

Biotin Status Affects Nickel Allergy via Regulation of Interleukin-1 β Production in Mice^{1,2}

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Abstract

Biotin, a water-soluble B complex vitamin, is possibly involved in chronic inflammatory diseases, although the detailed mechanisms are unclear. In this study, we investigated the effects of biotin status on nickel (Ni) allergy in mice. Mice were fed a basal or biotin-deficient (BD) diet for 8 wk and sensitized with an intraperitoneal injection of NiCl₂ and lipopolysaccharide. Ten days after sensitization, NiCl₂ was intradermally injected into pinnae and ear swelling was measured. For in vitro analysis, we cultured a murine macrophage cell line, J774.1, under a biotin-sufficient (C, meaning control) or BD condition for 4 wk and analyzed interleukin (IL)-1 production. Significantly higher ear swelling was induced in BD mice than C mice. Adaptive transfer of splenocytes from both C and BD mice induced Ni allergy in unsensitized mice. Regardless of donor mice, ear swelling was significantly higher in BD recipient mice than C recipient mice. Ni allergy was not induced in either C or BD IL-1^{-/-} mice. Splenocytes from BD mice produced a significantly higher amount of IL-1 β than those from C mice. Production and mRNA expression of IL-1 β were significantly higher in BD J774.1 cells than in C cells. Biotin supplementation inhibited the augmentation of IL-1 β production in vitro. In vivo supplementation of biotin in drinking water dose-dependently decreased ear swelling in C and BD mice. These results indicate that biotin status affects Ni allergy in the elicitation phase via the upregulation of IL-1 β production in mice, suggesting that biotin supplementation may have therapeutic effects on human metal allergy. J. Nutr. 139: 1031–1036, 2009.

Introduction

Biotin is a water-soluble B complex vitamin found in all organisms (1). It functions as a cofactor of 5 indispensable carboxylases: pyruvate carboxylase (EC 6.4.1.1), 2 forms of acetyl-CoA carboxylase (1 and 2, EC 6.4.1.2), propionyl-CoA carboxylase (PCC)⁷ (EC 6.4.1.3), and methylcrotonoyl-CoA carboxylase (EC 6.4.1.4). These enzymes catalyze the metabolism of glucose, amino acids, and fatty acids, indicating that biotin deficiency is potentially lethal (2). Holocarboxylase synthetase (EC 6.3.4.10)

catalyzes covalent attachment of biotin to specific lysine residues of carboxylases. Biotinidase (EC 3.5.1.12) catalyzes the release of biotin from biotinylated peptides during the turnover of carboxylases. Biotin released from old carboxylases is recycled in the biotinylation of new carboxylases. The defect of this biotin cycle causes multiple carboxylase deficiency, a neonatal form of a life-threatening metabolic disorder (2).

In addition to this classical function as a cofactor of carboxylases, biotin regulates the expression of biotin-dependent carboxylases and holocarboxylase synthetase through a cGMP-dependent signaling pathway (3). Moreover, biotin regulates the localization of transcriptional factors, such as specificity proteins 1 and 3 and nuclear factor- κ B (4,5). Chromatin structure and gene expression are regulated by the biotinylation of histones (6–8). The expressions of asialoglycoprotein receptor and insulin receptor are posttranscriptionally regulated by biotin (9). These reports clearly indicated that biotin regulates the various cellular events at the transcriptional and posttranscriptional levels.

Moderately severe biotin deficiency causes alopecia and scaly, erythematous dermatitis (10–12). Makino et al. (13) reported that serum biotin concentrations are significantly lower in atopic

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⁷ Abbreviations used: Ab, antibody; BD, biotin deficient; BS, biotin supplemented; C, control (biotin sufficient); FCS, fetal calf serum; IL, interleukin; LPS, lipopolysaccharide; PCC, propionyl-CoA carboxylase; TNF, tumor necrosis factor.

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dermatitis patients than in healthy subjects. We recently reported that serum tumor necrosis factor (TNF)- α concentrations were significantly higher in biotin-deficient (BD) mice than in biotin-sufficient (C, meaning control) mice after i.v. administration of lipopolysaccharide (LPS) (14). Moreover, biotin deficiency upregulated TNF α production by a murine macrophage cell line, J774.1, at the transcriptional level (14). TNF α induction by biotin deficiency was downregulated by biotin supplementation in vivo and in vitro (14). These observations suggest that biotin status is involved in inflammatory diseases.

