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Mast cell histamine-mediated transient inflammation following exposure to nickel promotes nickel allergy in mice

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Abstract

We previously reported that allergic responses to nickel (Ni) were minimal in mice deficient in the histamine-forming enzyme histidine decarboxylase (HDC-KO), suggesting an involvement of histamine in allergic responses to Ni. However, it remains unclear how histamine is involved in the process of Ni allergy. Here, we examined the role of histamine in Ni allergy using a murine model previously established by us. Mice were sensitized to Ni by intraperitoneal injection of a NiCl₂-lipopolysaccharide (LPS) mixture. Ten days later, allergic inflammation was elicited by challenging ear-pinnas intradermally with NiCl₂. Then, ear-swelling was measured. Pyrilamine (histamine H1-receptor antagonist) or cromoglicate (mast cell stabilizer) was intravenously injected 1 h before the sensitization or the challenge. In cell-transfer experiments, spleen cells from Ni-sensitized donor mice were intravenously transferred into non-sensitized recipient mice. In both sensitized and non-sensitized mice, 1 mM or more NiCl₂ (injected into ear-pinnas) induced transient non-allergic inflammation (Ni-TI) with accompanying mast cell degranulation. LPS did not affect the magnitude of this Ni-TI. Pyrilamine and cromoglicate reduced either the Ni-TI or the ensuing allergic inflammation when administered *before* Ni-TI (at either the sensitization or elicitation step), but not if administered when the Ni-TI had subsided. Experiments on HDC-KO and H1-receptor-KO mice, and also cell-transfer experiments using these mice, demonstrated histamine's involvement in both the sensitization and elicitation steps. These results suggest that mast cell histamine-mediated Ni-TI promotes subsequent allergic inflammatory responses to Ni, raising the possibility that control of Ni-TI by drugs may be effective at preventing or reducing Ni allergy.

Key words: pyrilamine, cromoglicate, histidine decarboxylase, H1-receptor, dermatitis

Introduction

Metals are widespread in our environment, and indeed essential for our life. However, metals are often recognized by the body as antigens and frequently induce allergic contact dermatitis (ACD), defined as a delayed-type hypersensitivity to low molecular weight substances (1,2). Actually, nickel (Ni) is the major cause of ACD (1-3) and induces various kinds of allergic symptoms, leading to a reduced quality of life for such patients (3). Because of a historical paucity of adequate animal models, most studies of metal allergies have been performed *in vitro* using sensitized human lymphocytes, although a few *in vivo* studies have been performed (4-6). The existing literature has led to the belief that T cells are essential for metal allergies, as well as for contact hypersensitivities to classical organic chemical haptens. However, the detailed mechanism underlying metal allergies remains obscure and treatment options for metal allergies remain inadequate. Thus, at present we cannot help but rely on limited strategies such as exhaustive avoidance of metal antigens (7-9) or usage of corticosteroids (10-12), even though these often create difficulties for the patients (compliance, effects on lifestyle, *etc.*).

It has always been considered difficult to induce metal allergies in mice, but our recent findings that lipopolysaccharide (LPS, a cell-wall component of Gram-negative bacteria) strongly promotes metal allergies allowed us to introduce a new murine model of metal allergies (13). Such LPS-promoted metal allergies can be reproducibly induced by low concentrations of metals that do not impose any detectable burden on the mice, favoring the use of this model for *in vivo* studies on metal allergies. Using this model, we found that (i) LPS markedly reduces the minimum allergy-inducing concentration of metals at both steps: *i.e.*, sensitization and elicitation, and (ii) although a relatively high concentration of a metal is

required for sensitizing mice to the metal, once sensitization has been established, metal allergies can easily be induced by low metal concentrations (13-15).

Histamine is synthesized by and released from various cells (mast cells, basophils, macrophages, endothelial cells, haematopoietic cells, *etc.*) *via* stimulation with cytokines or with antigens (16-19) and exerts its effects through binding to its receptors (H1, H2, H3 and H4 receptors) (20). Histamine is involved in many physiological functions (including cell proliferation and differentiation, haematopoiesis, embryonic development, regeneration and wound healing) (21,22) and it modifies allergic reactions (23,24) and regulates immune responses associated with T-helper type 1 (Th1) and Th2 cells (22,23,25). Thus, histamine may be involved in ACD, too. Indeed, Dudeck et al. recently reported that histamine is important in contact hypersensitivity to classical organic chemical haptens, such as dinitrofluorobenzene (DNFB) and fluorescein isothiocyanate (FITC) (26). However, there has been no study of the involvement of histamine in metal allergies. Although it is difficult to define allergenic epitopes, metal ions may form reversible coordination complexes with unidentified self-proteins, while classical haptens produce covalent complexes with self-proteins (1,27-30). Thus, the presentation process of metal antigens is not clear. Histidine decarboxylase (HDC) is the enzyme that forms histamine from histidine. Interestingly, Ni allergy has been shown to be minimal in mice deficient in HDC (HDC-KO mice) (13), suggesting an involvement of histamine in the onset of Ni allergy. However, it remains unclear how histamine might be involved in the process of Ni allergy.

