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Lipopolysaccharide promotes and augments metal allergies in mice, dependent on innate immunity and histidine decarboxylase

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Clinical and Experimental Allergy

Summary

Background Few adequate murine models exist for metal allergies, it being especially difficult to induce Ni allergy in mice.

Objective We examined the effect of lipopolysaccharide (LPS) on allergies to Ni and other metals in mice.

Methods Ten days after sensitization with a metal salt and LPS, the ears were challenged with the same metal salt.

Results LPS + NiCl₂ (1 mM) was effective at sensitizing mice to Ni, LPS being effective at very low concentrations whether injected intradermally or intraperitoneally. The ear-swelling response to Ni was more severe and more rapid in C57BL/6 mice than in BALB/c mice. In mast-cell-deficient mice, TNF- α -deficient mice, and interestingly even in nude (T cell deficient) mice, NiCl₂ + LPS induced a Ni allergy similar in degree to that in the respective control mice, but it induced Ni allergy only weakly in TLR4-mutant mice, macrophage-depleted mice, and IL-1-deficient mice. The activity of the histamine-forming enzyme histidine decarboxylase (HDC) in the ears increased in parallel with ear swelling, and HDC-deficient mice were resistant to ear swelling. Challenge with NiCl₂ + LPS augmented ear swelling (vs. NiCl₂ alone). LPS induced effective sensitization to other metals (Cr, Co, Pd, or Ag).

Conclusions These results indicate that in mice, LPS is a very important inducer of metal allergies, and potently promotes them (dependent on both innate immunity and HDC induction in cells other than mast cells). We discussed the idea that the bacterial environment is important for the establishment of metal allergies and for their provocation, and that the current thinking (including the contribution of T cells) should be reappraised in future studies.

Keywords contact hypersensitivity, histamine, histidine decarboxylase, IL-1, lipopolysaccharide, metal allergies, nickel allergy, TNF, toll-like receptor

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Introduction

Metals are thought to cause various types of allergic reactions (including dermatitis, lichen planus, palmoplantar pustulosis, asthma and rhinitis) [1–6], and even to cause carcinomas [4]. Alloys containing Ni are widely used in our environment and in medicine, exposing us to Ni through a variety of routes. Indeed, Ni is the most frequent contact allergen among the metals [2, 7]. Unlike classical haptens, metal ions form geometrically highly defined, but reversible, coordination complexes with

partner molecules. Thus, it is difficult to define the allergenic epitopes of such complexes, and the host recognizes metal ions in complicated ways [8]. The fact that the partner molecules that confer allergenicity to metals are 'intact' self-proteins led us to speculate that metal allergies might belong to the autoimmune diseases.

As described in various reviews [9–11], Ni allergy is thought to involve the maturation of dendritic cells (DCs), their migration in the periphery to the T cell areas of regional lymph nodes (where the matured DCs stimulate naïve T cells to mature or activate them), recruitment of

the matured or activated effector T cells to the site of Ni challenge, cytokine production by these T cells, and the induction of apoptosis in cells such as keratinocytes. During this sequence of events, two types of regulatory CD4⁺ T cells have been suggested to exert inhibitory effects: (a) CD4⁺ Tr1 cells, via the production of IL-10, and (b) CD4⁺CD25⁺ T cells, via cell-to-cell contact with either effector or naïve T cells (see the review by Cavani [9]). However, we should remember that this hypothesis is based largely on *in vitro* experiments, and thus, as pointed out by Büdinger and Hertl [7], *in vivo* studies are urgently needed to test it. Unfortunately, *in vivo* studies have been limited by the paucity of adequate murine models of Ni allergy. Indeed, it has been said that it is difficult to induce Ni allergy in mice [12]. However, Artik et al. [13] showed that it was possible to sensitize C57BL/6 mice to Ni (20 mM) by using 0.6 M H₂O₂ as an adjuvant [in contrast to the use of complete Freund's adjuvant (CFA) in current guinea-pig models].

Infections, or such bacterial components as lipopolysaccharide (LPS), reportedly either confer protection against allergic disorders or exacerbate them [14–16]. LPS, IL-1, TNF, and CD40 ligands all activate DCs to mature them [17–20], while LPS stimulates innate immunity via Toll-like receptor 4 (TLR4), and TLRs may modify adaptive immunity [21]. It has also been reported that LPS-stimulated DCs act via TLR4 to reduce the suppressive effect of regulatory T cells [22]. In this regard, it should be noted that LPS is common in our environment.

Histamine regulates immune responses associated with T-helper type 1 (Th1) and Th2 cells [23, 24] and modifies allergic reactions [25–27]. We have reported previously that a low dose of LPS or IL-1 induces the histamine-forming enzyme, histidine decarboxylase (HDC), in various tissues in mice [28, 29]. Although HDC induction by LPS is poor in those mice with a TLR4 mutation [30], HDC is fully induced in nude mice (deficient in matured T cells) [30] and even in mast-cell-deficient mice [31, 32].

Against this background, we designed the present study to examine (i) the *in vivo* effects of LPS on the establishment and development of the allergic responses to Ni and other metal, and (ii) the contributions made to these allergic responses by T cells, macrophages, IL-1, TNF, and HDC.