Metals cause various allergic reactions, such as contact dermatitis and lichen planus. Biotin is occasionally prescribed for metal allergy. Biotin has a therapeutic effect on pustulosis palmaris et plantaris, also known as acropustulosis, a type of chronic dermatitis that is restricted in the palms and soles and indicates a relationship with metal allergy (15,16); however, few reports are available on the biological and immunological effects of biotin on allergic inflammation. Recently, we reported an effective nickel (Ni) allergy mouse model, which is sensitized with NiCl₂ and LPS, as an adjuvant, suggesting that the bacterial environment is important for the establishment of metal allergies (17). Among various metals, Ni is a one of the most frequent contact allergens (18).

In this study, we investigated the effects of biotin deficiency on metal allergy using mice treated with Ni and LPS as a model to study metal allergy. We also investigated the in vitro effects of biotin deficiency on interleukin (IL)-1 β production by splenocytes and J774.1 cells. Moreover, we examined the therapeutic effects of biotin supplementation on Ni allergy.

Materials and Methods

Reagents. LPS from *Escherichia coli* O55:B5 prepared by Westphal's method was purchased from Difco Laboratories. Anti-mouse IL-1 β polyclonal antibody (Ab) was purchased from Medical and Biological Laboratories. All other reagents were purchased from Sigma-Aldrich unless otherwise indicated.

Mice. Female BALB/c mice (4 wk old), obtained from the Institute for Experimental Animals of the Tohoku University Graduate School of Medicine (Sendai, Japan), were used for the experiments. BALB/c IL-1^{-/-} mice (deficient in both IL-1 α and IL-1 β) were established from the original IL-1 α ^{-/-} and IL-1 β ^{-/-} mice (19). Mice were divided into 2 groups: C and BD. The groups received a basal diet (AIN-76; 0.8 mg/kg of *d*-biotin) (20) or a BD (biotin-free) AIN-76 diet (Nosan), respectively, for 8 wk. Preliminary experiments showed that 8-wk feeding led to the significant decrease in serum biotin concentrations in mice (14). The PCC activity in the liver did not differ between the C and BD groups (14). For in vivo biotin supplementation, mice were provided with biotin-supplemented (BS) drinking water (0.3–30 μ mol/L) for an additional 2 wk. The dose of biotin was based on the dose of human biotin therapy (10–40 mg/d) and the drinking volume of mice (5 mL/d) (21,22). The Ethical Board for Nonhuman Species of Tohoku University approved the experimental procedure followed in this study.

Mouse model to Ni allergy. The Ni allergy mouse model was constructed as previously reported (17). Briefly, mice were sensitized with an intraperitoneal injection of 250 μ L of solution containing NiCl₂ (0.5 mmol/L) and LPS (0.5 mg/L). Ten days after sensitization, the mice were challenged with 20 μ L of NiCl₂ (2.5 mmol/L) by an intradermal injection into the left and right pinnae near the root of the ear. The mice were anesthetized with ethyl ether just before the challenge 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). For adoptive cell transfer, T cells were prepared from splenocytes using a Pan T Cell Isolation kit (Miltenyi Biotec). Percentages of CD3⁺ T cells in isolated T cell and non-T cell fractions were >98 and <10%,

respectively (data not shown). Whole splenocytes, isolated T cells, or non-T cells (10⁶ cells) from sensitized mice were i.v. injected into unsensitized mice at 24 h before the challenge.

Biotin depletion from fetal calf serum. Biotin in fetal calf serum (FCS) was depleted with immobilized avidin-agarose (Pierce Biotechnology) as previously reported (14). Briefly, FCS (Tissue Culture Biologicals) was mixed with avidin-agarose and gently stirred overnight at 4°C. After centrifugation at 3000 $\times$ g; 20 min at 4°C, FCS was sterilized by filtration (0.2- μ m pore size) and stored at -30°C until use.

