic or tolerant T-cells with persistent suppressor activity [13].

A further public interest in models for studying skin sensitisation is the need to test the sensitisation risk of new domestic and industrial products. Many chemicals and metals are therefore under increasing scrutiny by regulators because of real or alleged effects on human health. The market release of such products has to be preceded by documenting that the product has no or limited sensitisation potential. For this, national and international agencies have published guidelines on how such sensitisation is to be tested and documented. However, the content of these guidelines varies, for which reason the same agencies are trying to develop new and improved testing guidelines with international consensus. As part of this attempt, the Swiss Federal Office of Public Health wanted to study different aspects of the murine model of nickel sensitisation, with the goal of testing its applicability in public guidelines for the industry. In the following contracted investigation, we applied the model described by Artik et al. [11] to address the given questions.

2. Methods

2.1.1. Chemicals

Nickel chloride hexahydrate and nickel sulphate hexahydrate were purchased from Fluka (Buchs, Switzerland), and hydrogen peroxide (30%) was purchased from Merck (Darmstadt, Germany). Sodium dodecyl phosphate (SDS), lipopolysaccharide (LPS) from *E. coli* 0111:B4, and ovalbumin were obtained from Sigma–Aldrich (Buchs, Switzerland). Phosphorothioate-modified CpG ODN 1668 was synthesized by Microsynth (Balgach, Switzerland). Ni(III) is unstable and therefore freshly prepared for each experiment by mixing Ni(II) with a 20-fold molar excess of hydrogen peroxide as described [11].

2.1.2. Mice

Female C57BL/6 mice were purchased from Harlan (Horst, the Netherlands) and used at 6–10 weeks of age. Female nude (B6.Cg-Foxn1nu/J) and T-cell receptor deficient (B6.129P2-Tcrbtm1Mom Tcrdtm1Mom/J) mice were purchased from Charles River Laboratories (Sulzfeld, Germany) and used at age 16–20 weeks. The mice were typically kept in conventional cages with certified AISI 304 stainless steel wire lids (Series 116) and bottle caps (Model ACCP2521) from Techniplast® as purchased from Indulab® (Gams, Switzerland); both lids and bottle caps were made from nickel-containing stainless steel. In selected experiments, in order to control for development of nickel tolerance, we bred C57BL/6 mice using PVC animal cages and water bottles which were not equipped with nickel-containing parts. The F1 offsprings were weaned after 3 weeks, kept in the same kind of nickel-free environment, and used for sensitisation experiments at the age of 8–9 weeks.

2.1.3. Sensitisation of mice

If not otherwise described, the mice were sensitised by intradermal injection (28G needles) of 10 mM Ni(II) or Ni(III) chloride; oxidised nickel was made by mixing equal volumes of 20 mM nickel chloride and 400 mM hydrogen peroxide [11]. The nickel salts were diluted in 0.9% saline and a volume of 50 µl was injected into each of the inguinal flanks as described [11]. In

selected experiments, the sensitising nickel solution was mixed with ovalbumin (20 µg per injection), LPS (1 µg), SDS (2 µg), or CpG ODN (20 nmole). Alternatively, the mice were sensitised by (i) epicutaneous application of 10 mM Ni(III) to the shaven abdominal skin, (ii) oral administration as Ni(II) or Ni(III) on days 0, 2 and 4 using syringe and needle for gavage feeding, or (iii) by topical administration of a 5% suspension of nickel chloride in vaseline (petrolatum) to the shaven abdominal skin on days 0, 2 and 4.

All animal experiments were reviewed and approved by an animal ethical review committee and performed according to regulations described by Swiss Veterinary authorities.

