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Disclosures

None.

References

1 Kohu K, Kubo M, Ichikawa H, Ohno S, Habu S, Sato T, Satake M. Pleitropic roles of Runx transcription factors in the differentiation and function of T lymphocytes. *Curr Immunol Rev* 2008; **4**:101–15.

2 Sato T, Ohno S, Hayashi T, Sato C, Kohu K, Satake M, Habu S. Dual functions of Runx proteins for reactivating CD8 and silencing CD4 at the commitment process into CD8 thymocytes. *Immunity* 2005; **22**:317–28.

3 Egawa T, Tillman RE, Naoe Y, Taniuchi I, Littman DR. The role of the Runx transcription factors in thymocyte differentiation and in homeostasis of naive T cells. *J Exp Med* 2007; **204**:1945–57.

4 Ichikawa M, Asai T, Saito T *et al.* AML-1 is required for megakaryocytic maturation and lymphocytic differentiation, but not for maintenance of hematopoietic stem cells in adult hematopoiesis. *Nat Med* 2004; **10**:299–304.

5 Hayashi K, Abe N, Watanabe T, Obinata M, Ito M, Sato T, Habu S, Satake M. Overexpression of AML1 transcription factor drives thymocytes into the CD8 single-positive lineage. *J Immunol* 2001; **167**:4957–65.

6 Setoguchi R, Tachibana M, Naoe Y, Muroi S, Akiyama K, Tezuka C, Okuda T, Taniuchi I. Repression of the transcription factor Th-POK by Runx complexes in cytotoxic T cell development. *Science* 2008; **319**:822–5.

7 Taniuchi I, Osato M, Egawa T, Sunshine MJ, Bae SC, Komori T, Ito Y, Littman DR. Differential requirements for Runx proteins in CD4 repression and epigenetic silencing during T lymphocyte development. *Cell* 2002; **111**:621–33.

8 Woolf E, Xiao C, Fainaru O *et al.* Runx3 and Runx1 are required for CD8 T cell development during thymopoiesis. *Proc Natl Acad Sci USA* 2003; **100**:7731–6.

9 Talebian L, Li Z, Guo Y *et al.* T-lymphoid, megakaryocyte, and granulocyte development are sensitive to decreases in CBFβ dosage. *Blood* 2007; **109**:11–21.

10 Ghozi MC, Bernstein Y, Negreanu V, Levanon D, Groner Y. Expression of the human acute myeloid leukemia gene AML1 is regulated by two promoter regions. *Proc Natl Acad Sci USA* 1996; **93**:1935–40.

11 Levanon D, Groner Y. Structure and regulated expression of mammalian *RUNX* genes. *Oncogene* 2004; **23**:4211–9.

12 Telfer JC, Rothenberg EV. Expression and function of a stem cell promoter for the murine *CBFalpha2* gene: distinct roles and regulation in natural killer and T cell development. *Dev Biol* 2001; **229**:363–82.

13 Araki K, Araki M, Miyazaki J, Vassalli P. Site-specific recombination of a transgene in fertilized eggs by transient expression of Cre recombinase. *Proc Natl Acad Sci USA* 1995; **92**:160–4.

14 Takahama Y, Ohishi K, Tokoro Y, Sugawara T, Yoshimura Y, Okabe M, Kinoshita T, Takeda J. Functional competence of T cells in the absence of glycosylphosphatidylinositol-anchored proteins caused by T cell-specific disruption of the *Pig-a* gene. *Eur J Immunol* 1998; **28**:2159–66.

15 Kanto S, Chiba N, Tanaka Y *et al.* The PEBP2β/CBFβ–SMMHC chimeric protein is localized both in the cell membrane and nuclear subfractions of leukemic cells carrying chromosomal inversion 16. *Leukemia* 2000; **14**:1253–9.

16 Okada H, Watanabe T, Niki M *et al.* AML1$^{-/-}$ embryos do not express certain hematopoiesis-related gene transcripts including those of the PU.1 gene. *Oncogene* 1998; **17**:2287–93.

17 Chiba N, Watanabe T, Nomura S, Tanaka Y, Minowa M, Niki M, Kanamaru R, Satake M. Differentiation dependent expression and distinct subcellular localization of the protooncogene product, PEBP2β/CBFβ, in muscle development. *Oncogene* 1997; **14**:2543–52.

18 Senoo M, Wang L, Suzuki D, Takeda N, Shinkai Y, Habu S. Increase of TCR Vβ accessibility within Eβ regulatory region influences its recombination frequency but not allelic exclusion. *J Immunol* 2003; **171**:829–35.

19 Suzuki D, Wang L, Senoo M, Habu S. The positional effect of Eβ on Vβ genes of TCRβ chain in the ordered rearrangement and allelic exclusion. *Int Immunol* 2005; **17**:1553–60.

20 Tanaka T, Kurokawa M, Ueki K *et al.* The extracellular signal-regulated kinase pathway phosphorylates AML1, an acute myeloid leukemia gene product, and potentially regulates its transactivation ability. *Mol Cell Biol* 1996; **16**:3967–79.

21 von Boehmer H, Aifantis I, Feinberg J, Lechner O, Saint-Ruf C, Walter U, Buer J, Azogui O. Pleiotropic changes controlled by the pre-T-cell receptor. *Curr Opin Immunol* 1999; **11**:135–42.

22 Taghon T, Yui MA, Pant R, Diamond RA, Rothenberg EV. Developmental and molecular characterization of emerging β- and γδ-selected pre-T cells in the adult mouse thymus. *Immunity* 2006; **24**:53–64.

23 Wolfer A, Wilson A, Nemir M, MacDonald HR, Radtke F. Inactivation of Notch1 impairs VDJβ rearrangement and allows pre-TCR-independent survival of early αβ lineage thymocytes. *Immunity* 2002; **16**:869–79.

24 Bell JJ, Bhandoola A. The earliest thymic progenitors for T cells possess myeloid lineage potential. *Nature* 2008; **452**:764–7.

25 Porritt HE, Rumfelt LL, Tabrizifard S, Schmitt TM, Zuniga-Pflucker JC, Petrie HT. Heterogeneity among DN1 prothymocytes reveals multiple progenitors with different capacities to generate T cell and non-T cell lineages. *Immunity* 2004; **20**:735–45.

26 Rothenberg EV. Cell lineage regulators in B and T cell development. *Nat Immunol* 2007; **8**:441–4.

27 Rothenberg EV, Moore JE, Yui MA. Launching the T-cell-lineage developmental programme. *Nat Rev Immunol* 2008; **8**:9–21.

28 Schmitt TM, Ciofani M, Petrie HT, Zuniga-Pflucker JC. Maintenance of T cell specification and differentiation requires recurrent notch receptor–ligand interactions. *J Exp Med* 2004; **200**:469–79.

29 Wada H, Masuda K, Satoh R, Kakugawa K, Ikawa T, Katsura Y, Kawamoto H. Adult T-cell progenitors retain myeloid potential. *Nature* 2008; **452**:768–72.

30 Blyth K, Slater N, Hanlon L, Bell M, Mackay N, Stewart M, Neil JC, Cameron ER. Runx1 promotes B-cell survival and lymphoma development. *Blood Cells Mol Dis* 2009; **43**:12–9.

31 Wotton S, Stewart M, Blyth K, Valliant F, Kilbey A, Neil JC, Cameron ER. Proviral insertion indicates a dominant oncogenic role for Runx1/AML-1 in T-cell lymphoma. *Cancer Res* 2002; **62**:7181–5.

32 Vaillant F, Blyth K, Andrew L, Neil JC, Cameron ER. Enforced expression of Runx2 perturbs T cell development at a stage coincident with β-selection. *J Immunol* 2002; **169**:2866–74.

33 Sato T, Ito R, Nunomura S, Ohno S, Hayashi K, Satake M, Habu S. Requirement of transcription factor AML1 in proliferation of developing thymocytes. *Immunol Lett* 2003; **89**:39–46.

34 Chung DD, Honda K, Cafuir L, McDuffie M, Wotton D. The Runx3 distal transcript encodes an additional transcriptional activation domain. *FEBS J* 2007; **274**:3429–39.

35 Kanatani N, Fujita T, Fukuyama R *et al.* Cbf β regulates Runx2 function isoform-dependently in postnatal bone development. *Dev Biol* 2006; **296**:48–61.

36 Xiao Z, Awad HA, Liu S, Mahlios J, Zhang S, Guilak F, Mayo MS, Quarles LD. Selective Runx2-II deficiency leads to low-turnover osteopenia in adult mice. *Dev Biol* 2005; **283**:345–56.