Against the above background, we performed the present study to examine the details of the involvement of histamine in the onset process of Ni allergy, using our previously described murine model.

Materials and Methods

Animals

C57BL/6 mice (purchased from CLEA Japan, Inc., Tokyo, Japan) were used throughout the experiments. HDC-KO mice (C57BL/6 background) and histamine-H₁-receptor (H1R)-KO mice (C57BL/6 background) were established as previously described (31,32). Females (6-7 weeks old) were used in the present study. The mice were raised in standard aluminium cages (with a lid made of stainless-steel wires), with water (supplied by the water service of our city) being accessible *ad libitum* from a plastic bottle through a stainless-steel tube. They were kept in an air-conditioned room at $23 \pm 1^{\circ}\text{C}$ and $55 \pm 5\%$ relative humidity with a standard cycle of 12 h light and 12 h dark (lights on at 07:00 a.m.). All experiments complied with the relevant Japanese law (Law no. 105; Notification no. 84) and with the Regulations for Animal Experiments and Related Activities at Tohoku University (approval number 2015DA-036, date 3-3-2015).

Reagents

First-grade NiCl₂ (purity > 95.0 %) was purchased from Wako Pure Chemical Ind. (Osaka, Japan). LPS from *Escherichia coli* O55:B5 prepared by the method of Westphal and Jann (phenol-water extraction), pyrilamine, and cromoglicate were purchased from Sigma (St. Louis, MO, USA). These reagents were dissolved in sterile saline. Pylamine (200 µg/mouse) and cromoglicate (500 µg/mouse) were each injected intravenously (i.v.) via a tail vein.

Sensitization to Ni

To sensitize mice to Ni, mice were injected intraperitoneally (i.p.) with an equivolume mixture of 1 mM NiCl₂ and 1 µg/mL LPS (0.25 mL/mouse) as a “standard sensitizing solution” (abbreviated as SSS-Ni).

Ni-challenge and measurement of ear swelling

Ten days after the injection of SSS-Ni, 1 mM NiCl₂ was injected intradermally (i.d.) into the left and right pinnae near the root of each ear (20 µL per ear) under sevoflurane anesthesia. 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 net swelling (*i.e.*, the difference between before and after the administration) was recorded.

Adoptive cell-transfer experiments

Donor mice were sensitized with SSS-Ni and, 10 days later, their spleen cells (obtained by excising the spleen and preparing a suspension of cells) were transferred intravenously via a tail vein into unanesthetized non-sensitized recipient mice (1×10⁶ cells/mouse). One day after the transfer, the recipient's ears were challenged with 1 mM NiCl₂ as described above.

Histology

Ear-pinnae were fixed in 4% paraformaldehyde and embedded in optimal cutting temperature compound. Frozen-sections (5 µm) were subjected to Giemsa staining.

Statistical analysis

Experimental values are given as the mean $\pm$ standard deviation (SD). The statistical significance of differences was assessed by using a Bonferroni multiple comparison test after analysis of variance (ANOVA) with the aid of InStat software (InStat, Scottsdale, AZ, USA). *P* values less than 0.01 were considered to indicate significance.

Results

Transient inflammation associated with Ni exposure

Recent studies have found that classical organic chemical haptens by themselves induce a transient *non-allergic* inflammation that is mediated by histamine and occurs soon after the exposure, and that this inflammation promotes the onset of contact hypersensitivities (26,33,34). We asked whether such a transient non-allergic inflammation might be involved in Ni allergy, too. Wild-type (WT) mice were i.d. injected with 0.1-5 mM NiCl₂ and then ear-swelling was measured at frequent intervals for 12 hours. As shown in Fig. 1a, NiCl₂, at 1 mM or more, induced transient ear-swelling soon after its injection (peaking at 1 h) in a dose-dependent manner. This transient inflammation, occurring largely within 6 h of the injection of NiCl₂, is hereafter termed “Ni-TI”.

We previously reported that LPS promotes Ni allergy (15). We therefore examined the effect of LPS on Ni-TI by injecting mixtures of LPS and NiCl₂ (Fig. 1a). LPS did not alter the present Ni-TI.