Cells and cell culture. J774.1 cells were obtained from the Cell Resource Center for Biomedical Research, Institute of Development, Aging and Cancer, Tohoku University (Sendai, Japan). The C medium was a commercially available complete RPMI 1640 containing *d*-biotin (0.2 mg/L) supplemented with 10% FCS. The BD medium was biotin-free RPMI 1640 (Invitrogen) supplemented with 10% biotin-free FCS. J774.1 cells were cultured with BD medium for 4 wk and then further incubated in medium with or without biotin for 2 wk. J774.1 cells were also cultured in C medium for 6 wk.

Preparation of cytoplasmic fraction. Cells (10⁶ cells) were lysed with a buffer (100 μ L) containing 50 mmol/L Tris-HCl (pH 7.4), 1% Triton X-100, 1 mmol/L phenylmethylsulfonyl fluoride, and 10 mg/L soybean trypsin inhibitor for 30 min on ice. After centrifugation at 12,000 $\times$ g; 20 min, the supernatant was collected and used as the cytoplasmic fraction.

Measurement of IL-1 β . Cells were suspended in RPMI 1640 with 10% FCS stimulated with or without LPS at 37°C for the indicated time. The amount of IL-1 β in the culture supernatant or cytoplasmic fraction was measured with an Opt EIA Mouse IL-1 β set (BD Bioscience).

Quantitative RT-PCR. Cells were lysed in 1 mL of ISOGEN (Nippon Gene) and total RNA was extracted. Total RNA was dissolved in 30 μ L of diethyl pyrocarbonate-treated water (Nippon Gene) and incubated at 65°C for 10 min. cDNA synthesis was conducted with a first-strand cDNA synthesis kit (GE Healthcare Bio-Science). Real-time PCR was performed with a LightCycler FastStart DNA Master SYBR Green I and a LightCycler 1.5 system (Roche Diagnostics). The primers used for PCR were as follows: IL-1 β : forward, 5'-TTCAGGCAGGCAGTATCA-3' and reverse, 5'-CCAGCAGGTTATCATCATC-3'; β -actin: forward, 5'-CGTTGACATCCGTAAAGACCTC-3' and reverse, 5'-AGC-CACCGATCCACACAGA-3' (Nihon Gene Research Laboratories). The PCR conditions were 35 cycles at 95°C for 10 s, 60°C for 10 s, and 72°C for 10 s. The product sizes for IL-1 β and β -actin were 166 and 173 bp, respectively. The mRNA expression levels were expressed as relative units after normalization by the β -actin level. The specificity of the PCR was confirmed by the molecular weight of the products and melting curve analysis for each data point. PCR products were electrophoresed using 3% agarose (Nusieve 3:1 agarose; BMA). After staining with ethidium bromide, amplified DNA bands were analyzed with a Chemi Imager (Alpha Innotech).

Western blotting. After SDS-PAGE, gel proteins were electrophoretically transferred to a polyvinylidene difluoride membrane (Bio-Rad Laboratories). The blot was blocked for 1 h with 3% (wt:v) skim milk and 0.05% Tween 20 in PBS and incubated with anti-mouse IL-1 β polyclonal Ab. Horseradish peroxidase-conjugated anti-rabbit IgG (Pierce Biotechnology) was used as the second Ab. After washing, the blot was analyzed with SuperSignal West Femto Maximum Sensitivity substrate (Pierce Biotechnology) and a Chemi Imager.

Measurement of caspase-1 activity. Caspase-1 activity was measured as originally described by Thornberry (23). Cytoplasmic fractions were incubated in a reaction mixture consisting of 50 mmol/L HEPES-NaOH (pH 7.5), 50 mmol/L NaCl, 0.1% 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate, 10 mmol/L EDTA, 5% glycerol, 5 mmol/L dithiothreitol, and 25 mmol/L substrate (Ac-Trp-Val-Ala-Asp-AMC, Merck KGaA). After incubation at room temperature for 5 h

in the dark, fluorescence was measured with excitation at 380 nm and emission at 460 nm.