37 Telfer JC, Hedblom EE, Anderson MK, Laurent MN, Rothenberg EV. Localization of the domains in Runx transcription factors required for the repression of CD4 in thymocytes. *J Immunol* 2004; **172**:4359–70.

Supporting Information

Additional Supporting information may be found in the online version of this article:

Figure S1. Northern blot analysis *of Runxl* transcripts in various mouse cell lines.

Figure S2. (a) Total numbers of Thy-1.2$^+$ gated thymocytes. (b) Comparison of the percentages and cell numbers of Thy-1.2$^+$ gated DN, DP, CD4 SP and CD8 SP subsets.

Figure S3. Expression profiles of TCR-γδ and Annexin-V in the DN and DP cells from *distal Runxl-tg;Lckl-Cre-tg* and *Lck-Cre-tg* thymi.

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Th2 Immune Response Plays a Critical Role in the Development of Nickel-Induced Allergic Contact Dermatitis

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Key Words
GATA-3 · Nickel · Transgenic mice · Th2 cytokine

Abstract
Background: The precise roles of T helper (Th)1-type and Th2-type cytokine responses in nickel (Ni)-induced allergic contact dermatitis have not yet been clearly defined. We investigated the involvement of Th2 cytokines in Ni-induced contact hypersensitivity reaction using GATA-3 transgenic (Tg) mice. ***Methods:*** A Ni-titanium (Ti) alloy was implanted under the skin of GATA-3 Tg mice. A Ni solution was then injected 1 month after sensitization. The ear swelling response was measured at several time points after the injection; the cytokine levels in the skin were measured at 48 h after injection, and the serum levels of IgE were measured 1 month after injection. In addition, purified CD4+ splenic cells obtained from the GATA-3 Tg mice sensitized with the Ni-Ti alloy were infused into Rag-2$^{-/-}$ mice, and the ear swelling response of these mice after a further challenge with Ni solution was also measured. ***Results:*** Marked ear swelling and elevated serum IgE levels and skin tissue levels of IL-4 were observed in Ni-Ti-sensitized GATA-3 Tg mice. The Rag-2$^{-/-}$ mice transfused with the CD4+ splenic cells from the Ni-Ti alloy sensitized GATA-3 Tg mice showed a significantly more pronounced ear swelling response than the control mice. ***Conclusion:*** We confirmed the participation of Th2-type immune reactions in Ni-induced allergy using GATA-3 Tg mice.

Introduction

A variety of metals are known to be present in the environment; in recent years, allergic contact dermatitis (ACD) caused by these metals has drawn attention. The use of nickel (Ni), in particular, in numerous industrial processes, such as Ni plating and the production of Ni alloys, provides numerous opportunities for sensitization, and the reported sharp increase in Ni allergy has become a serious cause for concern. Ni-induced ACD has been reported to be a delayed-type hypersensitivity reaction associated with a T cell-mediated response following the exposure of skin to metal [1]. It has been estimated that about 4–8% of all males and 18–30% of all females in the industrialized world are sensitized to Ni [2–4]. Ni-induced ACD involves the activation of Ni-specific T cells, followed by the proliferation and induction of cytokine

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production [5]. Earlier studies suggested that the delayed-type hypersensitivity reaction to Ni in humans predominantly involved interferon (IFN)-γ-producing T cells [6–8], but subsequent studies of Ni-specific T cell clones have shown the involvement of mixed T helper (Th)1- and Th2-type cytokine responses in this condition [9, 10]. Moreover, Yokozeki et al. [11] described a murine model of contact sensitization to paraphenylenediamine (PPD) induced by the application of PPD to mouse skin and demonstrated that Th2-like γδT cells played a role in the development of the ACD in response to exposure to PPD. However, the association between the Th1- and Th2-type cytokine responses involved in the development of ACD in vivo has not yet been clearly defined.

GATA-3 is selectively expressed in the T cell lineage from the early stage of the development of the thymus [12, 13]. Among mature peripheral T cells, a low level of GATA-3 expression is observed in CD4+ naive T cells, and while the expression increases in Th2 cells during the process of their differentiation in vivo, the Th1 cells do not express this gene [14, 15]. The exclusive expression of GATA-3 in Th2 cells is thought to play an important role in Th2-specific functions and/or cytokine gene expressions [16]. In vitro studies have demonstrated that the ectopic overexpression of GATA-3 in cell lines results in an increase in the products of Th2 cytokine genes or the enhancement of their promoter activities [17, 18]. We previously demonstrated that the exposure of actively sensitized lck-GATA-3-transgenic (GATA-3 Tg) mice to ovalbumin aerosol increased the expression of both interleukin (IL)-5 and IL-13, the number of eosinophils in the bronchoalveolar lavage fluid, and the serum IgE levels [19]. In the present study, we investigated the immune reactions involved in the ACD response to Ni in GATA-3 Tg mice, and our results suggested that Th2-type immune reactions might play an important role in Ni-induced contact hypersensitivity reactions.

Materials and Methods

Animals

Murine GATA-3 cDNA in pBlueScript SK was provided by Dr. M. Yamamoto (Tsukuba University School of Medicine, Tsukuba, Japan). Transgenic mice harboring GATA-3 have been described previously [19]. All animals were housed under specific-pathogen-free conditions and had free access to a commercial diet and water. In this study, the animals were used at 8–15 weeks of age. Each experimental group consisted of at least 5 mice. All experiments were conducted in accordance with the Guidelines for Animal Experimentation published by the Japanese Association for Laboratory Animal Science (1987).

Contact-Sensitizing Agent

Ni-titanium (Ti) alloy bars (Ni-49.2 %Ti) were cut into sections 5 mm in diameter and 3 mm thick. The sections were then ground and polished to an ash surface and ultrasonically cleaned with acetone and ethanol. The Ni-Ti alloy test pieces were prepared using isothermal oxidation treatment. Briefly, the polished samples were heated in a furnace under a flow of 16 ml/min nitrogen and 4 ml/min oxygen and kept at 500°C (#500) for 30 min, then cooled to room temperature.

Induction of Contact Hypersensitivity to Ni

For the implantation of the test pieces, the GATA-3 Tg mice and their wild-type (WT) littermate controls (C57/BL6 mice) were anesthetized, and the coat on their backs was shaved. Part of the exposed skin was incised, the test piece was implanted under the skin, and the wound was closed with surgical clips.

Challenge and Measurement

One month after the implantation of the test piece, 10 μl of an aqueous Ni solution [Wako Nickel Std. Soln. $Ni(NO_3)_2$ in 0.1 mol/l · HNO_3; Lot No. YPK9895] that had been purchased from Wako Pure Chemical Industries Ltd. was injected into the skin of the left pinna of the GATA-3 Tg mice and WT mice, and saline was injected into the skin of the right pinna.

To determine the time course of the changes in the ear-swelling responses, the ear skin thickness on either side was measured at 1, 3, 24, 48 and 72 h and at 3, 7, 14 and 28 days after the challenge using an engineer's micrometer (Peacock, Ozaki Engineering, Tokyo, Japan); then, the difference in the thickness of the two ears was calculated for each time point.

Measurement of the Serum Concentrations of IgE, IgG1 and IgG2a

The total serum levels of IgE, IgG1 and IgG2a antibody in the sensitized/challenged mice were measured using sandwich enzyme-linked immunosorbent assays (ELISA). Serial dilutions of the test sera and control sera were incubated in 96-well microplates coated with anti-mouse antibody directed against each isotype. Biotin-labeled rat antibody against mouse IgE, IgG1 and IgG2a was added before the second incubation. After a thorough washing, the plates were incubated with streptavidin-horseradish peroxidase (HRP); the color that developed during a eroxidase reaction in TMB 3, 3′, 5, 5′-tetramethylbenzidine substrate was measured at 450 nm using an ELISA plate reader.

Quantification of the Cytokine Levels in the Supernatants of the Skin Tissue Extracts

Extracts from the ear lobe tissue were prepared for ELISA as described by Ferguson et al. [20]. Briefly, the ears were excised at 48 h after Ni application and were immediately homogenized with 500 μl of 0.1% Tween 20 in phosphate-buffered saline (PBS). The samples were quickly frozen in liquid nitrogen, thawed in a 37°C water bath, sonicated for 15 s, and sedimented by centrifugation for 5 min at 13,000 *g*. The supernatants were stored at −80°C before the cytokine assays. The ELISAs for IL-4, IL-5 and IFN-γ were performed using the respective ELISA kits (Endogen Inc., Woburn, Mass., USA). The IL-13 assay was conducted using the mouse IL-13 Quantikine ELISA kit (R&D Systems, Minneapolis, Minn., USA) in accordance with the manufacturer's instructions.