2.1.4. Mouse ear-swelling test (MEST)

Typically, the mice were challenged 10 days after sensitisation by injecting 20 µl Ni(II) chloride into the pinna of the left ear. As a negative control, 20 µl saline was injected into the right ear. In the case of epicutaneous sensitisation, the mice were also challenged with Ni(II) by epicutaneous application to the ear, while topical and oral sensitisations were followed by intradermal Ni(II) challenge. Two days after the challenge, delayed-type hypersensitivity reactions were quantified by measuring the increase in ear thickness compared with pre-challenge values. Measurements were performed using a precision digital thickness gage from Miltutoya as provided by Brüttsch-Rüeggger Corp. (Urdorf, Switzerland). Swelling and erythema were also documented by photography and by hematoxylin and eosin (H&E) staining of skin sections of ears that had been collected 2 days after the challenge and snap frozen in liquid nitrogen.

2.1.5. Lymphocyte proliferation test

In addition to determining ear-swelling after recall with Ni(II), *in vitro* proliferation of auricular lymph node cells was measured. The mice were sensitised Ni(III) as described above. After 10 days, the sensitisation was repeated, and 6 days thereafter, the mice were challenged with 2 mM Ni(II) into one ear and with saline into the other ear. The auricular lymph node isolated 48 h later, the lymph node cells were counted and then analysed by flow cytometry for determination of CD4 T-cell numbers as well as by activation of these cells in terms of CD69 and CD44 expression. Moreover, 3×10^5 freshly prepared lymph node cells were incubated for 24 h in the presence or absence of 1–100 µM Ni(II) in DMDM medium supplemented with penicillin, streptomycin, 10% FCS, 50 µM 2-mercaptoethanol, and 10 U/ml mL-2. Then, cell proliferation was determined by incubating the cells for another 16 h with 1 µCi [³H]-thymidine per well. The cells were harvested onto nitrocellulose filters, and the incorporated radioactivity was measured by beta-scintillation. The results are expressed as stimulation index (SI) and calculated as the ratio of Ni-stimulated to non-stimulated cells.

3. Results

As illustrated in Fig. 1, the intradermal application with a nickel chloride solution produced cutaneous inflammation in mice. However, the severity of the skin reaction was independent of the oxidation state of nickel, because mice sensitised with Ni(II) and Ni(III) produced similar ear-swelling reactions (Fig. 1a). Surprisingly, these reactions were not significantly different from those obtained in mock-sensitised mice. Although the inflammation and swelling did not suggest a nickel-specific sensitisation, the observed skin reactions seemed dependent on nickel, as similar skin reactions could be produced with a