Mast cell degranulation by Ni

As described in Introduction, histamine is involved in the development of Ni allergy (13). Interestingly, by histochemical examination of ear-pinnae from mice that had exhibited Ni-TI, we found that 1 mM NiCl₂ induced a degranulation of mast cells within 1 h of its i.d.

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injection (Fig. 1b-upper). This occurred in Ni-sensitized mice, too (Fig. 1b-lower). Such a degranulation was not observed in ear-pinnas taken from either Ni-sensitized or non-sensitized mice at 48 h (the peak time of the allergic response) after the injection of 1 mM NiCl₂ (data not shown). In addition, LPS injected into ear-pinnas did not induce degranulation of mast cells (data not shown). These results suggest that (i) Ni ion by itself can release histamine from mast cells *via* their degranulation during Ni-TI, although it remains to be clarified how NiCl₂ induces this effect, and (ii) such a degranulation of mast cells does not occur during allergic ear-swelling in Ni allergy.

Involvement of histamine in Ni-TI and Ni allergy

Next, to confirm the involvement of histamine in Ni-TI and Ni allergy, we examined the effects of pyrilamine, a histamine H₁-receptor (H₁R) antagonist. In the following experiments, mice were i.d. injected with 1 mM NiCl₂. Non-sensitized or Ni-sensitized mice (10 days after SSS-Ni injection) were i.v. injected with pyrilamine 1 h before the NiCl₂ injection. In both non-sensitized and Ni-sensitized mice, pyrilamine inhibited Ni-TI (upper panels in Figs. 2a and 2b). Interestingly, in Ni-sensitized mice, pyrilamine markedly attenuated the allergic response to Ni (*i.e.*, swelling at 24 to 72 h), too (Fig. 2b-lower).

Next, we examined the effect of pyrilamine at the sensitization step. For this purpose, pyrilamine was administered 1 h before the injection of SSS-Ni and, 10 days later, ear-swelling was measured after challenge with 1 mM NiCl₂ (*i.e.*, after the 3rd injection) (Fig. 2c). In this case, pyrilamine did not reduce Ni-TI (Fig. 2c-upper), although the allergic response to Ni (*i.e.*, swelling at 24 to 72 h) was markedly reduced (Fig. 2c-lower). These results suggest that histamine is important for the induction of both Ni-TI and Ni allergy.

Effects of the mast cell stabilizer cromoglicate

Cromoglicate is a mast cell stabilizer used for preventing mast cell degranulation, although the mechanism of action is not completely known (35). Thus, we examined the effects of this agent on Ni-TI and Ni allergy. Like pyrilamine, cromoglicate reduced both Ni-TI and Ni allergy when it was injected 1 h before the injection of 1 mM NiCl₂ in non-sensitized and Ni-sensitized mice (Figs. 3a and 3b). In addition, degranulation of mast cells was rarely detectable in the ear-pinnas of mice given cromoglicate 1 h before the injection of 1 mM NiCl₂ (data not shown). These results indicate that mast cells are indeed involved in both Ni-TI and Ni allergy.

Effects of Ni-TI on Ni allergy in mice sensitized to Ni

The findings described above suggest that Ni-TI may have important effects on Ni allergy. Hence, we examined whether allergic ear-swelling might be affected by the timing of the pyrilamine or cromoglicate injection. Ni-TI peaked at 1h after the injection of 1 mM NiCl₂ (Fig. 1a) and had largely subsided within 6 h of the injection. Thus, we tested the effects of pyrilamine and cromoglicate when they were injected *after* Ni-TI had subsided. When injected 6 h *after* the injection of SSS-Ni, pyrilamine and cromoglicate were ineffective at reducing allergic ear-swelling (Fig. 3c-upper). These drugs were also ineffective at reducing allergic ear swelling when injected 6 h after the Ni challenge (Fig. 3c-lower). Under the conditions shown in Fig. 3c-upper, cromoglicate did not reduce the degranulation of mast cells (data not shown). These results, together with those described above, demonstrate that mast cell-derived histamine and/or histamine-mediated Ni-TI plays a critical role at the onset of Ni allergy and/or during the first few hours after exposure to Ni.

Ni-TI and Ni allergy in HDC-KO and H1R-KO mice

We next examined whether Ni-TI and Ni allergy can be induced in HDC-KO and H1R-KO mice. First, non-sensitized mice were injected with 1 mM NiCl₂. In HDC-KO and H1R-KO mice, the observed Ni-TI responses were weaker than those seen in their control WT mice (C57BL/6) (Fig. 4a), confirming that histamine plays a key role in Ni-TI. Next, we examined Ni-allergy in HDC-KO or H1R-KO mice. As shown in Fig. 4b, the ear-swelling response to Ni was markedly weaker in both HDC-KO and H1R-KO mice than the corresponding responses in their control WT mice, supporting the idea that histamine is indeed involved in Ni allergy (13).