Histological Examination

The ear skin specimens were excised and fixed in 10% formalin, then processed and stained with hematoxylin and eosin or toluidine blue. The numbers of mononuclear cells, neutrophils and eosinophils infiltrating the dermis were counted in using micrometer sections of tissue specimens stained with hematoxylin and eosin. The number of mast cells infiltrating the dermis was evaluated by staining sections of the tissue specimens with toluidine blue solution, and the tissue sections were examined at a magnification of ×400. At least 10 fields were examined per ear lobe. The number of cells was counted and expressed as the number of cells per square millimeter area.

Passive Transfer of the Contact Hypersensitivity Reaction

To determine the effect of purified CD4+ T cells isolated from the GATA-3 Tg mice on the development of the contact hypersensitivity reaction, the GATA-3 Tg mice and WT mice were sensitized with the Ni-Ti alloy and the spleen cells were isolated on day 30. A single-cell suspension was then prepared by gentle teasing; the suspension was washed 3 times with PBS, and the washed cells were passed through a column packed with nylon fibers (Wako Pure Chemicals, Osaka, Japan). CD4+ T cells were positively selected using magnetic sorting with Dynabeads and DTACHa-BEAD mouse CD4. The CD4+ T cell specimens were usually >95% pure according to a flow cytometry analysis. The CD4+ T cells (2×10^6/mouse) were intravenously infused into Rag-$2^{-/-}$ mice. The ears of the recipient mice were immediately challenged with Ni solution, and the ear swelling response was measured at 0.5, 1, 3, 24, 48 and 72 h after the challenge.

Indirect Immunohistochemical Staining

Indirect immunohistochemical staining was performed on 5-μm-thick frozen sections of the ear lobe skin. After they were thawed, the sections were air-dried for 1 h and then fixed with cold acetone for 10 min. Endogenous peroxidase activity was blocked by treatment with 3% hydrogen peroxide for 5 min at room temperature, and the slides were incubated with 5% fetal calf serum in PBS overnight at 4°C to block nonspecific protein binding. The slides were then incubated with anti-mouse CD4 (1:100; Bio Legend, San Diego, Calif., USA) for 60 min at room temperature, washed with PBS, and incubated with biotinylated rabbit anti-rat IgG (1:400; Dako, Glostrup, Denmark) for 15 min at room temperature. After the slides were washed with PBS, they were incubated with HRP-conjugated streptavidin solution (Dako) for 10 min at room temperature. Finally, immunoreactivity was visualized by staining with 8-amino-9-ethylcarbazole solution (Dako) and counterstaining with Mayer's hematoxylin (Wako Pure Chemical).

Ear Swelling Response in GATA-3 Tg Mice Implanted with the Ni-Ti (#500) Test Piece and Measurement of the Serum IgE Concentration

The Ni-Ti test pieces were subjected to oxygen diffusion processing and implanted subcutaneously in the mice. One month later, the ear skin of the mice was injected with Ni solution, and the ear swelling response was measured using a thickness gauge. The serum IgE antibody levels were measured one month later using an ELISA.

Examination of Ni Ion Elution from the Oxygen Diffusion Processed Ni-Ti Alloy Subjected to Oxygen Diffusion Processing

The test pieces subjected to oxygen diffusion processing were soaked in 3% sodium chloride (NaCl) solution at 37°C for 28 days, and the density of the Ni ions eluted into the solution was measured using atomic absorption photometry. The surface area of the test piece was assumed to be 113 mm^2, and the volume of the solution used to measure the Ni ion density was 5 ml.

Statistical Analysis

The experimental data were expressed as the mean ± standard deviation (SD). The statistical significance of differences in the data was determined using a Student t test. $p < 0.05$ was considered to denote significance.

Results

Increased Ear Swelling Response to Ni Challenge in GATA-3 Tg Mice Implanted with Ni-Ti Alloy

The GATA-3 Tg and WT mice were sensitized with a Ni-Ti alloy to evaluate the ability of the GATA-3 Tg mice to mount a Ni-induced ACD reaction. A significantly greater amount of swelling was recognized in the GATA-3 Tg mice than in the WT mice until 4 days after the Ni antigen challenge. Thereafter, however, the extent of the swelling was almost the same in both the GATA-3 Tg and WT mice. The reactions within 1 h after the local injection of Ni solution were considered to represent a physical response to the local injection, since no differences in the reactions between the GATA-3 Tg mice and WT mice were observed. The characteristics of the specific responses seen subsequently in the GATA-3 Tg mice suggested that Th2-type immune reactions were involved in the reactions. Also, as reported previously, the reactions that were observed from 4 days onward after the Ni antigen challenge were considered to represent Th1-type immune reactions. The ear swelling reactions occurring after the local injection of Ni solution into the non Ni-Ti alloy sensitized mice averaged 1–5 × 0.01 mm (data not shown). The values were the same in the GATA-3 Tg mice and WT mice at every time-point (fig. 1). The reaction of GATA-3 Tg mice and WT mice not sensitized to the Ni-Ti alloy test piece to a challenge with 10 μl of HNO_3 solution was almost the same as that following the administration of saline (data not shown).

Serum IgE Elevation in GATA-3 Tg Mice Challenged with Ni

IgE is known to play an important role in the pathogenesis of ACD [21, 22]. GATA-3 Tg mice and WT mice

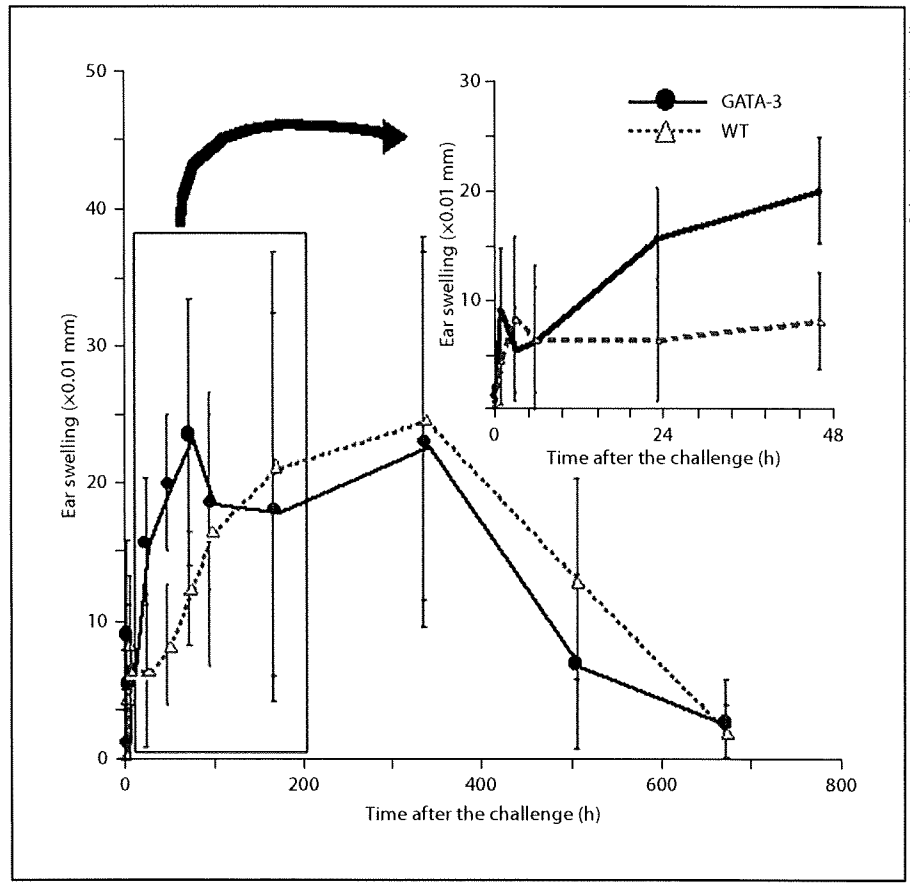

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Fig. 1. GATA-3 Tg mice exhibit enhanced allergic contact dermatitis. The ear skin thickness of the Ni-sensitized GATA-3 Tg and the WT mice was measured at the time points indicated after the subcutaneous injection of 10 μl of a solution of Ni (1,000 ppm) into the left ear and 10 μl of saline into the right ear. The differences in the thickness between the right and left ears after the challenge are shown. The data are the means ± SD of 5 animals per group and are representative of 3 independent experiments. $p < 0.05$ vs. WT mice.

were sensitized with Ni and then challenged with Ni or saline. Twenty-eight days after the Ni challenge, the serum concentrations of IgE, IgG1, and IgG2a were measured using ELISA. Neither the nonsensitized/challenged nor the nonsensitized/nonchallenged mice showed any detectable IgE in their sera (data not shown). The Ni-Ti alloy sensitized/Ni-challenged GATA-3 Tg mice showed significantly higher serum IgE concentrations than the Ni-Ti alloy sensitized/Ni-challenged WT mice (fig. 2a). On the other hand, the serum concentrations of IgG1 and IgG2a were comparable in the Ni-Ti alloy sensitized/Ni-challenged GATA-3 Tg and WT mice in this experiment (fig. 2a).