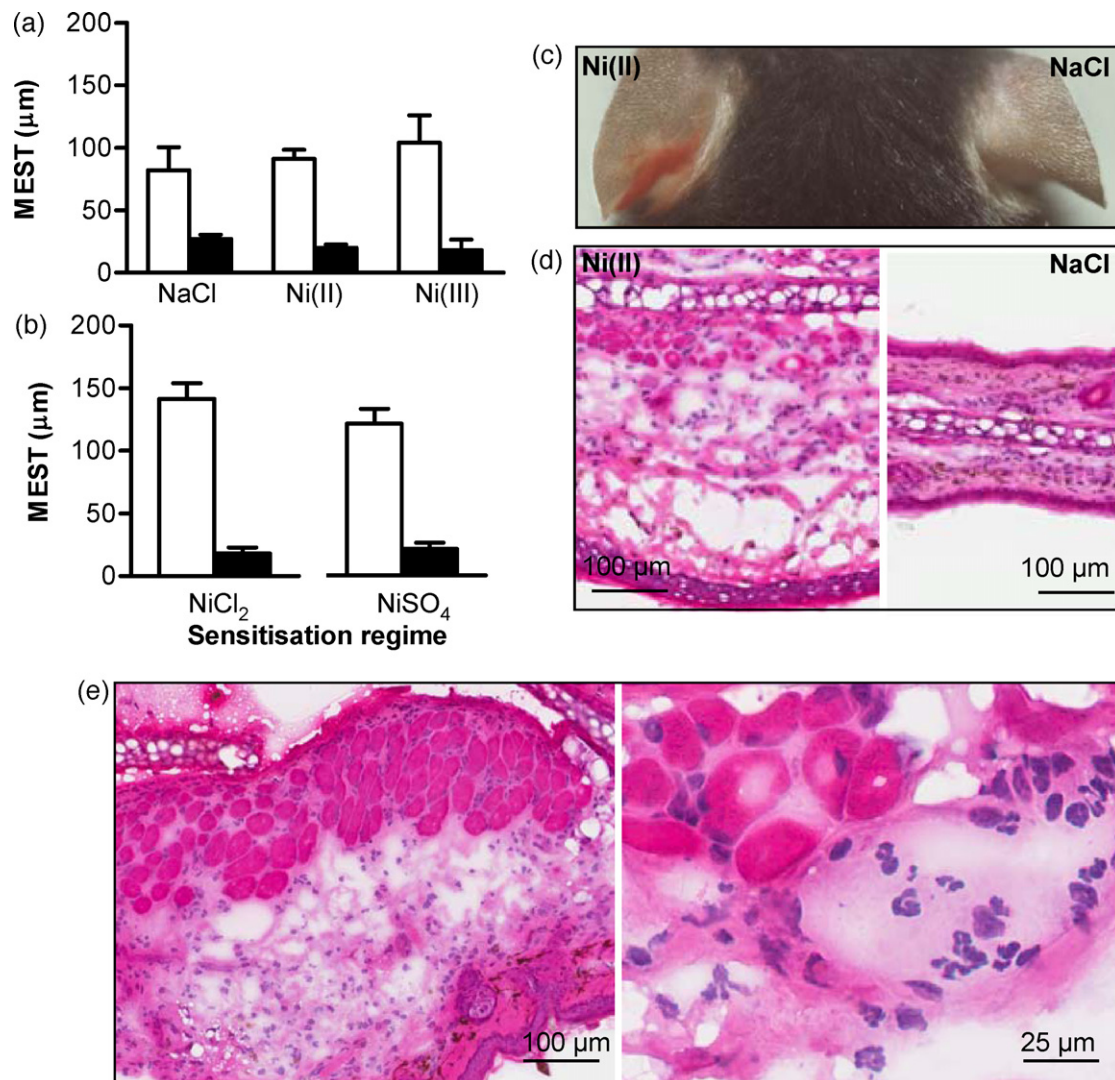


Fig. 1. Intradermal nickel administration causes cutaneous inflammation in mice skin. (a) C57BL/6 mice were sensitised by intradermal injections with 10 mM Ni(II) or Ni(III) or with saline ($n = 5$). Elicitation was provoked by an intradermal injection with 20 μ l 2 mM Ni(II) chloride (open bars) or 0.9% NaCl (filled bars) after 10 days. The swelling was measured by the mouse ear-swelling test (MEST) 2 days after the challenge. Each bar represents the mean MEST + standard error. The experiment was repeated four times with comparable results. (b) Alternatively, mice were sensitised with 10 mM Ni(II) chloride ($n = 9$) or sulphate ($n = 8$) and challenged intradermally with 10 mM Ni(II) of the same salts 10 days later. (c) Photograph showing erythema and swelling reactions 2 days after a challenge with Ni(II) chloride or with saline as described above. (d) H&E histology of mice ears 2 days after a challenge with Ni(II) chloride or saline showing an increase in ear thickness accompanied by the pathological features of oedema, hyperplasia, an inflammatory cell infiltration. (e) High resolution H&E histology of mice ear skin 2 days after a challenge with Ni(II) chloride showing inflammation by neutrophils and not lymphocytes.

sulphate salt of nickel (Fig. 1b). The skin reaction was accompanied by a prominent erythema (Fig. 1c), and the increase in ear thickness was associated with characteristic pathological features of oedema, and inflammatory cell infiltration (Fig. 1d and e). The H&E staining of histological skin sections suggested that the skin reactions were not mediated by nickel-specific lymphocytes but irritant in nature and characterised mainly by the infiltration with neutrophil granulocytes (Fig. 1e). Further experiments using various sensitisation intervals and doses of nickel, different oxidation stages, and different routes of administration (epicutaneous, topical and oral) all revealed that the skin reactions upon nickel challenge were independent of the sensitisation regimen and that the reactions were not adaptive (not shown).