Materials and methods

Animals

BALB/c IL-1 KO mice (deficient in both IL-1 α and IL-1 β) and BALB/c TNF- α KO mice (deficient in TNF- α) were established from original IL-1 α KO, IL-1 β KO, and TNF- α KO mice [33, 34]. C57BL/6 HDC KO mice were established by Ohtsu et al. [35]. Mast-cell-deficient W/W^v mice, their normal litter mate +/+ mice, and BALB/c nude mice were

purchased from SLC (Shizuoka, Japan). Females (6–7 weeks old) were used in the present study. Starting at least 1 week before use, mice were kept in plastic cages (with a top made of stainless-steel wires), with water available *ad libitum* from a glass bottle through a glass tube. All experiments complied with the Guidelines for Care and Use of Laboratory Animals in Tohoku University.

Reagents

Escherichia coli O55:B5 LPS (prepared by phenol extraction), clodronate (dichloromethylene diphosphonate), and CFA were purchased from Sigma (St. Louis, MO, USA), while CrCl₂, CoCl₂, NiCl₂, PdCl₂, and AgNO₃ were from Wako Pure Chemical Ind. (Osaka, Japan). LPS and metal salts (except AgNO₃) were dissolved in sterile saline. AgNO₃ was dissolved in sterile 0.1 M phosphate buffer (pH 7).

Sensitization to Ni

An adjuvant, NiCl₂, or a mixture thereof was injected intradermally (i.d.) or intraperitoneally (i.p.) as follows: (1) i.d. route: an equivolume mixture of NiCl₂ and LPS solutions, or of a NiCl₂ solution and CFA, was injected i.d. into both the right and left flanks (50 μ L each) of mice that had been anaesthetized with ethyl ether just before the injections. The concentrations of NiCl₂ and LPS are indicated in the relevant experiments. (2) i.p.–i.d. route: LPS was injected i.p. (0.25 mL/mouse), and 5 min later NiCl₂ was injected i.d. into both the right and left flanks (50 μ L each). (3) i.p. route: an equivolume mixture of a metal salt solution and an LPS solution was injected i.p. (0.25 mL/mouse), the concentrations being indicated in the relevant experiments. In some parts of this paper, we used an equivolume mixture of 1 mM NiCl₂ (or 1 mM of another metal salt) and 1 μ g/mL LPS (0.25 mL/mouse, i.p.) as a standard sensitizing solution, abbreviated as SSS-Ni (or SSS-metal).

Provocation of ear swelling

At various intervals (days) after a sensitizing injection, a metal salt solution or an equivolume mixture of a metal salt solution and an LPS solution was given as a challenge by i.d. injection into the left and right pinnae near the root of the ear (20 μ L each ear). The concentrations used are indicated in the relevant experiments. Saline was injected (20 μ L, i.d.) as a control for the challenging injection. However, this saline injection produced no significant ear swelling (so, these data are not shown in some figures). Mice were anaesthetized with ethyl ether just before the challenging injection. Ear swelling was measured at a site 2–3 mm distant from the challenge site at the indicated times (using a Peacock dial thickness gauge; Ozaki MFG

Co. Ltd, Tokyo, Japan), and the induced difference (after vs. before the challenge) was recorded.

Depletion and detection of macrophages

Clodronate-encapsulated liposomes (Clo-lip) selectively deplete phagocytic macrophages [36]. As described previously [37, 38], a suspension of Clo-lip was intravenously injected into mice, and depletion of macrophages was confirmed by immunostaining of liver using an F4/80 antibody.

Assay of histidine decarboxylase activity in ears

The ears were removed after decapitation, and HDC activity was assayed using our previously described method [32, 39]. HDC activity is expressed as nanomoles of histamine formed during a 1-h period of incubation by the enzyme contained in 1 g (wet weight) of tissue (nmol/h/g).

Flow cytometry

Flow cytometric analysis was performed using a FACScalibur flow cytometer and CELLQuest software (BD Biosciences, San Diego, CA, USA). Murine spleen cells were incubated on ice for 30 min with fluorescein isothiocyanate (FITC)-conjugated anti-mouse CD8a monoclonal antibody (clone 53-6.7, rat IgG2a; BD Biosciences) and r-phycoerythrin (R-PE)-conjugated anti-mouse CD4 monoclonal antibody (clone H129.19, rat IgG2a; BD Biosciences).

Statistical analysis

Experimental values are given as the mean $\pm$ standard deviation (SD). The statistical significance of differences was analysed using an analysis of variance (ANOVA), with a Bonferroni's multiple-comparison test performed *post hoc* (InStat software). *P*-values less than 0.01 were considered to indicate significance.