Cytokine Production in the Ear Tissue of the Ni-Challenged GATA-3 Tg Mice

The measurement of IL-4 in the supernatant obtained from the skin tissue of the Ni-Ti alloy sensitized/Ni-challenged GATA-3 Tg mice and WT mice revealed significantly higher levels of the cytokine in the ear-tissue supernatant obtained from the Ni-Ti alloy sensitized/Ni-challenged GATA-3 Tg mice (fig. 2b). The extracts were prepared using samples from the ears of the Ni-challenged GATA-3 Tg or WT mice obtained 30 days after the implantation of the Ni-Ti alloy. The IL-4 level in the Ni-Ti alloy sensitized/Ni-challenged GATA-3 Tg mice was twice as high as that in the control WT mice. In contrast, no changes in the levels of IL-5 or IL-13 were observed in the ear-tissue supernatant of either the Ni-Ti alloy sensitized/Ni-challenged GATA-3 Tg or WT mice (fig. 2b), although the IFN-γ levels were significantly lower in the Ni-Ti alloy sensitized/Ni-challenged GATA-3 Tg mice than in the Ni-Ti alloy sensitized/Ni-challenged WT mice (fig. 2b).

Histopathology of the Ni-Induced Contact Hypersensitivity Reaction in GATA-3 Tg Mice

Since the ACD to Ni in the GATA-3 Tg mice increased at 48–72 h after the challenge, we histologically examined the ear skin of the GATA-3 Tg and WT mice sensitized with the Ni-Ti alloy. The results showed severe edema in the challenged skin of the GATA-3 Tg mice (fig. 3a), but

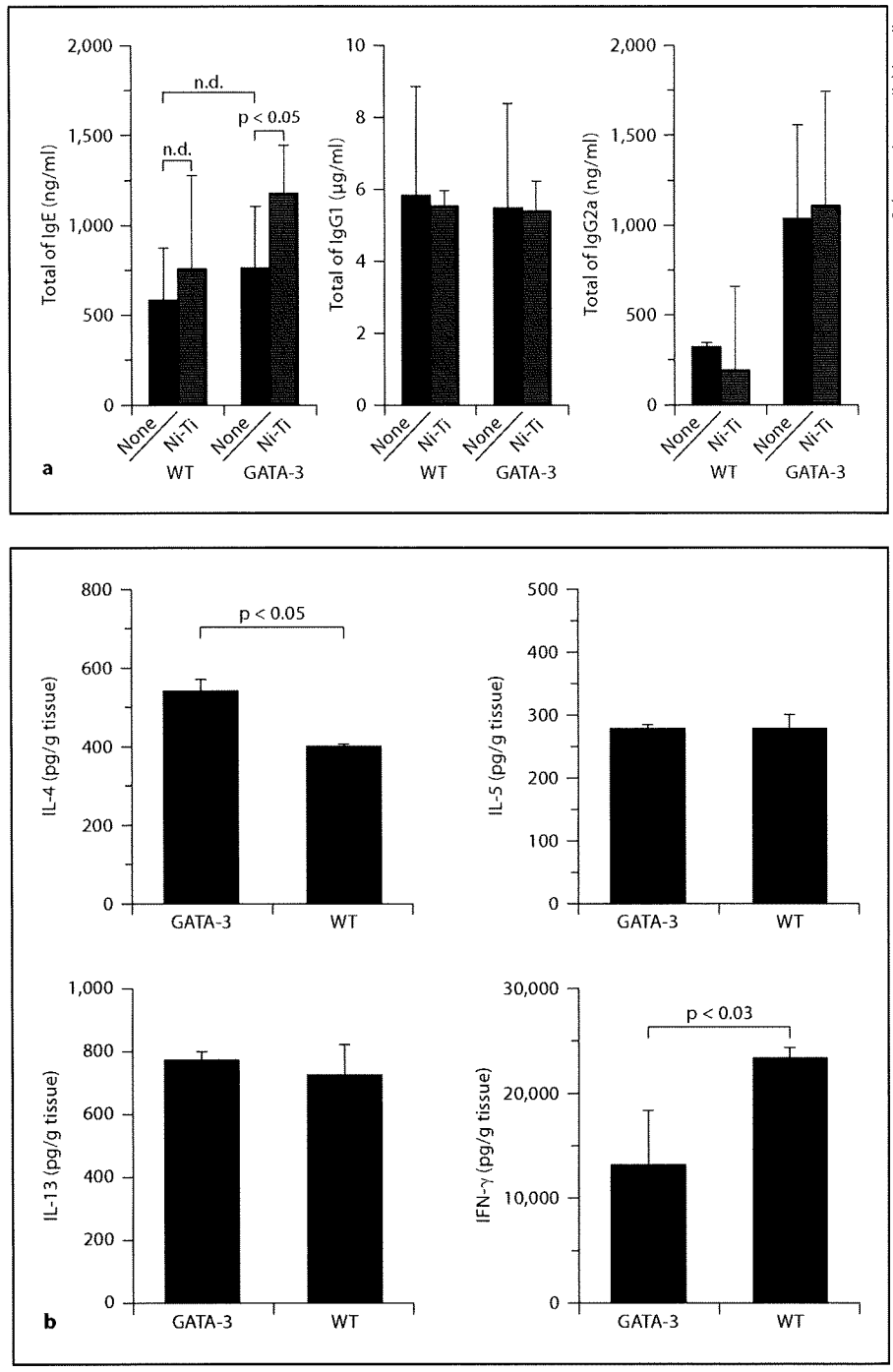

Fig. 2. Immunological response of the Ni-Ti alloy sensitized GATA-3 Tg mice to a Ni challenge. **a** Serum IgE, IgG1 and IgG2a levels in pre-immunized GATA-3 Tg and WT mice and 16 h after a Ni challenge in sensitized GATA-3 Tg and WT mice. No significant difference in the serum level of IgG1 or IgG2a was noted between the Ni-Ti alloy-sensitized/Ni-challenged GATA-3 Tg and WT mice. **b** Cytokine levels in the skin tissue supernatants in the Ni-challenged GATA-3 Tg and WT mice. Data are the means ± SD in 5 mice per group and are representative of 3 independent experiments. $p < 0.05$ vs. Ni-challenged WT mice.

the edema was not intense in the WT mice (fig. 3b). A very severe inflammatory response with intense infiltration by mononuclear cells, neutrophils and mast cells was observed in the GATA-3 Tg mice, whereas only a mild inflammatory response with little infiltration by mononuclear cells and neutrophils was observed in the challenged skin of the WT mice. When the number of mast cells infiltrating the dermis was evaluated by staining the tissue specimens with toluidine blue, the number in the Ni-Ti alloy sensitized/Ni-challenged GATA-3 Tg mice (24 ± 2.7) was 3-fold higher than that in the control WT mice (7 ± 5.0) (fig. 3c).