Cell-transfer between WT and HDC-KO or H1R-KO mice

Finally, we performed cell-transfer experiments between WT and HDC-KO or H1R-KO mice to determine at which step of Ni allergy (the sensitization or elicitation step) histamine is involved. In these experiments, donor mice were sensitized with SSS-Ni and, 10 days later, their spleen cells were i.v. transferred *via* a tail vein into non-sensitized recipient mice (1×10^6 cells/mouse). One day after the transfer, the recipient's ears were challenged with 1 mM NiCl₂. The results shown in Fig. 4c reveal that Ni allergy was induced *only* by such cell-transfer from SSS-Ni-treated WT mice to non-treated WT mice (*i.e.* Ni allergy was not induced by cell-transfer from SSS-Ni-treated HDC-KO mice to non-treated WT mice, from SSS-Ni-treated WT mice to non-treated HDC-KO mice, or from SSS-Ni-treated HDC-KO mice to non-treated HDC-KO mice). In a similar experiment using H1R-KO mice (Fig. 4d), the results were essentially the same. These results clearly indicate that histamine is involved in both steps: sensitization and elicitation.

Discussion

Summary of findings

Here, using a murine model devised previously by us, we made a detailed examination of the involvement of histamine in the onset process of Ni allergy. The findings may be summarized as follows. (i) In WT mice, NiCl₂ by itself, at 1 mM or more, induced non-allergic Ni-TI soon after its injection. No effects of LPS on such Ni-TI were observed. (ii) Degranulation of mast cells occurred in ear-pinnas during Ni-TI. (iii) Both pyrilamine and cromoglicate, when administered *before* Ni-TI, were highly effective at inhibiting both Ni-TI and the subsequent Ni-allergic reactions. However, they were not effective when administrated *6 h after* Ni-injection, at which time the Ni-TI had largely subsided. (iv) Experiments using HDC-KO or H1R-KO mice, and also cell-transfer experiments using these mice, strongly supported the involvement of histamine in both the sensitization and elicitation steps. We discuss these findings in the following paragraphs.

Transient inflammation following exposure to Ni ion

Little is known about the early responses following metal exposure. In the present study, we clarified that Ni ion can induce non-allergic transient inflammation, regardless of whether sensitization to Ni has or has not taken place, and that mast cell histamine and H1R are needed for such induction of Ni-TI. Similarly, it has been reported that classical organic chemical haptens (DNFB, FITC, *etc.*) can, by themselves, induce a transient inflammation (lasting for a few hours after their injection) that depends on mast cell histamine (26). Indeed, the profile of Ni-TI is analogous to the profiles of the transient inflammatory responses to classical organic haptens in terms of magnitude, duration, and time-to-peak. Degranulation from mast cells is generally caused by an IgE-mediated antigen-antibody reaction (36-38), but it remains to be clarified precisely how Ni ion induces degranulation of mast cells.

LPS was reported by de Filippo *et al.* to induce degranulation of mast cells *in vivo* (39), although such an effect of LPS was not observed in the present study. The previous authors discussed the idea that degranulation does not occur directly via TLR4 triggering or that there is a requirement for other factors derived from the environment in which the mast cells are embedded. We speculate that whether degranulation of mast cells is induced by LPS might depend on the experimental environment [peritoneal cavity (39) vs. ear-pinnas (our study)].

Roles of histamine in Ni-TI

Histamine is a well-known mediator of inflammation. In general, histamine constricts larger blood vessels (arteries and veins), but dilates smaller blood vessels (arterioles and venules), while histamine-mediated dilation of precapillary arterioles leads to an increase in capillary permeability. Through these effects, histamine may help to support the functions of the microcirculatory system, including the supply of nutrients, the removal of waste products, and the infiltration and activities of inflammatory and immune cells. Histamine by itself has a variety of effects on these cells *via* stimulation of its receptors (40-42). Further, the histamine-mediated increase in vascular permeability may permit an accumulation of immunoglobulins to occur at sites of inflammation, favoring the clearance of pathogens (43).