Data analysis. All of the experiments in this study were performed at least 3 times to confirm the reproducibility of the results. The data shown are representative results. When the IL-1 β concentration was <30 ng/L, the detection limit of the assay, 30 ng/L was used in the statistical analyses. Experimental values are given as the mean $\pm$ SEM of replicate assays. Differences of ear swelling between each time point in each group were analyzed by 1-way repeated ANOVA. Differences of ear swelling between each group in each time point were also analyzed by 1-way repeated ANOVA. The main effects of the biotin status, the dose of LPS, and the biotin status $\times$ dose of LPS interaction for IL-1 β concentration were analyzed by 2-way ANOVA. Fisher's protected least significant difference procedure was used for post hoc testing. StatView 5.0.1 (SAS Institute) was used to perform calculations and $P < 0.05$ was considered significant.

Results

Exacerbation of Ni allergy in BD mice. Higher ($P < 0.05$) ear swelling was induced after 12 h and peaked at 48 h in both groups (Fig. 1). In C mice, ear swelling was almost resolved at 96 h after challenge, whereas ear swelling in BD mice was significantly higher than that in C mice and continued to differ significantly until 96 h. These results clearly indicated that biotin deficiency exacerbates Ni allergy.

Augmentation of elicitation but not sensitization phase by biotin deficiency. Adaptive cell transfer of splenocytes from both C and BD mice induced Ni allergy in both C and BD recipient mice (Fig. 2), indicating that this allergy model depended on cell-mediated immunity. Regardless of donor mice, ear swelling was higher ($P < 0.05$) in BD recipient mice than C recipient mice (Fig. 2). These results indicated that biotin deficiency was involved in the elicitation but not sensitization phase in Ni allergy. Adaptive cell transfer of whole splenocytes and isolated T cells from sensitized BD mice induced similar levels of ear swelling (Fig. 3). On the other hand, non-T cells from sensitized BD mice induced lower ($P < 0.05$) levels of ear swelling than whole splenocytes and isolated T cells. These results indicated that the cell-mediated immunity in this mouse model was mainly mediated by T cells.

IL-1 dependency of Ni allergy in BD mice. We recently reported that the Ni allergy mouse model used in this study depends on IL-1 (17). Although slight ear swelling was detected at 12 h after challenge, Ni allergy was not induced in either C or BD IL-1 $^{-/-}$ mice (data not shown). These results indicated that IL-1 was a key cytokine for Ni allergy in BD mice as well as C mice.

Augmentation of IL-1 β production in BD cells. Stimulation with LPS increased the level of IL-1 β in the culture supernatant

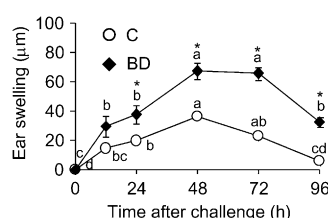


FIGURE 1 Ear swelling in response to Ni in C and BD mice. Results are means $\pm$ SEM, $n = 4$. Means without a common letter in each group differ, $P < 0.05$. *Different from C mice at that time, $P < 0.05$.

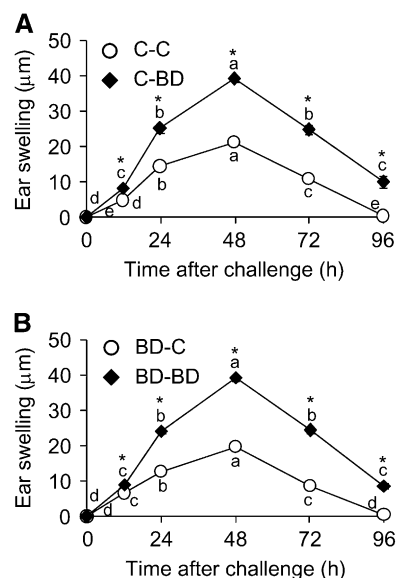


FIGURE 2 Adaptive cell transfer of 10^6 splenocytes from Ni-sensitized C (A) or BD (B) mice to unsensitized mice. Results are means $\pm$ SEM, $n = 4$. Means without a common letter in each group differ, $P < 0.05$. *Different from C mice at that time, $P < 0.05$.

of splenocytes from both C and BD mice (Table 1). The concentration of IL-1 β in the culture supernatants of splenocytes from BD mice was higher ($P < 0.05$) than that of C mice.