Results

Comparison of adjuvant effects of complete Freund's adjuvant, H_2O_2 , and lipopolysaccharide

In these experiments, a 5 mM $NiCl_2$ challenge was delivered at 10 days after a sensitizing injection (i.d. or i.p.). (i) i.d. injection of a mixture of an adjuvant and $NiCl_2$. An equivolume mixture of an adjuvant (saline, CFA, 0.6 M H_2O_2 , or 10 μ g/mL LPS) and 4 mM $NiCl_2$ was injected i.d. into both the right and left flanks in BALB/c mice. Saline+ $NiCl_2$ was ineffective in inducing ear swelling (Fig. 1a), and it produced no detectable lesions at the injection sites. The adjuvant effect of CFA was negligible (Fig. 1b), although it produced keloidal lesions at the injection sites in all mice. Incidentally, when CFA was

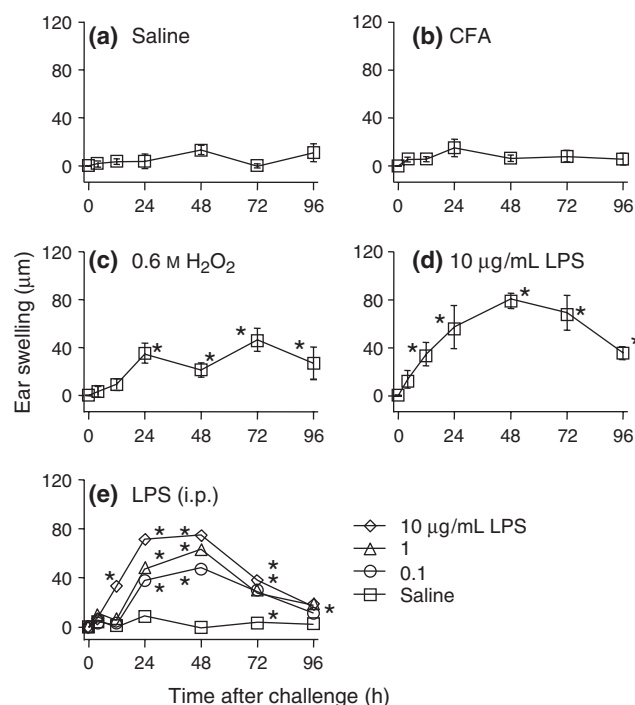


Fig. 1. Effects of intradermally (i.d.)-injected lipopolysaccharide (LPS), complete Freund's adjuvant (CFA), and H_2O_2 on sensitization to Ni in BALB/c mice. An equivolume mixture of $NiCl_2$ (4 mM) and an adjuvant [(a) saline, (b) CFA, (c) H_2O_2 (0.6 M), or (d) LPS (10 μ g/mL)] was injected i.d. into both the right and left flanks (50 μ L each) of BALB/c mice. In the experiment shown in (e), $NiCl_2$ (2 mM) was injected i.d. into right and left flanks of BALB/c mice 5 min after i.p. injection of various concentrations of LPS. In all experiments (a–e), each ear was challenged with 5 mM $NiCl_2$ (20 μ L) at 10 days after sensitization. Each value is the mean $\pm$ standard deviation (SD) from three mice (i.e. $n = 6$ ears). In (e), to reduce complexity, mean values are shown without SD. * $P < 0.01$ vs. time 0. Each experiment (a–e) was repeated a further two times, and the results obtained were similar to those shown.

mixed with 40 mM $NiCl_2$, marked ear swelling occurred in C57BL/6 mice too (Figs 7b and c). The adjuvant effect of H_2O_2 was significant (Fig. 1c), but variable, and it too produced keloidal lesions at the injection sites. In contrast, LPS displayed powerful adjuvant activity (Fig. 1d) without producing detectable lesions at the injection sites. (ii) i.p. injection of LPS and i.d. injection of $NiCl_2$. Next, we examined the adjuvant effect of i.p.-injected LPS. Soon (5 min) after i.p. injection of LPS (at various concentrations), $NiCl_2$ (2 mM) was injected i.d. into both the right and left flanks in BALB/c mice (Fig. 1e). LPS displayed dose-dependent adjuvant activity, and it was effective even at 0.1 μ g/mL. (iii) i.p. injection of a mixture of LPS and $NiCl_2$. i.p. injection of $NiCl_2$ alone at 20 mM made mice moribund within a few hours, and some died within 24 h, while others recovered after 48 h. At 10 mM, $NiCl_2$ reduced the movement of mice within 30 min, and although some fell into a moribund state within a few hours, they recovered after 24 h. The sensitizing effect of $NiCl_2$ alone was significant at 10 mM, but not at 5 mM (Fig. 2a),