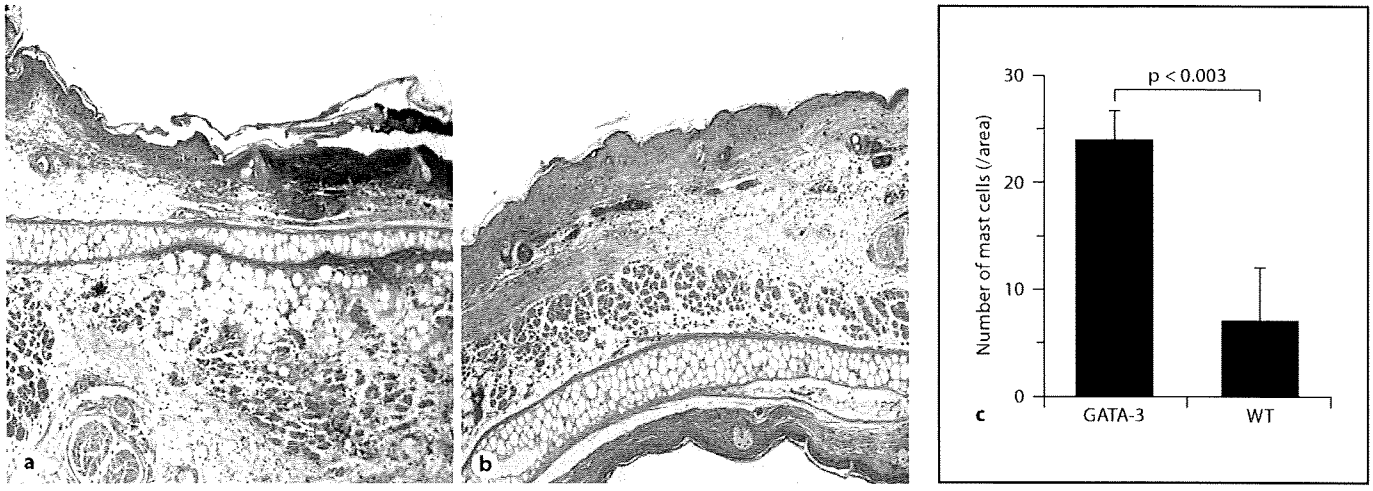

Fig. 3. Histological examination. Ear skin specimens were excised and fixed in 10% formalin, then processed and stained with hematoxylin and eosin. **a** The dermis of the challenged skin in the GATA-3 Tg mice showed a very severe inflammatory response, with infiltration by mononuclear cells, neutrophils and mast cells. **b** WT mouse. **c** The number of mast cells infiltrating the dermis was evaluated by staining the tissue specimens with toluidine blue. The sections were examined at a magnification of ×400. At least 10 fields per section were examined for each lobe. The cells were counted and expressed as the number of cells per square millimeter area. The bars represent the number of mast cells. Data are the means ± SD of the counts in groups of 10 mice per group. $p < 0.003$ vs. Ni-challenged WT mice. The photographs of the ears were taken 2 days after the antigen challenge.

Effect of GATA-3 Tg-Purified CD4+ T Cells in Transferring the Ni-Induced Contact Hypersensitivity Reaction

Rag-$2^{-/-}$ mice were transfused with CD4+ T cells from the Ni-Ti alloy-sensitized GATA-3 Tg mice or WT mice. Rag-$2^{-/-}$ mice transfused with CD4+ splenic cells isolated from the Ni-Ti alloy sensitized GATA-3 Tg mice showed significantly greater ear swelling responses than the WT mice at various time-points after the challenge. The peak ear swelling response was observed at 48–72 h after the Ni challenge in both the GATA-3 Tg mice and WT mice (fig. 4a). These results suggest that the CD4+ splenic T cells of the Ni-Ti alloy sensitized GATA-3 Tg mice regulated the ear swelling response more significantly than the CD4+ splenic T cells from the Ni-Ti alloy sensitized WT mice. After the CD4+ splenic T cells derived from non-Ni-Ti alloy sensitized GATA-3 or WT mice were transfused into Rag-$2^{-/-}$ mice, the ear swelling response following challenge with Ni solution followed a similar time-course to that in the Rag-$2^{-/-}$ mice not infused with the cells (data not shown).

Indirect Immunohistochemical Staining for CD4+

Immunohistochemical staining showed severe infiltration by CD4+ cells in the ear skin lesions of the Rag-$2^{-/-}$ mice infused with the CD4+ splenic T cells isolated from the Ni-Ti alloy sensitized GATA-3 Tg mice, but not in the Rag-$2^{-/-}$ mice infused with CD4+ T cells isolated from Ni-Ti alloy sensitized WT mice (fig. 4b).

Reduced Ear Swelling Response to Ni Challenge and Reduced Serum IgE Level in GATA-3 Tg Mice Sensitized with the Ni-Ti (#500) Test Piece Subjected to Oxygen Diffusion Processing

The ear swelling response to the implantation of a Ni-Ti (#500) test piece subjected to oxygen diffusion processing was about 1/30th to 1/20th of that elicited in response to the implantation of an untreated Ni-Ti test piece (fig. 5). The IgE antibody in the serum of the treated Ni-Ti (#500) test piece-implanted GATA-3 Tg mice was about 60% lower than in the GATA-3 Tg mice implanted with the untreated test piece (table 1).

Degree of Ni Ion Elution from the Oxygen Diffusion-Processed Ni-Ti Alloy

The degree of Ni ion elution from the untreated Ni-Ti test piece was 2–2.5 times greater than that from the Ni-Ti (#500) test piece subjected to oxygen diffusion processing (fig. 6). This finding suggested a correlation between the solubility of the Ni ion and the degree of the ear swelling response.

Discussion

Contact hypersensitivity reactions are mainly thought to be associated with the activation of Th1 cells. However, since evidence of the involvement of Th2 cells and Th2 cytokines has also been reported in the development of contact hypersensitivity [23, 24], in this study, we investigated whether Th2-type immune responses might also be involved in the pathogenesis of ACD. We recently established a GATA-3 Tg mouse model to study the role of Th2-type immune responses in the absence of the suppression of Th1-type immune responses [19]. In this study, we used the GATA-3 Tg mouse model to study the role of the Th2 cytokines IL-4, IL-5, and IL-13 in the development of ACD. At 48–72 h after antigen challenge, the ear swelling response in the GATA-3 Tg mice was significantly greater than that in the WT mice, and the maximal ear swelling response persisted until 320 h after the challenge. The kinetics of the ear swelling responses in the GATA-3 and WT mice were similar, but the magnitude of the response was about three-fold greater in the GATA-3 Tg mice. We also assessed, in another study, the development of 2,4,6-trinitrochlorobenzene-induced contact dermatitis in the GATA-3 Tg mice. Our results also revealed the involvement of Th2-type immune reactions in 2,4,6-trinitrochlorobenzene-induced contact dermatitis, consistent with the results of the present study. The ear swelling reaction in the aforementioned study, however, ended at 48 h after stimulation with the antigen, unlike in the present study. In the experiment involving CD4+ cell implantation into Rag-2$^{-/-}$ mice, the response ended at 48 h after the challenge, consistent with other results of the present study. The reasons for the differences are not clear, but the following two explanations should be considered: (1) the number of the cells implanted was not adequate to induce Th1-type cytokine production, or (2) the antigen test substance was present in vivo even during the determination of the responses in the present experiment. Taking into consideration the adequate production of IFN-γ in the ear tissue at 48 h after stimulation with the antigen, however, the persistent in vivo existence of the antigen used for sensitization was considered to be the major reason for the difference in the duration of the response. Further detailed studies are, however, needed to solve this question. Clarifying this difference is also important from a clinical standpoint and for understanding the possibility of differences in the duration of responses among patients with the same disease, namely, metallic allergy. Our results for the kinetics of ear swell-