In the following, we sensitised mice with Ni(III) combined with different adjuvants such as CpG ODN and LPS or other excipients such as ovalbumin and SDS in order to test if these regimes could facilitate specific sensitisation to nickel. As shown in Table 1, the

ear-swelling 48 h after the challenge did not differ between sensitised and not sensitised mice, and the adjuvants and the excipients did not affect the ear-swelling and erythema when compared to mice sensitised with Ni(III) alone. Furthermore, when isolating and analysing the auricular lymph nodes, we found that the number of CD4-positive T-cells (Table 1) as well as their activation status (not shown) were not dependent on the sensitisation regime. In fact, non-sensitised and sensitised mice had the similar frequencies of CD4 T-cells, as well as of double-positive CD4 CD44 or CD4 CD69 T-cells. Likewise, the in vitro proliferation of auricular lymphocytes did not suggest that sensitisation with nickel induced specific CD4 T-cell responses (Table 1).

The discrepancy between the positive ear-swelling reactions and the negative lymph node reactions further indicated that the observed skin inflammation was not a result of nickel-specific adaptive immune responses. Therefore, we administrated titrated amounts of Ni(II) to the ears of mice sensitised with 10 mM Ni(III)

Table 1

Erythema, ear-swelling, and lymphocyte activation in mice sensitised by intradermal injections of nickel chloride.

Sensitisation	Readout and challenge regime							
	Erythema ^a		MEST ^b		CD4 ⁺ cells in LNs ^c		Stimulation index ^d	
	Ni(II)	NaCl	Ni(II)	NaCl	Ni(II)	NaCl	Ni(II)	NaCl
NaCl	1.25	0.25	97	4	3.2×10^5	4.6×10^5	1.8	1.7
Ni(III)	1.25	0	91	9	3.1×10^5	2.5×10^5	1.5	1.5
Ni(III)+OVA	1.25	0	66	7	2.6×10^5	2.4×10^5	1.2	1.3
Ni(III)+LPS	0.75	0	85	6	2.9×10^5	3.4×10^5	1.5	2.0
Ni(III)+SDS	0.50	0	70	3	1.6×10^5	3.6×10^5	1.1	1.3
Ni(III)+CpG	1.25	0	81	4	3.4×10^5	4.5×10^5	2.2	1.8

^a Mice were individually scored with 0 (no erythema), 1 (notable erythema), or 2 strong erythema and an average was calculated.

^b The average MEST (swelling of ear compared to baseline) is indicated micrometers.

^c Number of CD4-positive lymphocytes in auricular lymph nodes was calculated based on the number of lymph node cells (counted in hemocytometer) and the percentage of CD4 T-cell subsets (analysed by flow cytometry). No statistical differences were detected using the Kruskal–Wallis non-parametric *H*-test with Dunn's multiple comparison test ($n = 5$).

^d Stimulation index of lymph cells re-stimulated in vitro with 100 μ M Ni(II) in the presence of [³H]-thymidine for 16 h. No statistical differences were detected using the Kruskal–Wallis non-parametric *H*-test with Dunn's multiple comparison test ($n = 5$).

chloride and did the same with non-sensitised mice. As shown in Fig. 2, this experiment revealed a dose-dependent swelling of the ear, and importantly, no difference was found between sensitised and non-sensitised mice ($p > 0.05$ by two-way ANOVA). This suggests that the observed elicitation reactions were likely a result of irritant reactions induced by nickel to the mouse ear skin rather than a result of secondary immunological reactions mediated by nickel-specific T lymphocytes. Indeed, the cellular infiltrate in the swollen ears was identical in sensitised and non-sensitised mice as measured by histology (not shown). The exclusion of any lymphocytic involvement in the skin reactions was further supported by similar experiments performed in lymphocyte deficient mice. The cutaneous inflammation observed in T-cell

receptor knock-out mice and in athymic nude mice did not differ from that observed in wild type mice (Fig. 3).