We also analyzed IL-1 β production of C, BD, and BS J774.1 cells. All cells produced IL-1 β in response to LPS in a dose-dependent manner (Fig. 4A). The concentration of IL-1 β in the culture supernatant of BD cells was higher ($P < 0.05$) than that of C cells. On the other hand, the concentration of IL-1 β in the culture supernatant of BS cells was lower ($P < 0.05$) than that of BD cells. mRNA expression levels of IL-1 β were also higher ($P < 0.05$) in BD cells than C cells (Fig. 4B). Moreover, a markedly higher amount of IL-1 β precursor (pro-IL-1 β) was detected with Western blotting analysis in the whole-cell lysate of BD cells than C cells (Fig. 4C). Caspase-1 activity did not differ between C and BD cells (data not shown). These results indicated that biotin deficiency augmented IL-1 β production and biotin supplementation restored the augmentable effect.

Therapeutic effects of biotin supplementation on Ni allergy. Finally, we examined the therapeutic effects of biotin supplementa-

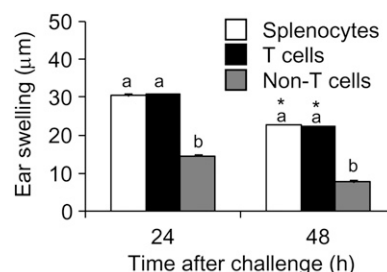


FIGURE 3 Adaptive cell transfer of T or non-T cells from Ni-sensitized BD mice to unsensitized BD mice. Whole splenocytes, isolated T cells, or non-T cells (10^6 cells) from sensitized BD mice were i.v. injected into unsensitized BD mice. Results are means $\pm$ SEM, $n = 4$. Means without a common letter in each time point differ, $P < 0.05$. *Different from value at 24 h in each group, $P < 0.05$.

TABLE 1 IL-1 β production by unstimulated and LPS-stimulated splenocytes from C and BD mice¹

Fraction ²		Medium	LPS
Culture supernatant ³	C	ND ^{4,a}	30.3 $\pm$ 8.3 ^b
	BD	ND ^a	54.9 $\pm$ 5.8 ^c
Cytoplasmic fraction	C	316 $\pm$ 49.6	1240 $\pm$ 75.6
	BD	490.9 $\pm$ 145	2140 $\pm$ 308

¹ Values are means $\pm$ SEM, n = 4 (supernatant) or 3 (cytoplasm). Within a fraction, labeled means without a common letter differ, P < 0.05.

² Splenocytes (10⁶/200 μ L/well for supernatant, 5 $\times$ 10⁶/500 μ L for cytoplasm) were stimulated with LPS (10 μ g/L) for 24 h (for supernatant) or 4 h (for cytoplasm).

³ Effects of biotin, LPS, and LPS $\times$ biotin were significant, P < 0.05.

⁴ ND, Not detected (<30 ng/L).

tation on Ni allergy. When BD mice were provided with BS (15 μ mol/L) drinking water, ear swelling was reduced (P < 0.05) to similar levels as in C mice (Table 2). Moreover, even in C mice, biotin supplementation decreased (P < 0.05) ear swelling. BS drinking water decreased ear swelling in a dose-dependent

manner in both C and BD mice (Table 3). When both groups of mice were provided with 30 μ mol/L of BS drinking water, ear swelling was reduced by more than one-half of that without supplementation at 48 h after challenge. These results indicated that biotin supplementation had therapeutic effects on Ni allergy in mice.

Discussion

In this study, we clearly demonstrated that Ni allergy was significantly exacerbated in BD mice. Ni allergy was not induced in BD IL-1^{-/-} mice and biotin deficiency augmented IL-1 β production. Biotin supplementation inhibited the augmentation of IL-1 β production in vitro. Furthermore, in vivo biotin supplementation improved Ni allergy. These results suggest that biotin is involved in metal allergy through the upregulation of IL-1 production and biotin supplementation has therapeutic effects on metal allergy.