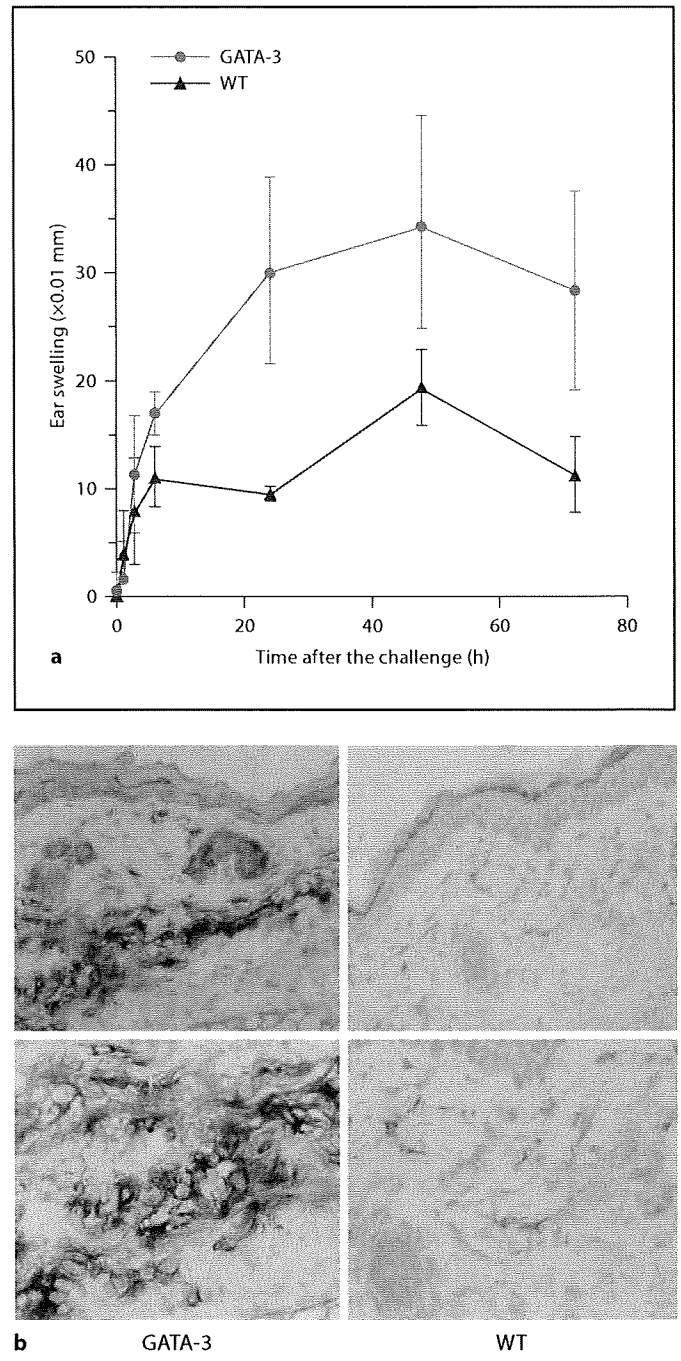

Fig. 4. Immune response of Rag-2$^{-/-}$ mice implanted with CD4+ cells isolated from the spleen of the Ni-Ti sensitized GATA-3 Tg mice. **a** The increases in the ear skin thickness of the Rag-2$^{-/-}$ mice implanted with CD4+ splenic cells isolated from the GATA-3 Tg or WT mice were measured at 0.5, 1, 3, 24, 48 and 72 h after Ni challenge. Data are the means ± SD of 5 animals per group and are representative of three independent experiments. $p < 0.05$ vs. WT mice. **b** Sections of Ni-challenged ears from the Rag-2$^{-/-}$ Ni-Ti alloy sensitized GATA-3 Tg and WT mice and nontreated Rag-2$^{-/-}$ mice were analyzed by staining for anti-mouse CD4. The sections were observed at magnifications of ×200 and ×400.

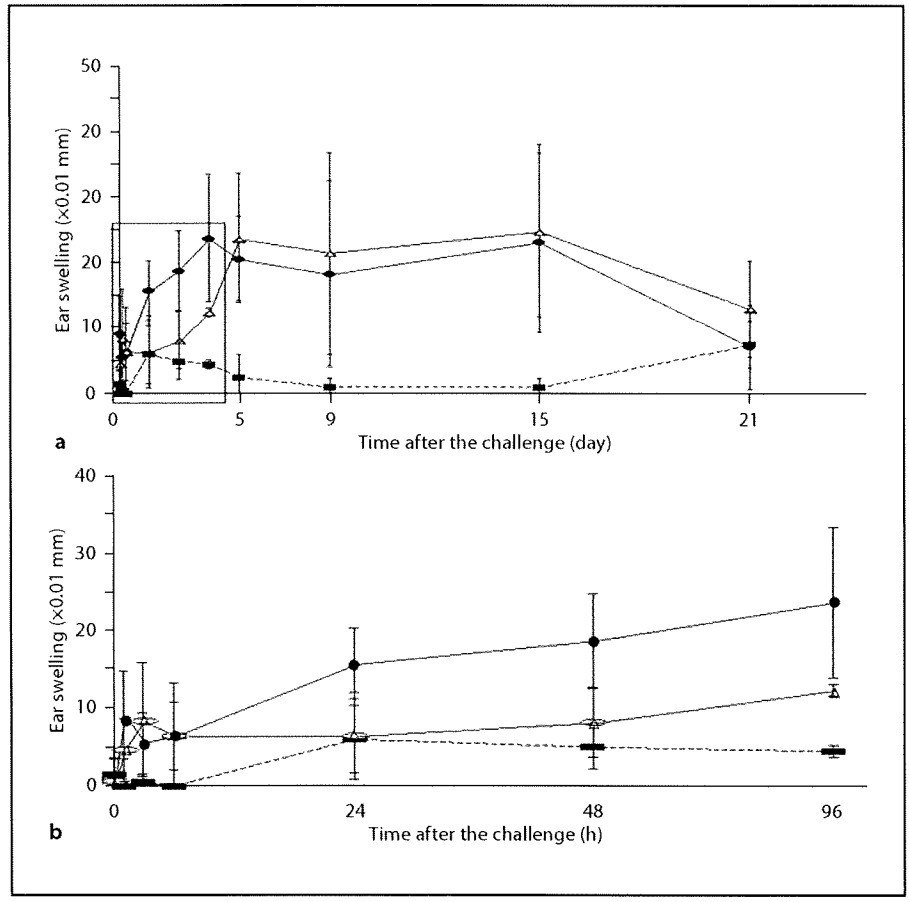

Fig. 5. Immune response (ear swelling) in GATA-3 Tg mice transplanted with the Ni-Ti (#500) test piece. The ear skin thickness of the GATA-3 Tg mice implanted with the Ni-Ti (#500) test piece subjected to oxygen diffusion processing was measured at the time-points indicated after the subcutaneous injection of 10 μl of a solution of Ni (1,000 ppm) into the left ear and 10 μl of saline into the right ear. The differences in the thickness between the right and left ears after the challenge are shown. Data are the means ± SD of 5 animals per group and are representative of 3 independent experiments. $p < 0.05$ vs. WT mice implanted with nontreated Ni-Ti alloy. ● = GATA-3 Tg mice implanted with non-treated Ni-Ti alloy; □ = WT mice implanted with nontreated Ni-Ti alloy; ■ = GATA-3 Tg mice implanted with the Ni-Ti alloy subjected to oxygen diffusion.

Table 1. Serum IgE levels in the GATA-3 Tg mice implanted with the Ni-Ti (#500) test piece subjected to oxygen diffusion processing

Ni-Ti (treatment)	Total IgE, ng/ml	Reduction, %
–	762 ± 343.7	
+ (none)	1,489.8 ± 843.1	0
+ (#500)	605.3 ± 592.0	59.3

Data are means ± SD for groups of 5 mice and are representative of 3 independent experiments.

$p < 0.05$ vs. GATA-3 mice not implanted with the test piece.

ing in the GATA-3 Tg mice after antigen challenge reflected a Th2-type immune response (IL-4) to the Ni-Ti alloy sensitization as well as a Th1-type response (IFN-γ) suppressed by the Th2-type response. These findings suggest that while both Th1- and Th2-mediated immune responses were key factors in the development of the ACD in the GATA-3 Tg mice, the Th2 cytokines (for example IL-4) were particularly important for the induction of ACD in this model. The Th1-type immune response is also thought to be ultimately important for the development of dermatitis induced by Ni. In every one of our experimental systems, the amount of IL-4 produced in vivo was only 1/40 to 1/20 of the amount of IFN-γ produced. Since the amount of IL-4 produced was markedly lower than that of IFN-γ, the effects of the former could also be expected to be weaker. However, the effects of the cytokines became clear only after a comparison of not only the amount of production, but also the functions of IL-4 and IFN-γ. In the present study, the level of IL-4 produced in the GATA-3 Tg mice was approximately twice that produced in the WT mice at 48 h after stimulation with Ni solution, and the level of IFN-γ produced in the same mice was half of that in the WT mice. However, from 96 h onward after the stimulation, the tissue production of IFN-γ was sustained at 20,000–

25,000 pg/g tissue until the reaction ended, while the production of IL-4 decreased markedly in both the GATA-3 Tg mice and WT mice.

As shown in figure 4a, the Rag-2$^{-/-}$ mice infused with the CD4+ splenic cells isolated from the GATA-3 Tg mice showed marked ear swelling. The following mechanisms underlying this result have been proposed: (1) the induction of numerous memory cells to the spleens of the GATA-3 Tg mice, or (2) a higher activity level of the CD4+ splenic cells in the GATA-3 Tg mice than in the WT mice following Ni challenge, etc. However, the exact mechanisms remain unclear at the present time, and this issue will require further detailed studies in the future. Histopathological examination of the site of inflammation showing infiltration by the CD4+ cells and mast cells suggested that a Th2-type immune response was also secondarily involved in the development of the ACD. We confirmed the accumulation of the CD4+ splenic cells in the Ni-Ti alloy sensitized GATA-3 Tg mice following the Ni challenge, with an approximately four-fold higher number of these cells in the GATA-3 Tg mice than in the WT mice (data not shown). The results of the histopathological examination of the GATA-3 Tg mice also indicated that the decrease in IFN-γ production promoted fibrosis. These observations suggest that the Th1- type response was suppressed by the increase in IL-4 production as a part of the Th2-type immune response. IL-4 is known to be produced not only by T cells, but also by natural killer T cells, basophilic cells and mast cells. However, the results of an experimental system using the Rag-2$^{-/-}$ mice in this study showed the close involvement of the CD4+ splenic cells in the immediate-phase responses (24–48 h) after stimulation with the Ni solution in the Ni-Ti alloy sensitized GATA-3 Tg mice. This finding indicates that the T cells are the main IL-4-producing cells, even though this cytokine might also have been produced by other cells. In another experimental system using the lck distal promoter with the aim of specifically expressing transgenes in the T cells, a marked CD4+ cell response was seen in the GATA-3 Tg mice, indicating the major importance of the T cells. In the GATA-3 Tg mice, IL-4 production was significantly promoted by stimulation with the Ni solution, resulting in the suppression of IFN-γ production by the action of the former on macrophages. The production of IL-4 itself is considered to decrease with time, leading to the elimination of its suppressive effect on IFN-γ production. As a result, the levels might reach almost the same levels as those seen in WT mice over time, and IFN-γ and IL-4 might show similar time-course changes. The decrease