Since the conclusion of the above experiment was in contrast with published reports on nickel allergy in C57BL/6 mice, and since these mice have been reported to develop nickel tolerance when kept in a nickel-containing environment [14], we bred and kept mice under nickel-free conditions and repeated the nickel sensitisation experiments on these animals. There was no difference in the ear-swelling reactions in mice bred under conventional conditions (Fig. 4a) and in mice bred and kept under nickel-free conditions (Fig. 4b). Moreover, for mice kept under nickel-free conditions, the skin reactions following a challenge with Ni(II) chloride were similar in nickel-sensitised and in non-sensitised mice (Fig. 4b).

4. Discussion

As mentioned above, sensitisation to nickel has a high incidence in the female population [3,4] the ten times lower incidence in men most likely reflect the fact that piercing and wearing of jewellery is less frequent among males. At present, there is no immunotherapy available for treating type-IV hypersensitivity, however, the fact that people living in close proximity to nickel factories are less prone to develop nickel allergies than a comparable population living at further distances from the same factories, suggest that tolerance induction to nickel may be possible [6]. Likewise, a few immunotherapeutic clinical trials suggested a possible tolerating effect of oral administration of nickel salts [7,9,15–17].

For research purposes, animal models of allergic contact dermatitis have been suggested and described. However, the nickel-hypersensitivity model in mice has been hampered by the lack of reproducibility. Strong differences in the mouse strains are suggested, reports vary on the method of mounting DTH reactions, and environmental factors such as natural nickel exposure have been suggested to affect the sensitisation potential in mice. In this regard, Gleichmann and co-workers have developed a murine nickel allergy model and applied this for studies of sensitisation as well as of tolerance induction. In a series of papers, it was shown that sensitisation could only be induced with metals of higher oxidation state than the natural Ni(II) [11]. This was explained by the fact that in inflamed skin, antigen-presenting cells produced reactive oxygen species, which oxidised Ni(II) to a higher state. Furthermore, they also reported the allergic mice could be desensitised by oral administration of Ni(II) [13], and that this tolerance was mediated by nickel-specific CD4 CD25 T-regulatory cells [18] as well fas-ligand expressing invariant NK T-cells that induce apoptosis of nickel-carrying B-cells [19].

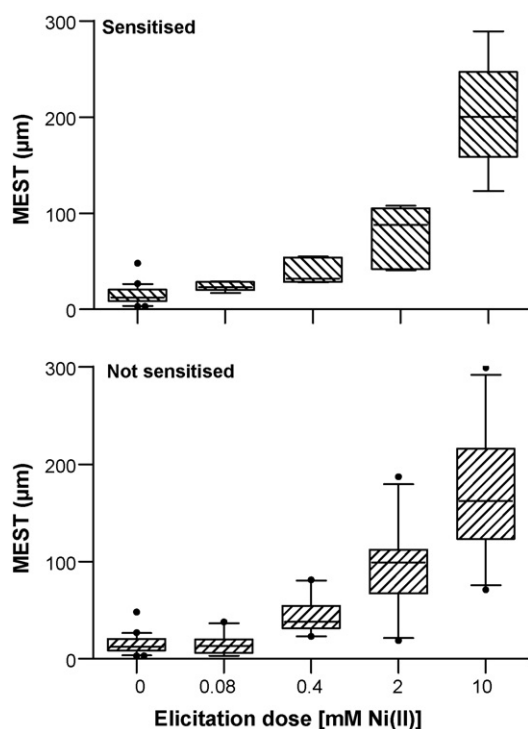


Fig. 2. Nickel causes a dose-dependent irritant skin reaction in mice independent of previous nickel contact. Groups of mice were sensitised by intradermal injections of 10 mM Ni(III) chloride (top panel) or saline (bottom panel) as described in Fig. 1 ($n = 6$). After 10 days, elicitation was provoked by intradermal injections different concentrations of Ni(II) chloride into the pinna of the ears, and the ear-swelling was measured after another 48 h. No statistical difference between sensitised and non-sensitised mice was detected using two-way ANOVA ($p > 0.05$).