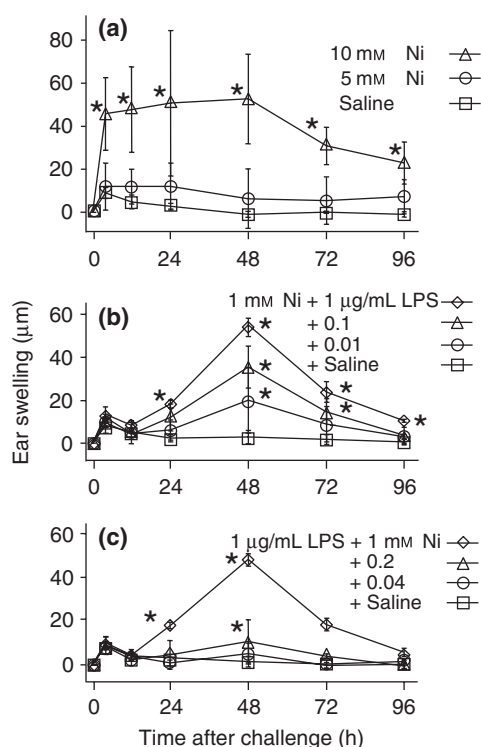


Fig. 2. Effects of intraperitoneal (i.p.) injection of lipopolysaccharide (LPS)+NiCl₂ on sensitization to Ni in BALB/c mice. (a) Saline or NiCl₂ (5 or 10 mM) was injected (0.25 mL, i.p.). (b) An equivolume mixture of 1 mM NiCl₂ and LPS (0.01–1 µg/mL) was injected (0.25 mL, i.p.). (c) An equivolume mixture of NiCl₂ (0.04–1 mM) and LPS (1 µg/mL) was injected (0.25 mL, i.p.). In (a–c), each ear was challenged 10 days later with 5 mM NiCl₂ (20 µL). Each value is the mean ± SD from four mice (i.e. $n = 8$). * $P < 0.01$ vs. time 0. Each experiment was repeated a further two times, and the results obtained were similar to those shown.

although the ear-swelling responses varied markedly from mouse to mouse. Injection of an equivolume mixture of LPS (0.01–1 µg/mL) and 1 mM NiCl₂ induced ear swelling in a manner that depended on the concentration of LPS (Fig. 2b). An equivolume mixture of 1 µg/mL LPS and NiCl₂ induced slight, but significant, ear swelling at 48 h at 0.2 mM NiCl₂ (Fig. 2c). Under these conditions, the activity of the mice was normal and they seemed healthy. In the following experiments, we sensitized mice by an i.p. injection of SSS-Ni (see 'Materials and methods'). Incidentally, when BALB/c mice were challenged with 5 mM NiCl₂ at 4, 7, 10, or 14 days after sensitization with SSS-Ni, both the magnitude and the time course of the ear swelling were similar among 7, 10, and 14 days, whereas the challenge at 4 days induced no detectable ear swelling (data not shown).

Effects of lipopolysaccharide at the time of Ni challenge

In mice sensitized with SSS-Ni (i.p.), the magnitude of the ear-swelling response depended on the concentration of the challenging NiCl₂ (Fig. 3a), with 0.08 mM or more of

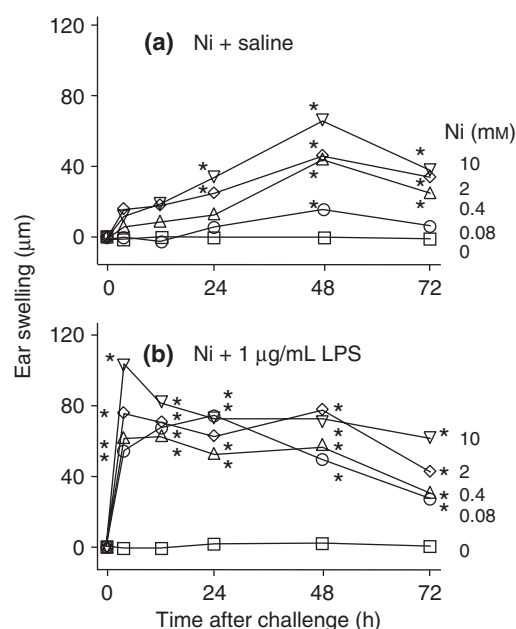


Fig. 3. Effects of lipopolysaccharide (LPS) at the time of Ni challenge in BALB/c mice. At 10 days after SSS-Ni injection (see text), ears were challenged with 20 µL of (a) an equivolume mixture of NiCl₂ (0.08–10 mM) and saline or (b) an equivolume mixture of NiCl₂ (0.08–10 mM) and LPS (1 µg/mL). To reduce complexity, mean values (from four mice, i.e. $n = 8$) are shown without standard deviation. * $P < 0.01$ vs. time 0. Each experiment was repeated a further two times, and the results obtained were similar to those shown.

NiCl₂ inducing significant ear swelling. Incidentally, in the H₂O₂ model employed by Artik et al. [13], mice are challenged with a much higher concentration (10 mM) of NiCl₂. Surprisingly, when we combined LPS with the challenging NiCl₂ (i.e. when we used an equivolume mixture of NiCl₂ and 1 µg/mL LPS), maximal ear swelling (even at 0.08 mM NiCl₂) occurred at as early as 4–12 h, and the swelling lasted for a long time (Fig. 3b).

Involvement of innate immunity in Ni allergy in mice

In the following experiments, mice were treated with SSS-Ni (i.p.) and challenged with 5 mM NiCl₂. This Ni allergy is hereafter termed 'Ni(+LPS) allergy'. We obtained the following results. (i) Unexpectedly, Ni(+LPS) allergy was fully induced in nude mice (Fig. 4a). We confirmed that nude mice mostly lack CD4⁺ T cells and CD8⁺ T cells (Fig. 5). (ii) Unlike in control C3H/HeN mice, Ni(+LPS) allergy was negligible in C3H/HeJ mice with a mutant TLR4 (Fig. 4b). (iii) Ni(+LPS) allergy was very weak in macrophage-depleted mice (– Mφ) (Fig. 4c). (iv) Interestingly, IL-1 KO mice were resistant, but TNF-α KO mice were sensitive to Ni(+LPS) allergy (Figs 4d and e). (v) In C57BL/6 mice, ear swelling developed more rapidly (reaching peak at 24 h) and was of a greater magnitude than in the other mice (Fig. 4f).