Discrepancy in the contribution of mast cells

Here, we provide evidence that mast cells participate in the onset of Ni allergy through Ni-TI. However, we reported previously that Ni allergy occurs in mast cell-deficient W/W^v mice, too (13). A similar discrepancy has been reported with regard to the contact hypersensitivities induced by classical organic haptens (26,44). W/W^v mice are known to possess mutated c-kit [the receptor for stem cell factor (SCF)], with the mutation causing complex alterations in many hematopoietic compartments because c-kit is also expressed in cells other than mast

cells (45). Hence, the above discrepancy is considered to be due to an alteration in the immune system beyond mast cell deficiency (26,44).

Possible roles of non-mast cell histamine produced via induction of HDC

It is known that HDC is induced in various tissues in mice that lack mast cells (16,18). Various inflammatory substances can induce HDC (46,47) and these exhibit adjuvant activities helping to promote Ni allergy at both steps: *i.e.*, sensitization and elicitation (48,49). Inflammatory cytokines can also induce HDC (17-19), with IL-1 (both IL-1 α and IL-1 β) being the strongest cytokine tested so far at inducing HDC (17). In fact, Ni allergy is marginal in mice deficient in IL-1 (13). Inflammatory chemicals also exhibit adjuvant activities, possibly via production of IL-1 (48,50). Thus, we speculate (a) that histamine -- irrespective of whether it is derived from mast cells or from the induction of HDC -- may promote Ni allergy and act as an adjuvant and (b) in mast cell-deficient W/W^v mice, it is histamine derived from the induction of HDC, as well as IL-1, that acts as an adjuvant to promote Ni allergy.

Relationship between the adjuvant effect of LPS and mast cell-derived histamine

LPS, a potent stimulator of TLR4, acts as an adjuvant in Ni allergy in mice (13), and TLR4 stimulation is necessary for inducing Ni allergy in humans, too (51). Interestingly, Ni by itself stimulates TLR4 in humans, although not in mice, this being due to the presence of non-conserved histidine residues in human TLR4 (51). Thus, Ni, like LPS, acts as an adjuvant in humans (*i.e.*, Ni acts as both an adjuvant and antigen in humans). In mice, LPS does not exhibit its adjuvant effects in C3H/HeJ mice, which have mutated non-functional TLR4 (13). The present results suggest that mast cell-derived histamine is involved in the induction of Ni allergy in mice, indicating that histamine may act as an adjuvant. We found

that in both C3H/HeJ and C3H/HeN mice (the control for C3H/HeJ), 1 mM NiCl₂ induced Ni-TI (data not shown), suggesting that TLR4 is not involved in degranulation of mast cells in mice. Thus, the adjuvant effect of LPS (*i.e.*, TLR4 stimulation) would appear to be independent of the degranulation of mast cells. However, it should be noted that LPS can induce HDC via stimulation of TLR4 (52), and that LPS can induce HDC in cells other than mast cells (18). Thus, NiCl₂ can release histamine via degranulation of mast cells, while LPS can produce histamine via induction of HDC. At present, exactly how such effects might contribute to the induction of Ni allergy remains to be clarified, and indeed their contributions might also depend on the experimental conditions.

Alternative explanations for the role of mast cells

In the present study, cromoglicate not only blocked the transient non-allergic ear-swelling, but also reduced the subsequent allergic ear-swelling. Similar effects are reported to exist in the responses to organic haptens (26). However, it has also been reported that cromoglicate does not inhibit mast cell-dependent responses in mice, although this reagent can inhibit IgE-dependent mast cell activation (53). We suspect that this discrepancy might be due to the use of different administration methods: Oka et al. (53) injected cromoglicate intravenously in a mixture with an antigen, whereas we and Dudeck et al. (26) injected it intravenously 1 h before antigen challenge. However, we cannot rule out the possibility that the effects of cromoglicate observed in our study were due not to its mast cell-stabilizing effect, but to one or more of its other activities. Indeed, cromoglicate reportedly has a variety of activities, including inhibition of leukocyte functions and suppression of the activating effects of chemotactic peptides on neutrophils, eosinophils, and monocytes (54). It is also possible that factors other than histamine within mast cells (such as proteases) might play important roles. Indeed, it has been reported that granular proteases are tremendously reduced in HDC-KO

mice (55), and that histamine synthesis is required for granule maturation in murine mast cells (56).

Therapeutic strategies for Ni allergy

As described in Introduction, the existing treatment strategies for metal allergies are inadequate in some respects. In the present study, we demonstrated that the H1-antagonist pyrilamine and the mast-cell stabilizer cromoglicate were highly effective at reducing the allergic response to Ni shown by mice. If these findings hold true in humans, clinical application of such drugs may provide novel options for improving the quality of life of patients suffering from metal allergies. Indeed, the present findings offer experimental support for the observation made by Pigatto *et al.* some 27 years ago that cromoglicate is effective at alleviating human Ni allergy (57).