Ni allergy was induced at the same levels as adaptive cell transfer of splenocytes from both C and BD mice. In contrast, BD recipient mice had significantly higher Ni allergy (ear swelling) regardless of the biotin status of donor mice (Fig. 2). These results suggest that biotin deficiency augments the elicitation phase. The adaptive transfer of isolated T cells from sensitized-mice induced Ni allergy in unsensitized recipient mice (Fig. 3). Ni allergy in non-T cell transferred mice was significantly lower than whole splenocytes or isolated T cell transferred mice (Fig. 3). These results indicate that the Ni allergy used in this study mainly depends on T cell-dependent, cell-mediated immunity.

Ni allergy was not induced in BD IL-1^{-/-} mice (data not shown). The IL-1^{-/-} mice used in this study were deficient in both IL-1 α and - β . Shornick et al. (24) reported that IL-1 β -deficient mice had impaired contact hypersensitivity to classical hapten. Inflammasome, a cytosolic protein complex regulating the activation of caspase-1 followed by IL-1 β secretion, is involved in contact hypersensitivity (25). These studies suggest that IL-1 β rather than IL-1 α contributes to Ni allergy. In this study, we clearly demonstrated that IL-1 β production is augmented in BD cells, both primary splenocytes and the murine macrophage cell line (Table 1; Fig. 4). Kondo et al. (26) reported that local injection of IL-1 receptor antagonist suppressed contact hypersensitivity in both elicitation and sensitization phases. These observations suggest that the augmentation of IL-1 β production is involved in the pathogenesis of Ni allergy.

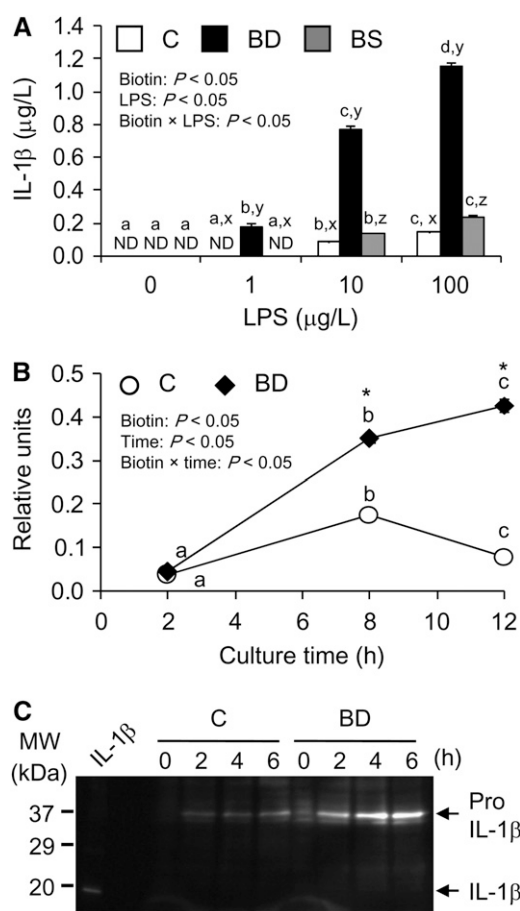


FIGURE 4 IL-1 β production of C, BD, and BS J774.1 cells stimulated with LPS. (A) Concentrations of IL-1 β in supernatants of cells cultured without or with LPS at 2 $\times$ 10⁵/200 μ L/well. ND, Not detected. Values are means $\pm$ SEM, n = 3. Means within a cell type without a common letter (a, b, c, d) differ, P < 0.05; means within an LPS dose without a common letter (x, y, z) differ, P < 0.05. (B) IL-1 β mRNA expression levels of J774.1 cells cultured with LPS at 5 $\times$ 10⁵/500 μ L/well. Values are means $\pm$ SEM, n = 3. Means within a cell type without a common letter, P < 0.05. *Different from C at that time, P < 0.05. (C) Whole cell lysates of cells cultured with LPS at 5 $\times$ 10⁵/mL in each tube were subjected to Western blotting with anti-mouse IL-1 β polyclonal Ab.