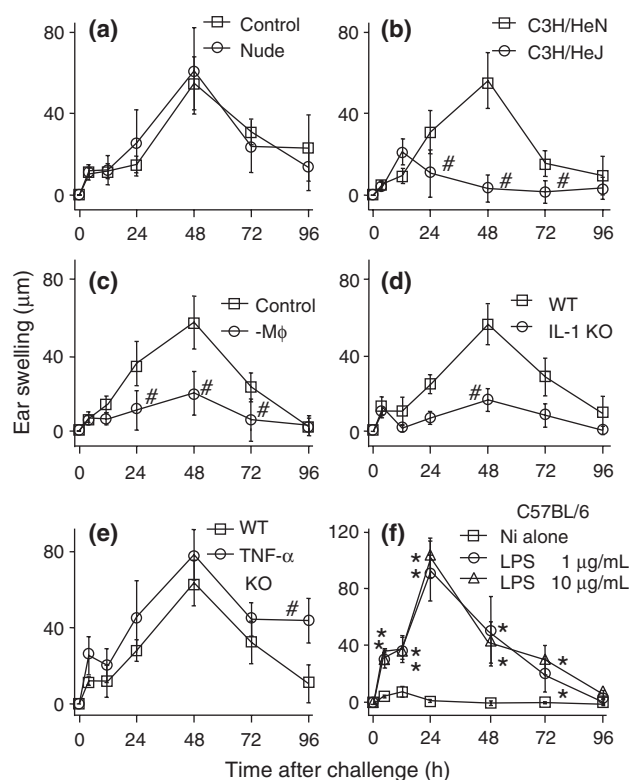


Fig. 4. Induction of Ni(+lipopolysaccharide (LPS)) allergy in (a) athymic (nude) BALB/c and control BALB/c mice, (b) TLR4-mutant C3H/HeJ and control C3H/HeN mice, (c) macrophage-depleted BALB/c and control BALB mice, (d) IL-1 KO BALB/c and control wild-type (WT) BALB mice, (e) TNF- α KO BALB/c and control WT BALB/c mice, and (f) C57BL/6 mice. Note different ordinate scale in (f). Mice were treated with SSS-Ni (in f, an SSS-Ni containing 10 μ g/mL LPS was also used), and 10 days later their ears were challenged with 20 μ L of 5 mM NiCl₂. In (c), SSS-Ni was given 24 h after Clo-lip injection. Each value is the mean $\pm$ standard deviation from four mice (i.e. $n=8$). # $P < 0.01$ and vs. control or WT mice. Each experiment was repeated a further two times, and the results obtained were similar to those shown.

Involvement of histamine in Ni(+LPS) allergy

(i) Ni(+LPS) allergy in mast-cell-deficient mice. W/W^v and +/+ mice were treated with SSS-Ni (i.p.), and 10 days later their ears were challenged with NiCl₂. However, as ear swelling was very slight in both +/+ and W/W^v mice (data not shown), they were again treated with SSS-Ni (at 12 days after the first SSS-Ni injection), and 10 days later again challenged with NiCl₂. This time, both +/+ and W/W^v mice exhibited marked ear swelling (Fig. 6a). Unexpectedly, the ear swelling lasted much longer in mast-cell-deficient W/W^v mice.

(ii) Contribution of HDC to Ni(+LPS) allergy. In contrast to our finding in W/W^v mice, the Ni(+LPS) allergy was markedly weaker in HDC KO mice than that in their control (wild-type C57BL/6 mice) (Fig. 6b). In BALB/c mice, HDC activity in the ears increased roughly in parallel with the development of ear swelling (Fig. 7a), as

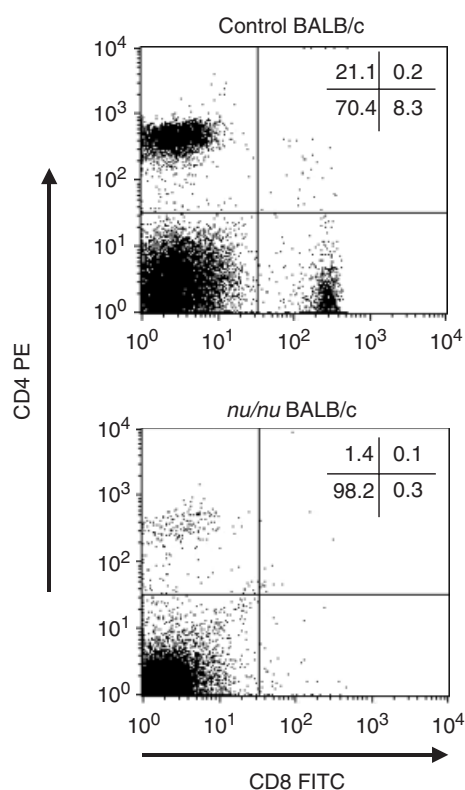


Fig. 5. Flow cytometric analysis of T cells in the spleen in control and nude BALB/c mice (see 'Materials and methods'). Similar results were obtained in one additional experiment.

it did in C57BL/6 mice sensitized (i.d.) with CFA + 40 mM NiCl₂ (Fig. 7b). Moreover, ear swelling and HDC activity each showed dependence on the concentration of NiCl₂ used for the challenge (Fig. 7c). These results indicate that HDC activity in the ear correlates with ear swelling, irrespective of differences in adjuvant or strain of mice.