Conclusion

In conclusion, the findings made here in mice suggest that Ni ion by itself can induce a non-allergic transient inflammation mediated by histamine released from mast cells, and moreover that this inflammation may promote subsequent Ni allergy at both the sensitization and elicitation steps. For the future treatment or prevention of Ni allergy (and perhaps other metal allergies), a possible strategy might be to regulate this transient non-allergic inflammation using antihistamines, HDC inhibitors, and/or mast-cell stabilizers.

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Conflict of interest

The authors declare no conflict of interest.

Author contribution

M.K. and Y.E. designed the experiments; M.K., Y.E., K.B., and D.S. performed the experiments and analyzed the data; M.K. and Y.E. wrote the paper; T.K., Y.N., H.O., T.T-Y., and S.S. conducted the study and provided critical comments. All authors approved the final manuscript version.

Conflict of interest

The authors have declared no conflicting interests.

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Legends to figures

Fig. 1. Transient inflammation associated with Ni exposure. (a) Ni injection, with or without LPS, in non-sensitized mice. Each ear-pinna of a given non-sensitized mouse was injected with a solution containing the indicated concentration of NiCl_2 and/or $0.5 \mu\text{g/mL}$ LPS ($20 \mu\text{L/ear}$). Each value is the mean $\pm$ SD from three mice (*i.e.* six ears). $*P < 0.01$ vs. Saline group at same time-point. (b) Histological features of mast cell degranulation as revealed by Giemsa-stained sections of ear-pinna skins. For Ni-sensitization, mice were sensitized with SSS-Ni. Non-sensitized or Ni-sensitized mice (10 days after SSS-Ni injection) were challenged with 1 mM NiCl_2 ($20 \mu\text{L/ear}$). One hour after this challenge (at the peak of Ni-TI), ears were removed, fixed in 4% paraformaldehyde and embedded in optimal cutting temperature compound. Frozen sections ($5 \mu\text{m}$) were Giemsa-stained for the detection of mast cells. The results shown were confirmed by at least 1 repetition.

Fig. 2. Effects of pyrilamine (H1R antagonist) on Ni-TI and Ni-allergy. (a) Effects of pyrilamine in non-sensitized mice. Saline or pyrilamine was intravenously injected ($200 \mu\text{g/mouse}$) 1 h before an injection of 1 mM NiCl_2 ($20 \mu\text{L/ear}$) or saline. $*P < 0.01$ vs. Saline $\rightarrow 1 \text{ mM}$ NiCl_2 at the same time-point. (b) Effects of pyrilamine injected 1 h before Ni-challenge in Ni-sensitized mice. Ni-sensitized C57BL/6 mice (10 days after SSS-Ni injection) were injected with saline or pyrilamine ($200 \mu\text{g/mouse}$) 1 h before the injection of 1 mM NiCl_2 ($20 \mu\text{L/ear}$). Each value is the mean $\pm$ SD from three mice (*i.e.* six ears). $*P < 0.01$ vs. SSS-Ni $\rightarrow$ Saline $\rightarrow 1 \text{ mM}$ NiCl_2 at the same time-point. (c) Effects of pyrilamine injected 1 h before SSS-Ni injection in mice. Mice were intravenously injected with pyrilamine 1 h before SSS-Ni injection. Then, 10 days after the SSS-Ni injection, their ear-pinnas were challenged with 1 mM NiCl_2 ($20 \mu\text{L/ear}$). Note that the scales are different between upper and lower panels. Each value is the mean $\pm$ SD from three mice (*i.e.* six ears).

* $P < 0.01$ vs. Saline $\rightarrow$ SSS-Ni $\rightarrow$ 1 mM NiCl₂ group at the same time-point.

Fig. 3. Effects of cromoglicate (mast cell stabilizer) on Ni-TI and Ni allergy. (a) Effects of cromoglicate in non-sensitized mice. Mice were injected intravenously with cromoglicate (500 μ g/mouse, ca. 20 mg/kg) 1 h before an injection of 1 mM NiCl₂ (20 μ L/ear). Each value is the mean $\pm$ SD from three mice (*i.e.* six ears). * $P < 0.01$ vs. Saline $\rightarrow$ 1 mM NiCl₂ at the same time-point. Note that the scales are different between upper and lower panels. (b) Effects of cromoglicate injected 1 h before Ni-challenge in Ni-sensitized mice. Ni-sensitized mice (10 days after SSS-Ni injection) were injected intravenously with saline or cromoglicate (500 μ g/mouse) 1 h before the injection of 1 mM NiCl₂ (20 μ L/ear). * $P < 0.01$ vs. SSS-Ni $\rightarrow$ Saline $\rightarrow$ 1 mM NiCl₂ at the same time-point. Note that the scales are different between upper and lower panels. (c) Effects of pyrilamine and cromoglicate injected when Ni-TI has largely subsided. Saline, pyrilamine (200 μ g/mouse) or cromoglicate (500 μ g/mouse) was i.v. injected into mice 6 h after SSS-Ni injection (upper panel) or 6 h after challenge with 1 mM NiCl₂ (20 μ L/ear) (lower panel). Note that the scales in the upper panels of (a) and (b) are different from the other panels. Each value is the mean $\pm$ SD from three mice (*i.e.* six ears).