TABLE 2 Ear swelling of C and BD mice that were (+15) or were not provided with 15 μ mol biotin/L in drinking water¹

Time after challenge, h	C	C + 15 ²	BD	BD + 15
		μ m		
0	0.0 $\pm$ 0.0 ^a	0.0 $\pm$ 0.0 ^a	0.0 $\pm$ 0.0 ^a	0.0 $\pm$ 0.0 ^a
12	16.5 $\pm$ 1.9 ^b	8.7 $\pm$ 4.1 ^a	17.4 $\pm$ 3.4 ^b	13.5 $\pm$ 1.3 ^{bc}
24	23.8 $\pm$ 2.2 ^{c,xy}	3.2 $\pm$ 1.5 ^{a,z}	32.3 $\pm$ 4.4 ^{c,x}	18.8 $\pm$ 2.2 ^{b,y}
48	37.3 $\pm$ 2.0 ^{d,x}	21.9 $\pm$ 3.5 ^{b,y}	76.2 $\pm$ 2.8 ^{d,z}	36.1 $\pm$ 2.5 ^{d,x}
72	14.4 $\pm$ 2.2 ^{b,x}	2.4 $\pm$ 1.9 ^{a,y}	40.1 $\pm$ 3.4 ^{c,z}	10.4 $\pm$ 2.5 ^{ae,xy}
96	4.3 $\pm$ 1.4 ^{a,x}	1.1 $\pm$ 0.8 ^{a,x}	11.4 $\pm$ 1.7 ^{b,y}	3.6 $\pm$ 1.0 ^{ae,x}

¹ Values are means $\pm$ SEM, n = 4. Labeled means in a row without a common letter (x, y, z) differ, P < 0.05. Labeled means in a column without a common letter (a, b, c, d, e) differ, P < 0.05, between different letters in each group. P < 0.05, between different symbols in each time point.

² + 15, mice were provided with BS (15 μ mol/L) drinking water.

TABLE 3 Ear swelling of C and BD mice provided with 0, 0.3, 3, or 30 μmol biotin/L in BS drinking water¹

Time after challenge, h	C	C + 0.3 ²	C + 3	C + 30	BD	BD + 0.3	BD + 3	BD + 30
	μm							
0	0.0 $\pm$ 0.0 ^a	0.0 $\pm$ 0.0 ^a	0.0 $\pm$ 0.0 ^a	0.0 $\pm$ 0.0 ^a	0.0 $\pm$ 0.0 ^a	0.0 $\pm$ 0.0 ^a	0.0 $\pm$ 0.0 ^a	0.0 $\pm$ 0.0 ^a
12	3.4 $\pm$ 0.3 ^{b,x}	3.1 $\pm$ 0.3 ^{b,xy}	2.4 $\pm$ 0.3 ^{b,yz}	1.8 $\pm$ 0.3 ^{ab,z}	4.8 $\pm$ 0.3 ^{b,x}	3.8 $\pm$ 0.3 ^{b,y}	3.0 $\pm$ 0.3 ^{b,yz}	2.5 $\pm$ 0.2 ^{b,z}
24	14.0 $\pm$ 0.4 ^{c,w}	12.1 $\pm$ 0.6 ^{c,x}	7.3 $\pm$ 0.5 ^{c,y}	2.6 $\pm$ 0.3 ^{b,z}	20.9 $\pm$ 0.5 ^{c,w}	16.3 $\pm$ 0.5 ^{c,x}	11.6 $\pm$ 1.5 ^{c,y}	6.3 $\pm$ 0.3 ^{c,z}
48	40.0 $\pm$ 0.7 ^{d,w}	30.4 $\pm$ 0.8 ^{d,x}	20.1 $\pm$ 0.4 ^{d,y}	12.5 $\pm$ 0.7 ^{c,z}	54.3 $\pm$ 0.6 ^{d,w}	38.4 $\pm$ 0.7 ^{d,x}	28.4 $\pm$ 0.6 ^{d,y}	22.0 $\pm$ 0.4 ^{d,z}
72	26.0 $\pm$ 0.7 ^{e,w}	14.8 $\pm$ 0.8 ^{c,x}	7.3 $\pm$ 0.8 ^{c,y}	3.1 $\pm$ 0.8 ^{b,z}	35.8 $\pm$ 1.1 ^{e,w}	22.6 $\pm$ 0.5 ^{e,x}	13.5 $\pm$ 0.5 ^{e,y}	10.0 $\pm$ 0.7 ^{e,z}
96	15.0 $\pm$ 0.8 ^{c,x}	3.6 $\pm$ 0.5 ^{b,y}	0.9 $\pm$ 0.2 ^{ab,z}	0.1 $\pm$ 0.4 ^{a,z}	17.9 $\pm$ 1.2 ^{c,x}	7.8 $\pm$ 0.5 ^{f,y}	1.1 $\pm$ 0.4 ^{ab,z}	0.9 $\pm$ 0.4 ^{a,z}