Sensitization to other metals

BALB/c mice were treated (i.p.) with an equivolume mixture of 1 μ g/mL LPS (or saline) and 1 mM CrCl₂, CoCl₂, PdCl₂, or AgNO₃, and 10 days later their ears were challenged with 5 mM of the same metal salt. LPS was effective at sensitizing the mice to each of these metals (Figs 8a–d), with Pd inducing the greatest ear-swelling response. Like Ni(+LPS) allergy, Pd(+LPS) allergy was weak in IL-1 KO and HDC KO mice, but was fully induced in TNF- α KO mice (Figs 8e and f).

Discussion

Adjuvant effects of lipopolysaccharide

CFA is used as an adjuvant to sensitize guinea-pigs to Ni [40], while H₂O₂ (0.6 M) is used as an adjuvant to sensitize C57BL/6 mice to Ni [13]. In these models, CFA and H₂O₂

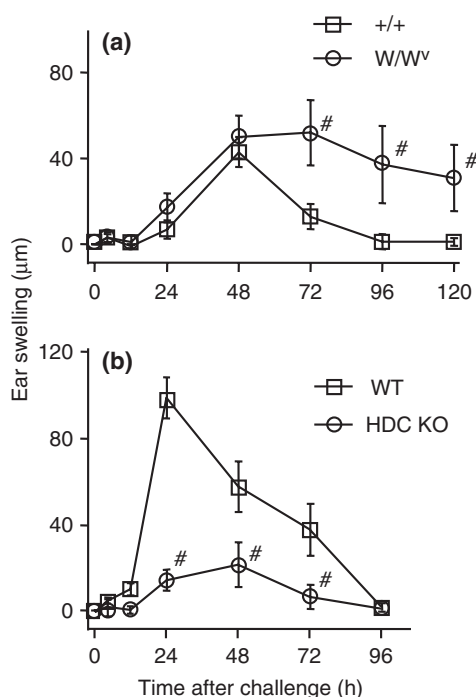


Fig. 6. Induction of Ni allergy in histamine-deficient mice: (a) mast-cell-deficient W/W^v and their normal litter mate +/+ mice, and (b) HDC KO C57BL/6 and control wild-type (WT) C57BL/6 mice. Mice were treated with SSS-Ni, and 10 days later their ears were challenged with 20 μL of 5 mM NiCl₂. Although a challenge at 10 days induced only minor ear swelling in both +/+ and W/W^v mice (data not shown), marked ear swelling was observed following a second sensitization and challenge, as shown in this figure (see 'Results' for details). Each value is the mean ± standard deviation from four mice (i.e. *n* = 8). **P* < 0.01 vs. +/+ or WT. Each experiment was repeated a further two times, and the results obtained were similar to those shown.

are applied topically to the skin, and the range of Ni concentrations employed for sensitization is 20–200 mM. However, we noted that both CFA and H₂O₂ (0.6 M) are very injurious to mice. The present study indicates that LPS is a potent adjuvant for the sensitization of mice to Ni (even when a NiCl₂ concentration of 1 mM or less is employed) without producing any detectable lesions at the injection sites. Further, we found that i.d., i.p., or i.p.-i.d. injection of LPS+Ni easily sensitizes mice to Ni. In preliminary experiments, we observed that LPS obtained from *Prevotella intermedia* (an oral bacterium) (1 μg/mL) was also active as an adjuvant (data not shown). This is potentially important as surgical dental procedures and daily oral hygiene procedures can expose us systemically to oral bacteria [41], and the LPS produced in oral tissues may be transported easily to extraoral tissues [42]. Interestingly, LPS (from *Escherichia coli*) promoted sensitization to all the metals tested (Cr, Co, Pd, and Ag). In addition, it augmented ear swelling when injected at the time of the Ni challenge. These results suggest that in real life, LPS may be a factor promoting metal allergy via various routes.