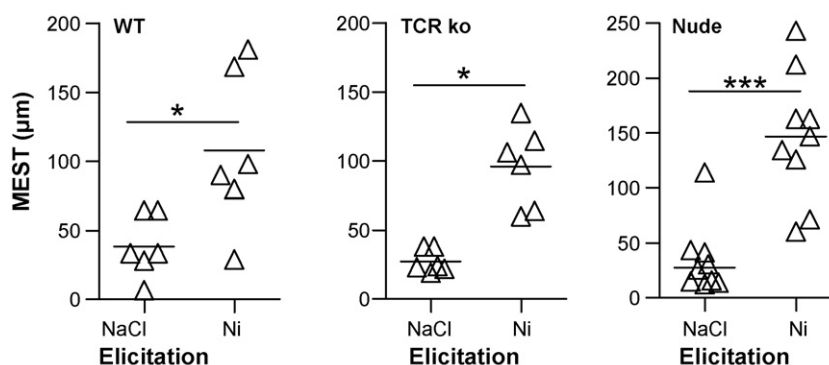


Fig. 3. The skin irritation responses against nickel were not dependent on T-cells. Groups of wild type, T-cell receptor deficient mice, or nude mice were sensitised by intradermal injections of 10 mM Ni(III) chloride as described above ($n = 6-9$). After 10 days, elicitation was provoked by intradermal injection of 10 mM Ni(II) chloride into the pinna of the ears, and the ear-swelling was measured after another 48 h. Statistical differences were determined by the Mann–Whitney U -test. * $p < 0.05$, *** $p < 0.001$.

The purpose of our study was 2-fold. Firstly, we wanted to apply the above-described murine nickel allergy model in the development of immunotherapeutic strategies against nickel and similar type-IV allergies. Secondly, we aimed to study different aspects of the murine model of nickel sensitisation, with the goal of testing its applicability in public guidelines for the industry with regard to testing and documentation of domestic and industrial products prior to their market release. Surprisingly, the results revealed that we were not able to sensitise mice to nickel despite the exact reproduction of the published protocols. Intradermal, epicutaneous, as well as oral sensitisation with different nickel salts in different oxidation states caused an apparent elicitation of the immune response upon challenge, but the same elicitation was observed in mice that were not allegedly sensitised. Moreover, while elicitation manifested as erythema and cutaneous infiltration upon intradermal administration of nickel, this was not dependent on adaptive immune responses. Even the use of carrier proteins such as ovalbumin, skins irritants such as SDS or adjuvants such as CpG or LPS did not induce nickel-specific sensitisation or specific stimulation of lymphocytes in the draining lymph nodes. In contrast, Gleichmann and co-workers reported in the original paper that Ni(III) evoked primary responses, which allowed elicitation of nickel-specific recall responses *in vivo* and *in vitro*, and that this sensitisation was caused by Ni(III) *per se* and not by the adjuvanticity of the hydrogen peroxide [11]. To verify the latter, we injected mice with Ni(II) with and without hydrogen peroxide, or mice were injected with peroxide alone without

nickel. In our hands, injection of the same or lower amounts of hydrogen peroxide intradermally into the flanks of mice resulted in severe “skin burns” and death of the animal within a few minutes, without exceptions (results not shown). Finally, the fact that the same skin reactions could be produced in lymphocyte deficient and in T-cell receptor knock-out mice indicates that the observed reactions did not represent a true and adaptive type-IV contact allergy, but rather an irritant reaction by nickel.