Fig. 4. Induction of Ni-TI and Ni allergy in HDC-KO and H1R-KO mice. (a) Effect of NiCl₂ in non-sensitized HDC-KO and H1R-KO mice. The ear-pinnas of C57BL/6 WT, HDC-KO and H1R-KO mice were injected with a solution containing 1 mM NiCl₂ (20 μ L/ear). Each value is the mean $\pm$ SD from three mice (*i.e.* six ears). * $P < 0.01$ vs. WT group at the same time-point. (b) Ni-allergy in WT, HDC-KO, and H1R-KO mice. Mice were injected with saline or SSS-Ni. Then, 10 days later, their ear-pinnas were challenged with 1 mM NiCl₂ (20 μ L/ear). Each value is the mean $\pm$ SD from three mice (*i.e.* six ears). * $P < 0.01$ vs. WT group at the same time-point. (c,d) Cell-transfer between WT and HDC-KO (c) or H1R-KO mice

(d). Donor mice were sensitized with SSS-Ni (250 μ L/mouse). Then, 10 days later, their spleen cells (1×10^6 cells) were transferred intravenously into non-sensitized recipient mice. Each ear of the recipient mice was challenged with 1mM NiCl₂ (20 μ L/ear) 1 d after the cell-transfer. Note that the scales in (a) are different from the other panels. Each value is the mean $\pm$ SD from three mice (*i.e.* six ears). * $P < 0.01$ vs. SSS-Ni-WT $\rightarrow$ WT group at the same time-point.