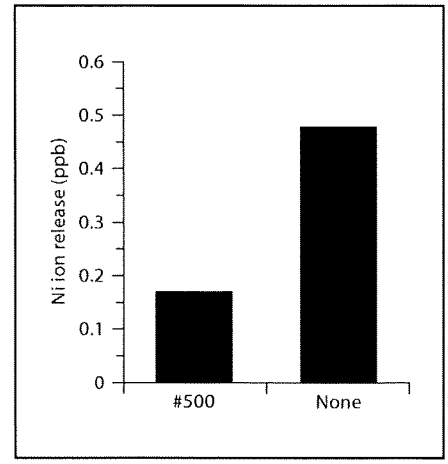

Fig. 6. Examination of Ni ion elution after oxygen diffusion processing. The test piece was subjected to oxygen diffusion processing and soaked in 3% NaCl solution maintained at 37°C for 28 days. The density of the eluted Ni ions in the solution was then measured using atomic absorption photometry.

is regarded as a specific reaction occurring in part as a result of the stimulation with the Ni solution, since no inflammatory cell infiltration was recognized in a histopathological examination of the tissues after Ni-Ti alloy implantation performed after a challenge with the Ni solution.

An investigation of the cytokines in the tissues showed that the immediate-phase responses in the Ni-Ti alloy-implanted GATA-3 Tg mice were in fact Th2-type immune reactions. We think that with time, the Th1-type immune reactions also contributed to the production of a major swelling reaction, because both GATA-3 Tg mice and WT mice started showing similar swelling reactions by 96 h after stimulation with Ni. Pathological examination of the tissues after the reactions subsided revealed marked fibrosis of the ear tissue in the GATA-3 Tg mice, which showed significant IL-4 production during the immediate-phase responses (data not shown). In the WT mice, which showed a significantly larger production of IFN-γ than of IL-4, no fibrotic changes were observed, and the changes were restored to the findings observed in the normal tissues. These data indicate that the IL-4 produced during the immediate-phase responses induces dermal fibrosis and that its production is suppressed by IFN-γ. The extent of the participation of the Th2-type immune reactions in the development of allergy has been suggested to influence the prognosis of Ni-induced allergy. Under this circumstance, the use

of Th2-type immune reactions as an indicator might influence the treatment policies used for subjects with metal allergies. Some reports have shown that IL-4 is important for the occurrence of fibrosis of the liver during infection with parasites and bleomycin-induced pneumonia [25, 26]. A previous report has also shown that IL-13 is more important than IL-4 [27]; however, no difference in IL-13 production was observed between the GATA-3 Tg mice and the WT mice in the present study, suggesting that IL-4 production plays a major role in the development of fibrosis in the present experimental system.

Th2 cell differentiation is mediated by signal transducer and activator of transcription (STAT) 6 activation induced by the engagement of the IL-4 with its receptor [28–30]. Asherson et al. [31] reported that IL-4 plays an important role in the pathogenesis of contact hypersensitivity. They speculated that the IL-4 secreted by mast cells or $B220^+$ cells binds to γ/δ T cells and induces these cells to secrete more IL-4 or other cytokines that increase the expression of adhesion molecules on the endothelial cells, which in turn are believed to play a critical role in the induction of contact hypersensitivity. In contrast, Berg et al. [32] demonstrated the induction of contact hypersensitivity to 4-ethoxyl methylene-2-phenyl-2-oxazolin-5-one (Oxa) in IL-4-deficient mice. Traidl et al. [33] found that the complete loss of endogenous IL-4 expression in BALB/C mice was associated with an impaired manifestation of contact hypersensitivity to 2,4-dinitrochlorobenzene, but not to Oxa. Yokozeki et al. [23] recently established a strain of STAT6-deficient mice and demonstrated that STAT6 plays a central role in IL-4- and IL-13-mediated biological responses. They found that the contact hypersensitivity responses to 2,4-dinitrochlorobenzene and Oxa were significantly attenuated in STAT6-deficient mice and that the peak response was also delayed. The STAT6 signal induces the expression of the transcription factors GATA-3 and c-Maf [34], which are reportedly selectively expressed in a Th2-specific fashion [14, 15, 30]. In a recent study of STAT6-deficient cells, it was shown that although IL-4 and STAT6 signaling might initially direct the development of the Th2-type response, GATA-3 and c-Maf are also capable of inducing a stable Th2 commitment, independent of STAT6 [35]. In vitro differentiation into Th2 cells induces the remodeling of the IL-4/IL-5/IL-13 locus. GATA-3 plays an essential role in the earliest stages of T-cell development [36, 37] and has also been recognized as a Th2 differentiation factor [16]. GATA-3 has been detected in naive CD4+ T cells, and its expression level continues to increase substantially during Th2 differentiation [14, 15]. GATA-3 expression is reportedly indispensable for Th2 development and is down-regulated in response to IL-12-mediated STAT4 activation [14, 38]. GATA-3 strongly transactivates the IL-5 and IL-13 promoters but appears to have only limited effects on IL-4 gene transcription [14, 17, 38]. The results of experiments conducted to date suggest the possibility that the GATA-3 gene expression level varies with the amount of the antigen, the method of immunity induction, and the duration of stimulation, although it is not clear whether the gene controls the level of IL-4 production. The results of the present experiment are consistent with the aforementioned suggestions.

In the present study, we demonstrated that Th2 cells and Th2-type cytokines are involved in the pathogenesis of the Ni-induced ACD response. In the GATA-3 Tg mice, the late-phase ear swelling response to a Ni challenge was significantly more pronounced than the immediate-phase ear swelling response. At the site of the challenge in the GATA-3 Tg mice, the local production of IL-4 was increased and INF-γ production was suppressed. These findings suggest the involvement of an IL-4-dominant Th2 response in the development of ACD to Ni. This murine model is characterized by the predominant production of IgE antibody and no IgG1 or IgG2a production, and the induction of the ACD to Ni was markedly suppressed when a Ni-Ti alloy subjected to oxygen diffusion processing was implanted. Whether the antibody titers were Ni-specific remains uncertain. However, the IgE antibody titers did not increase following the transplantation of the Ni-Ti alloy test piece in the GATA-3 Tg mice. In recent years, many reports have shown that allergens not only induce antigen-specific IgE antibody production, but also act like a Th2-type adjuvant, inducing non-antigen-specific immune reactions [39, 40]. Under this circumstance, Ni might represent a nonspecific response to Th2-type adjuvant in Ni-induced ACD, although the mechanism remains unclear. However, we believe that Ni-specific antibody was involved. These results suggest that Th2 cells and Th2 cytokines are important for the ear swelling response during the late phase of the development of ACD. The GATA-3 Tg murine model might be useful for increasing our understanding of the physiological significance of the Th2 cells and Th2 cytokines in ACD induced by Ni. When a Ni-Ti alloy subjected to oxygen diffusion processing was used, the ear swelling response was suppressed, indicating that the ear swelling response is increased independent of the concentration of

the Ni ions transplanted into the living body and that the suppression of the response is an indicator of the decrease in the allergy-inducing activity of the contact sensitizer. These findings suggest that the use of the GATA-3 Tg mice established by us may be of great use in the development of biomaterials with low allergy-inducing activity.