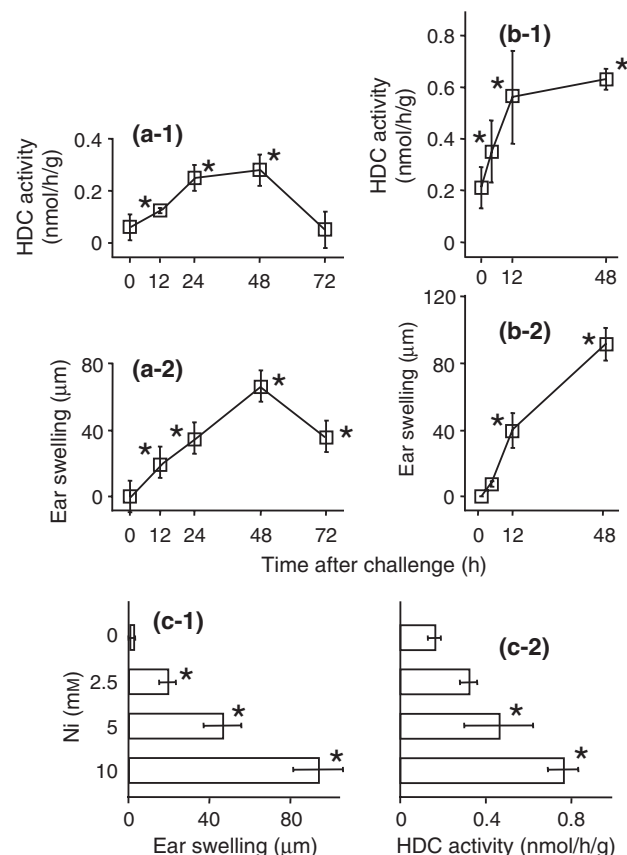


Fig. 7. Elevation of histidine decarboxylase (HDC) activity in the ears following Ni challenge in BALB and C57BL/6 mice. (a) BALB/c mice were divided into five groups (four mice per group), and then treated with SSS-Ni (i.p.). (b) C57BL/6 mice were divided into four groups and sensitized with an equivolume mixture of complete Freund's adjuvant (CFA) and NiCl₂ (40 mM), which was injected i.d. into both right and left flanks (50 μL each). (c) C57BL/6 mice were divided into four groups and sensitized with an equivolume mixture of CFA and NiCl₂ (40 mM), which was injected i.d. into both the right and left flanks (50 μL each). Ears were challenged 10 days later with 20 μL of 5 mM (a), 10 mM (b), or various concentrations (c) of NiCl₂, and ear swelling was measured at the indicated times (a and b) or at 48 h (c) after the Ni challenge. Just after measurement of ear swelling, the mice were decapitated and their ears removed for the assay of HDC activity. Each value is the mean ± standard deviation from four mice (i.e. *n* = 8 for ear swelling and *n* = 4 for HDC activity). **P* < 0.01 vs. time 0 or dose 0. Each experiment was repeated a further two times, and the results obtained were similar to those shown.

Contribution of T cells

As described in 'Introduction', T cells are believed to play critical roles in the establishment of Ni allergy. However, Ni(+LPS) allergy occurred fully in nude mice (mostly lacking mature T cells), suggesting that T cells may not be essential (or possibly that very low levels of T cells are sufficient) for the establishment of Ni allergy. However, we cannot exclude the possibility that in mice, Ni(+LPS) allergy is a different type of allergy from the so-called Ni allergy. C57BL/6 mice are Th1-dominant mice, whereas BALB/c mice are Th2 dominant [43]. Ear swelling was

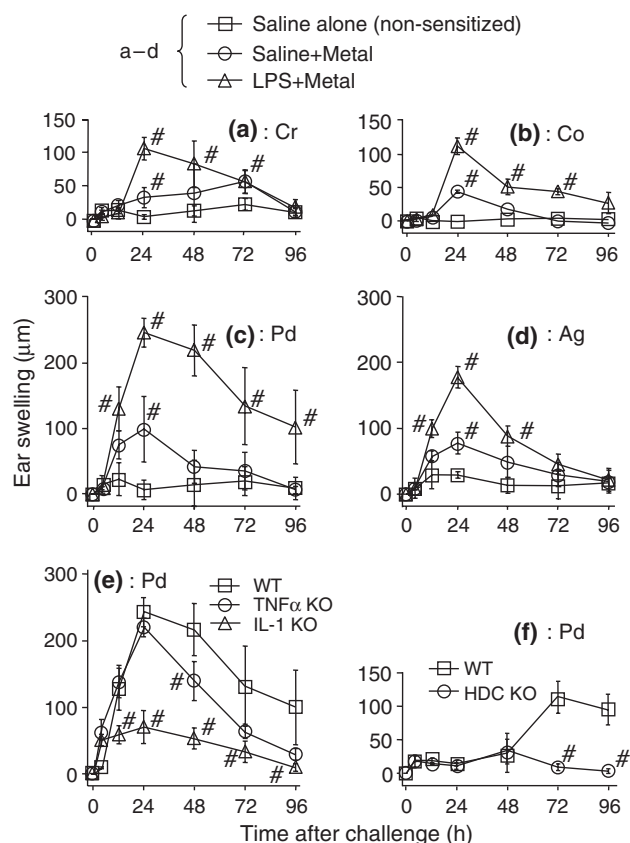


Fig. 8. Induction of allergies to metals other than Ni. (a–d) Lipopolysaccharide (LPS)-induced sensitization of BALB/c mice to other metals (CrCl_2 , CoCl_2 , PdCl_2 , or AgNO_3). Saline alone, an equivolume mixture of saline and 1 mM of a metal salt, or an equivolume mixture of 1 $\mu\text{g}/\text{mL}$ of LPS and 1 mM of a metal salt was injected into BALB/c mice [0.25 mL/mouse, intraperitoneal (i.p.)]. Ten days later, ears were challenged with 5 mM of the same metal salt (20 $\mu\text{L}/\text{ear}$). Each value is the mean $\pm$ standard deviation (SD) from four mice (i.e. $n=8$). $\#P < 0.01$ vs. saline+metal. (e and f) Pd(+LPS) allergy in IL-1 KO mice, TNF- α KO mice, and HDC KO mice. These mice and their control wild-type (WT) mice were injected i.p. with an equivolume mixture of 1 $\mu\text{g}/\text{mL}$ LPS and 1 mM PdCl_2 . Ten days later, ears were challenged with 5 mM PdCl_2 (20 $\mu\text{L}/\text{ear}$). Each value is the mean $\pm$ SD from four mice (i.e. $n=8$). $\#P < 0.01$ vs. WT. Each experiment was repeated a further two times, and the results obtained were similar to those shown.

more rapid and of greater magnitude in C57BL/6 than in BALB/c mice, although the difference was not marked, suggesting that Th1 immune responses may contribute more to Ni(+LPS) allergy. Be that as it may, careful studies will be required to evaluate the contribution made by T cells to Ni(+LPS) allergy in mice.