Fig. 1

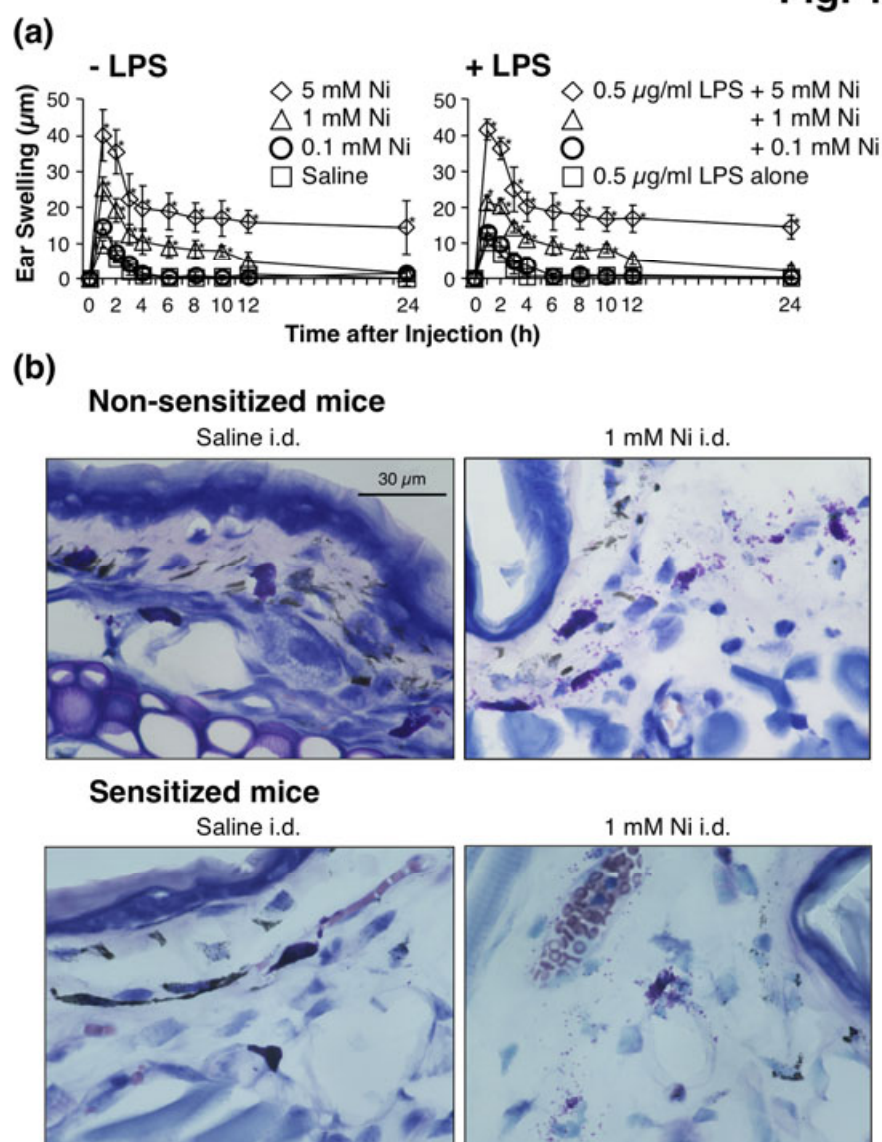


Fig. 2

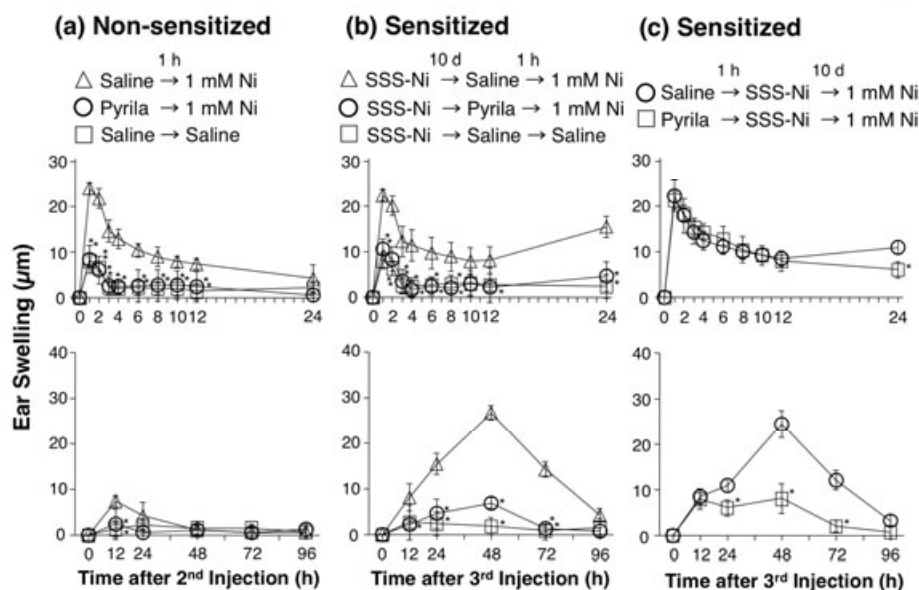


Fig. 3

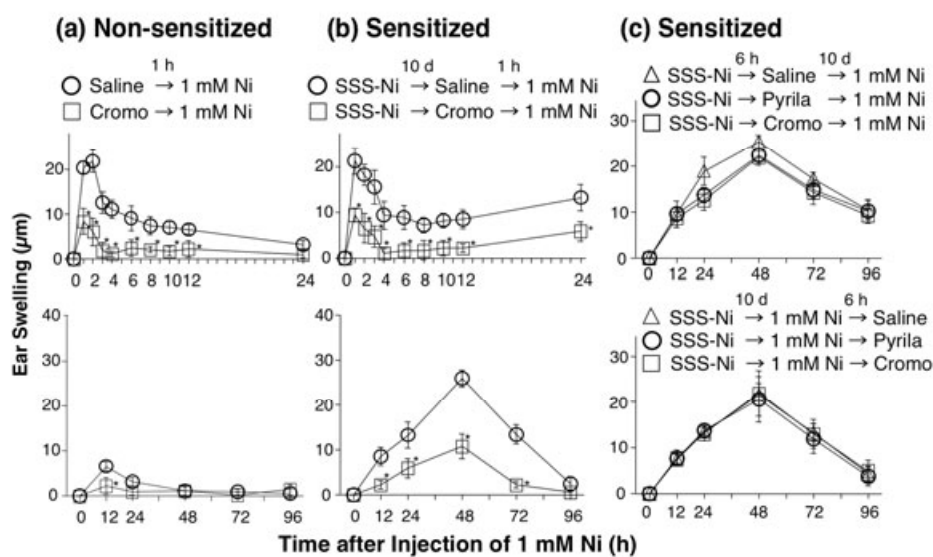


Fig. 4