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References

1 Grabbe S, Schwarz T: Immunoregulatory mechanisms involved in elicitation of allergic contact hypersensitivity. Immunol Today 1998;19:37–44.
2 Nielsen NH, Menne T: Allergic contact sensitization in an unselected Danish population. The Glostrup Allergy Study, Denmark. Acta Derm Venereol 1992;72:456–460.
3 Liden C: Occupational contact dermatitis due to nickel allergy. Sci Total Environ 1994; 148:283–285.
4 Brasch J, Geier J: Patch test results in schoolchildren. Results from the Information Network of Departments of Dermatology (IVDK) and the German Contact Dermatitis Research Group (DKG). Contact Dermatitis 1997;37:286–293.
5 Borg L, Christensen JM, Kristiansen J, Nielsen NH, Menne T, Poulsen LK: Nickel-induced cytokine production from mononuclear cells in nickel-sensitive individuals and controls. Cytokine profiles in nickel-sensitive individuals with nickel allergy-related hand eczema before and after nickel challenge. Arch Dermatol Res 2000;292:285–291.
6 Sinigaglia F, Scheidegger D, Garotta G, Scheper R, Pletscher M, Lanzavecchia A: Isolation and characterization of Ni-specific T cell clones from patients with Ni-contact dermatitis. J Immunol 1985;135:3929–3932.
7 Silvennoinen-Kassinen S, Poikonen K, Ikaheimo I: Characterization of nickel-specific T cell clones. Scand J Immunol 1991;33:429–434.
8 Kapsenberg ML, Wierenga EA, Stiekema FE, Tiggelman AM, Bos JD: Th1 lymphokine production profiles of nickel-specific $CD4^+$ T-lymphocyte clones from nickel contact allergic and non-allergic individuals. J Invest Dermatol 1992;98:59–63.
9 Probst P, Kuntzlin D, Fleischer B: TH2-Type infiltrating T cells in nickel-induced contact dermatitis. Cell Immunol 1995;165:134–140.
10 Werfel T, Hentschel M, Kapp A, Renz H: Dichotomy of blood- and skin-derived IL-4-producing allergen-specific T cells and restricted V beta repertoire in nickel-mediated contact dermatitis. J Immunol 1997;158: 2500–2505.
11 Yokozeki H, Watanabe K, Igawa K, Miyazaki Y, Katayama I, Nishioka K: γδT cells assist αβT cells in the adoptive transfer of contact hypersensitivity to para-phenylenediamine. Clin Exp Immunol 2001;125:351–359.
12 Pandolfi PP, Roth ME, Karis A, Leonard MW, Dzierzak E, Grosvels FG, Engel JD, Lindenbaum MH: Targeted disruption of the GATA-3 gene cause severe abnormalities in the nervous system and in fetal live hematopoiesis. Nat Genet 1995;11:40–44.
13 Nawijn MC, Ferreira R, Dingjan GM, Kahre O, Drabek D, Karis A, Grosveld F, Hendriks RW: Enforced expression of GATA-3 during T cell development inhibits maturation of CD8 single-positive cells and induces thymic lymphoma in transgenic mice. J Immunol 2001;167:715–723.
14 Zheng WP, Flavell RA: The transcription factor GATA-3 in necessary and sufficient for Th2 cytokine gene expression in CD4 T cells. Cell 1997;89:587–596.
15 Zhang DH, Cohn L, Ray P, Bottomly K, Ray A: Transcription factor GATA-3 is differentially expressed in murine Th1 and Th2 cells and controls Th2-specific expression of the interleukin-5 gene. J Biol Chem 1997;272: 21597–21603.
16 Farrar JD, Ouyang W, Lohning M, Assenmacher M, Radbruch A, Kanagawa O, Murphy KM: An instructive component in T heoper cell type 2 (Th2) development mediated by GATA-3. J Exp Med 2001;193:643–650.
17 Zhang DH, Yang L, Ray A: Differential responsiveness of the IL-5 and IL-4 gene to transcription factor GATA-3. J Immunol 1998;161:3817–3821.
18 Ferber IA, Lee HJ, Zonin F, Heath V, Mui A, Arai N, O'Garra A: GATA-3 significantly downregulates IFN-gamma production from developing Th1 cells in addition to inducing IL-4 and IL-5 levels. Clin Immunol 1999;91:134–144.
19 Tamauchi H, Terashima M, Ito M, Maruyama H, Ikewaki N, Inoue M, Gao X, Hozumi K, Habu S: Evidence of GATA-3-dependent Th2 commitment during the in vivo immune response. Int Immunol 2004;16:179–187.
20 Ferguson T, Dube P, Griffith TS: Regulation of contact hypersensitivity by interleukin 10. J Exp Med 1994;179:1597–1674.
21 Ptak W, Geba GP, Askenase PW: Initiation of delayed-type hypersensitivity by low doses of monoclonal IgE antibody. Mediation by serotonine and inhibition by histamine. J Immunol 1991;146:3929–3936.
22 Matsuda H, Ushio H, Paliwal V, Ptak W, Askenase PW: Adoptive cell transfer of contact sensitivity-initiation mediated by non-immune cells sensitized with monoclonal IgE antibodies. J Immunol 1995;154:5080–5092.
23 Yokozeki H, Ghoreishi M, Takagawa S, Takayama K, Satoh T, Katayama I, Takeda K, Akira S, Nishioka K: Signal transducer and activator of transcription 6 is essential in the induction of contact hypersensitivity. J Exp Med 2000;194:995–1004.
24 Muller KM, Jaunin F, Masouye I, Saurat JH, Hauser C: Th2 cells mediate IL-4-dependent local tissue inflammation. J Immunol 1993; 150:5576–5584.
25 Gharaee-Kermani M, Nozaki Y, Hatano K, Phan SH: Lung interleukin-4 gene expression in a murine model of bleomycin-induced pulmonary fibrosis. Cytokine 2001; 15:138–147.
26 Huaux F, Liu T, McGarry B, Ullenbruch M, Phan SH: Dual roles of IL-4 in lung injury and fibrosis. J Immunol 2003;170:2083–2092.
27 Fallon PG, Richardson EJ, McKenzie GJ, McKenzie ANJ: Schistosome infection of transgenic mice defines distinct and contrasting pathogenic roles for IL-4 and IL-13: IL-13 is a profibrotic agent. J Immunol 2000; 164:2585–2591.
28 Takeda K, Tanaka T, Shi W, Matsumoto M, Minami M, Kashiwamura S, Nakanishi K, Yoshida N, Kishimoto T, Akira S: Essential role of Stat6 in IL-4 signalling. Nature 1996; 380:627–630.
29 Shimoda K, van Deursen J, Sangster MY, Sarawar SR, Carson RT, Tripp RA, Chu C, Quelle FW, Nosaka T, Vignali DAA, Doherty PC, Grosveld G, Paul WE, Ihle JN: Lack of IL-4-induced Th2 response and IgE class switching in mice with disrupted Stat6 gene. Nature 1996;380:630–633.

30 Ho IC, Lo D, Glimcher LH: c-Maf promotes T helper cell type 2 (Th2) and attenuates Th1 differentiation by both interleukin 4-dependent and -independent mechanisms. J Exp Med 1998;188:1859–1866.
31 Asherson GL, Dieli F, Sireci G, Salerno A: Role of IL-4 in delayed type hypersensitivity. Clin Exp Immunol 1996;103:1–4.
32 Berg DJ, Leach MW, Kuhn R, Rajewsky K, Muller W, Davidson NJ, Rennick D: Interleukin 10 but not interleukin 4 is a natural suppressant of cutaneous inflammatory responses. J Exp Med 1995;182:99–108.
33 Traidl C, Jugert F, Krieg T, Merk H, Hunzelmann N: Inhibition of allergic contact dermatitis to DNCB but not to oxazolone in interleukin-4-deficient mice. J Invest Dermatol 1999;112:476–482.
34 Kurata H, Lee HJ, O'Garra A, Arai N: Ectopic expression of activated STAT-6 induces the expression of Th2-specific cytokines and transcription factors in developing Th1 cells. Immunity 1999;11:677–688.
35 Ouyang W, Lohning M, Gao Z, Assenmacher M, Ranganath S, Radbruch A, Murphy KM: Stat6-independent GATA-3 autoactivation directs IL-4-independent Th2 development and commitment. Immunity 2000;12:27–37.
36 Ting CN, Olson MC, Barton KP, Leiden JM: Transcription factor GATA-3 is required for development of the T-cell lineage. Nature 1996;384:474–478.
37 Hattori N, Kawamoto H, Fujimoto S, Kuno K, Katsura Y: Involvement of transcription factors TCF-1 and GATA-3 in the initiation of the earliest step of T cell development in the thymus. J Exp Med 1996;184:1137–1147.
38 Lavenu-Bombled C, Trainor CD, Makeh I, Romeo PH, Max-Audit I: Interleukin-13 gene expression is regulated by GATA-3 in T cells: Role of a critical association of a GATA and two GATG motifs. J Biol Chem 2002;277:18313–18321.
39 Uemura Y, Liu T-Y, Narita Y, Suzuki M, Matsushita S: 17β-estradiol (E2) plus tumor necrosis factor-α induces a distorted maturation of human monocyte-derived dendritic cells and promotes their capacity to initiate T-helper 2 responses. Hum Immunol 2008;69:149–157.
40 Trompette A, Divanovic S, Visintin A, Blanchard C, Hegde RS, Madan R, Thorne PS, Wills-Karp M, Gioannini TL, Weiss JP, Karp CL: Allergenicity resulting from functional mimicry of a Toll-like receptor complex protein. Nature 2009;457:585–588.