When reviewing the literature, it becomes evident that nickel sensitisation in mice has been difficult to standardise. Vreeburg et al. were unable to sensitise BALB/c mice against epicutaneous nickel sulphate, even under occlusive conditions [20]. Möller reported that contact allergy in NMRI albino mice only could be achieved by repeated epicutaneous but not intracutaneous application of high-dose nickel sulphate or chloride [21]. On the other hand, experiments by Kimber et al. revealed that BALB/c and CBA/Ca mice could be sensitised with approximately 50% efficacy by epicutaneous and occlusive nickel sulphate [22], while Sato et al. reported C57BL/6 mice could be sensitised against nickel chloride, provided that TLR-4 is stimulated [23].

Nickel has generally ranked as a relatively weak sensitizer, and the DTH methods as well as *in vitro* and *in vivo* lymphocyte proliferation tests have proven valid only under certain experimental conditions. This difficulty has led national and international governmental agencies to standardise methods for testing the sensitisation potential of nickel and other haptens. The Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) was established and in 1999, it recommended the use of the local lymph node assay (LLNA) for testing potential contact sensitizers [24,25]. It should be noted that while the LLNA methods was proven valid for some metals and many organic compounds, nickel still represented a borderline case as it produced unreliable results [26] with little applicability due to the low stimulation index it produces in the test [27]. Similarly, an independent scientific peer review panel in 2002 concluded that the LLNA performed as well as the accepted guinea pig methods (guinea pig maximization test and the Buehler assay) for the hazard identification of strong to moderate chemical sensitizing agents, but that the test might not correctly identify all weak sensitizers or metals (potential false negative response) or all strong irritants (potential false positive response) [28]. Nonetheless, the mice recommended for such testing were female CBA and this is in line with most other positive studies on nickel sensitisation in mice where this strain or the syngeneic H-2^d BALB/c mice has been used. In contrast, C57BL/6 mice are infrequently used for allergen sensitisation studies. C57BL/6 mice typically produce strong innate and antimicrobial immune responses, and a recent study by Kim et al.

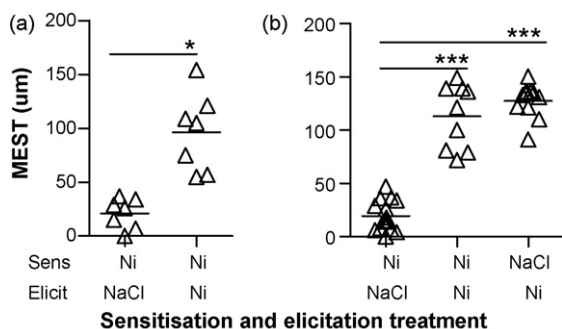


Fig. 4. Nickel exposure does not cause tolerance in C57BL/6 mice. Mice were bred and kept under conventional conditions (a) or under conditions totally void of environmental nickel or nickel-containing materials (b). The mice were then sensitised (Sens) by intradermal injections of 10 mM Ni(III) or saline as described above, and challenged (Elicit) 10 days later with Ni(II) or saline ($n = 7-10$). The swelling was measured by MEST 2 days after the challenge. Statistical differences were determined by the Mann–Whitney U -test (a) or by Kruskal–Wallis H -test with Dunn's multiple comparison post hoc test (b). * $p < 0.05$, *** $p < 0.001$.

suggested that immune responses to nickel in C57BL/6 mice were innate and not adaptive [29], and the same was observed in athymic BALB/c nude mice by Sato et al. [23]. Similarly, von Andrian and co-workers reported that mice devoid of T-cells and B-cells demonstrated substantial contact hypersensitivity responses against dinitrofluorobenzene [30].