¹ Values are means $\pm$ SEM, $n = 4$. Within C and D groups, labeled means in a row without a common letter (x, y, z) differ, $P < 0.05$. Labeled means in a column without a common letter (a, b, c, d, e) differ, $P < 0.05$.

² + 0.3, 3, and 30, mice were provided with 0.3, 3, and 30 μmol /L of BS drinking water.

The concentrations of IL-1 β were significantly higher in the culture supernatant of BD-J774.1 cells than in C cells. Because IL-1 β is produced as a biologically inactive precursor (pro-IL-1 β) and cleaved by caspase-1 as an active mature IL-1 β , the activity of caspase-1 is a rate-limiting factor of mature IL-1 β secretion (27); however, the activities of caspase-1 did not differ between C and BD cells. On the other hand, mRNA expression levels of IL-1 β were significantly higher in BD cells than in C cells. The accumulation of pro-IL-1 β in the cytoplasmic fraction was significantly higher in BD cells than in C cells. These results suggest that IL-1 β production was upregulated by biotin deficiency at the transcriptional level.

Kramer et al. (28) reported that biotin deficiency decreases (ω -6) PUFA in rat liver. It was discussed that biotin deficiency induces downregulation of PCC followed by abnormalities of lipid metabolisms. Mock (11) reported that supplementation of linoleic acid [ω -6) PUFA] improves cutaneous manifestation of BD rats. Although we could not detect differences in liver PCC activity between C and BD mice (14), abnormalities of lipid metabolism are possibly involved in the deterioration of Ni allergy in BD mice.

To our knowledge, the major methods of preventing metal allergy are to reduce or avoid metal exposure (18). In this study, Ni allergy was improved by biotin supplementation of at least 0.3 μmol /L biotin (Table 3). Patients with holocarboxylase synthetase or biotinidase deficiency are treated with oral administration of pharmacological doses of biotin (10–40 mg/d) (21). Considering the body weights ($\sim$ 25 g) and drinking volume (5 mL/d) of mice (22), the concentration of BS drinking water is reasonable. Moreover, biotin supplementation significantly decreased ear swelling in both C and BD mice (Tables 2 and 3). These observations suggest that biotin has not only therapeutic but also preventive effects on metal allergy. Further studies are needed to clarify this point.

We previously reported that biotin deficiency augments TNF α production in vivo and in vitro (14). Although TNF α is a one of the most important cytokines in inflammatory responses, Sato et al. (17) reported that TNF α is not a critical cytokine in the murine model used in this study. We also analyzed the production of IL-6, another important inflammatory cytokine, by J774.1 cells, but C and BD cells did not differ (data not shown). To clarify the involvement of other inflammatory cytokines, more detailed and comprehensive analyses are needed.

In conclusion, the present study demonstrated that biotin deficiency exacerbates Ni allergy and that biotin supplementation improves allergic inflammation. Because this study was conducted in a murine model, detailed and careful investigations are needed in human systems. Zemleni et al. (29) reported that the proliferation and IL-1 β production of human peripheral blood

mononuclear cells are decreased by in vivo biotin supplementation at a pharmacological dose. Moreover, Wiedmann et al. (30) reported that various genes of human peripheral blood mononuclear cells respond to in vivo biotin supplementation. Among biotin-responsive genes, TNF superfamily 15, a cytokine that induces proinflammatory cytokine production by human mononuclear cells, was downregulated by biotin supplementation (12). These studies suggest that biotin supplementation modulates immune/inflammatory systems in humans as well as in mice. All of these observations encourage further investigations of biotin application for other inflammatory diseases in which decreases of serum biotin concentrations are reported.

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