Contribution of innate immunity

In contrast to our finding in nude mice, Ni(+LPS) allergy was very weak both in C3H/HeJ mice and in macrophage-depleted mice, suggesting that TLR4 and macrophages are important in the establishment of Ni allergy. IL-1 and TNF are produced by various immune-competent cells and

share many biological activities [44]. The major difference between them is that IL-1 shares TLR4-signal cascades, whereas TNF stimulates apoptotic cascades. Interestingly, IL-1 KO mice (but not TNF- α KO mice) were resistant to the establishment of Ni(+LPS) allergy. Pd(+LPS) allergy was also very weak in IL-1 KO mice. These results support the idea that innate immunity is essential for the establishment of metal allergies, and also that TLR- or IL-1-related innate signals are more important than TNF-related signals for metal allergies.

Roles of histamine

Although histamine levels in the tissues (such as skin) are lower in W/W^v mice than in either $+/+$ or BALB/c mice [31, 32], HDC is normally induced in various tissues in W/W^v mice in response to LPS or IL-1 [32]. In W/W^v mice, the ear-swelling response to Ni was not only fully induced, but lasted much longer than that in control $+/+$ mice. Histamine levels in HDC KO mice are very low [45], and we confirmed that we could not detect HDC activity in their tissues even after LPS injection (data not shown). In contrast to the situation in W/W^v mice, Ni(+LPS) allergy was markedly lower in HDC KO mice than in their control mice. Further, HDC activity increased in the ears of both BALB/c and C57BL/6 mice in parallel with the swelling (Fig. 7). Pd(+LPS) allergy was also very weak in IL-1 KO mice. NC/Nga mice develop dermatitis in conventional (but not in specific pathogen-free) environments [46], and we previously reported that HDC activity increases in their skin in parallel with the development of the dermatitis [47]. As described in Introduction, histamine modifies Th1 and Th2 responses. Although, as is well known, mast cells store histamine and release it upon stimulation by allergens, cells other than mast cells also release histamine through HDC induction in response to inflammatory stimuli [including LPS and inflammatory cytokines (such as IL-1, TNF, IL-12, and IL-18)] [29, 48–50]. These results suggest that the histamine newly formed following HDC induction in non-mast cells (via TLRs or cytokines) may be involved in the establishment of Ni(+LPS) allergy. However, it remains to be clarified whether the newly formed histamine is involved in the course of sensitization or in the elicitation of inflammation, and whether histamine acts on blood vessels (inducing plasma extravasation) [45] or on immune-competent cells [23, 24].

Comparison with classical contact hypersensitivity models

Unlike the classical haptens (such as dinitro- or trinitro-phenyl derivatives), which covalently modify self-proteins, metal ions produce reversible complexes with unidentified self-proteins [8]. Hence, it is possible that the mechanism underlying contact hypersensitivity (CHS) may differ between that induced by metals and that

induced by the classical haptens [8]. The present findings highlight some similarities and dissimilarities between these two types of CHS responses as follows: our results suggest that TLRs are important in metal allergies. Similar promoting effects of TLR ligands (such as TLR7 and TLR9 ligands) have been reported in models entailing CHS induction by the classical haptens, and in these models TLR ligands enhance the antigen-presenting functions of dendritic cells (via up-regulation of HMC class II, CD80, and CD86 molecules or an enhanced production of IL-12) [51–53]. In addition, IL-1 β has been implicated as an important player in the classical hapten-induced CHS responses (as both mice treated with a neutralizing antibody to IL-1 β and mice deficient in IL-1 β display impaired CHS responses) [54, 55]. Moreover, it has been reported (a) that activation of caspase-1 (the enzyme converting pro-IL-1 β to IL-1) via NALP3 (a member of the Nod family) is essential for the classical CHS responses [56, 57], and (b) that NALP3 is required for caspase-1 activation in TLR-stimulated macrophages [57]. However, in contrast to metal(+LPS) allergies, the CHS induced by the classical haptens is reported to be similar or more severe in HDC KO mice than in WT mice [45, 58]. Although at present we have no data to help explain this difference, it is likely that the CHS models induced by the classical haptens involve a mechanism different from that involved in metal(+LPS) allergies.

Conclusion

In conclusion, we found LPS to be a potent adjuvant in mice for the establishment of allergic responses to Ni and to some other metals. LPS also promoted the development of allergic responses when given at the time of challenge. Metal(+LPS) allergies may depend both on innate immunity and on the *de novo* formation of histamine (via HDC induction) in non-mast cells. We need further careful studies, using *in vivo* models, to assess the contribution made by T cells to the establishment of allergies to Ni and other metals. Interestingly, O'Leary *et al.* [59] have recently reported that natural killer cells can mediate allergies induced by classical haptens.

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