Another explanation for the resistance to nickel sensitisation in our study could be that mice tolerated nickel due to early-life natural exposure to the metal. This possibility was based on old work by van Hoogstraten et al. showing that BALB/c could be sensitised by intradermal administration of nickel in complete Freund's adjuvant, but only when kept in a nickel-free environment [31]. When mice were kept in nickel-releasing cages, unresponsiveness to nickel challenge was observed. Also Gleichmann and co-workers have more recently reported similar findings, suggesting a dose-dependency of oral tolerance to nickel [14]. While mice deprived from contact with nickel had a high sensitisation potential, mice kept in conventional environment with cage lids and water bottle tips made from nickel-containing stainless steel as well as mice that were fed with soluble nickel in the drinking water showed an intermediate and a low sensitisation potential, respectively. However, the C57BL/6 mice used in our study were from the same source as those reported in the original sensitisation experiments by Gleichmann and co-workers, and they were used at 6–10 weeks of age. Hence, the mice had little time to develop a potential oral tolerance. Moreover, the use of conventional nickel-containing cage lids and water bottle tips did not prevent strong sensitisation in the original reports by Gleichmann and co-workers [11,13]. Although the AISI 304-certified stainless steel cage lids and bottle caps are nickel plated, their release of nickel when emerged in artificial saliva and sweat has been reported. Most stainless steel alloys release $<0.5 \mu\text{g}/\text{cm}^2$ per week [32–34] a level that is safe for nickel-sensitive individuals to use in direct and prolonged contact to the human skin [33]. In contrast, van Hoogstraten et al. reported that 3 weeks oral supply of water with 10 mg/ml nickel was required to induce tolerance [31], and Gleichmann similarly reported that 4 weeks with 10 mM (1.29 mg/ml) nickel was needed [13]. Hence, assuming that the surface of the bottle cap is $<10 \text{ cm}^2$, the mice are exposed to a dose of nickel being approximately 1/1000 to a 1/100,000 of the dose reported to induce tolerance. It is therefore not likely that the cage lids and bottle caps can cause development of tolerance. Nonetheless, and as to prove this claim, we bred mice in a nickel-free environment and were able to show that such mice did not differ from mice in conventional husbandry in terms of nickel sensitisation potential.

In closing, the present investigation demonstrated that C57BL/6 mice could not be immunologically sensitised to nickel, and that non-sensitised mice experienced the same local skin reactions upon intradermal administration of nickel as did mice sensitised to nickel. This let us conclude that the observed skin reaction upon nickel exposure should be characterised as an irritant rather than an allergic contact dermatitis due to nickel. Hence, our results question the validity of the described murine nickel allergy model.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jdermsci.2010.03.011.

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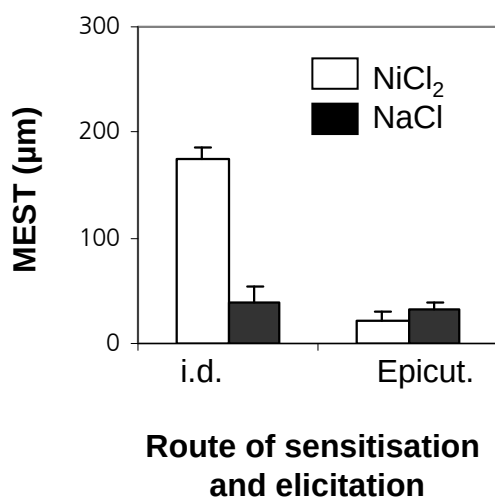
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Suppl. figure 1 for reviewers – Johansen et al.

Not for publication

Mice were sensitised by i.d. or epicutaneous application of Ni(III) chloride and challenged 10 days later by i.d. or epicutaneous application of Ni(II) or saline. Mouse.ear-swelling test was performed two days after the challenge as described in manuscript.



Suppl. figure 2 for reviewers – Johansen et al.

Not for publication

Mice were sensitised by i.d. application of Ni(III) chloride (left panel) or saline (right panel) and challenged 10 days later by i.d. or epicutaneous application of Ni(II). H&E staining of mouse ear section.

