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Short communication

Inhibitory function of NKT cells during early induction phase of nickel allergy

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ABSTRACT

Until now, metal allergies have been regarded as a Th1-type immune response. However, because the contribution of a Th2-type immune response has been suggested by clinical findings, we previously examined the Th2-type immune response during the development of metal allergies using a GATA-3 transgenic (GATA-3 Tg) mouse model. As a result, a Th2-type immunization reaction was suggested to be involved in the early phase of metal allergies.

Recently, the involvement of NKT cells in metal allergies has been suggested. We examined this possibility using the activation of NKT cells and an NKT cell-deficient mouse model to determine the contribution of NKT cells to nickel allergy in the present study. In NKT cell-deficient mice, ear swelling was remarkably increased, compared with that in control mice. Also, in mice that had been treated with α-galactosylceramide (α-GalCer) to activate NKT cells, the ear swelling response was remarkably inhibited, compared with that in untreated mice. These facts show that NKT cells are involved in the inhibition of nickel allergy-induced ear swelling responses.

1. Introduction

Allergies to nickel (Ni) are thought to be mainly mediated by Th1-type immune responses. Earlier studies have suggested that the delayed-type hypersensitivity reaction to Ni in humans involves mainly IFN-γ-producing T cells, but subsequent studies of Ni-specific T cell clones have shown the involvement of mixed Th1-type and Th2-type cytokine responses in this condition. For these reasons, we further examined this response using GATA-3 transgenic (GATA-3 Tg) mice to determine whether not only the Th1-type immune response, but also the Th2-type immune response is involved in Ni allergies. The transcription factor GATA-3 is selectively expressed in the T cell lineage from the early stage of the development of the thymus. The exclusive expression of GATA-3 in Th2 cells is thought to play an important role in Th2-specific functions and/or cytokine gene expression. We previously demonstrated that the exposure of actively sensitized GATA-3 Tg mice to ovalbumin aerosol increased the expressions of IL-5 and IL-13, the number of eosinophils in bronchoalveolar lavage fluid, and the serum IgE levels. As a result, the Th2-type immune response of $CD4^+$ cells was shown to be involved during the early phase of Ni allergy induction. However, we examined the contribution of NKT cells to Ni allergy in the present study because the transcription factor GATA-3 has been suggested to be associated with the differentiation of NKT cells (Cen et al., 2009; Kim et al., 2006).

The CD1 protein family is comprised of Ag-presenting molecules found on the cell surface of mainly hematopoietic-derived cells (Brossay et al., 1997). Based on shared sequence homology, the five types of CD1 molecules are divided into group 1 (CD1a, CD1b, CD1c) and group 2 (CD1d) (Behar et al., 1999; Calabi et al., 1989), with CD1e not being classified in either group because CD1e is present in a soluble form (de la Salle et al., 2005). The group 1CD1 molecules

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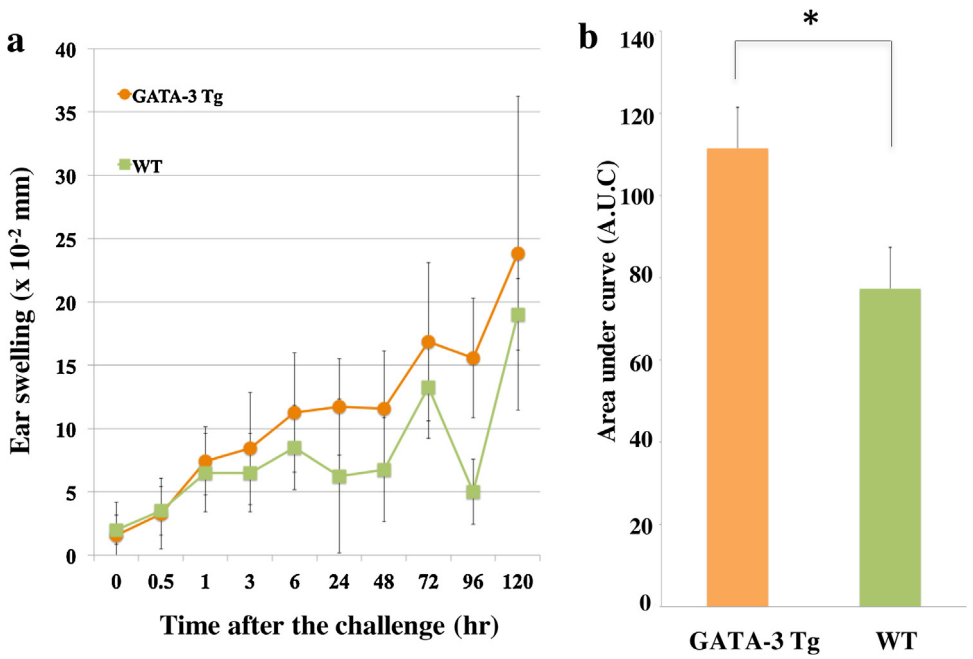

Fig. 1. GATA-3 Tg mice exhibit enhanced Ni-induced allergic contact dermatitis. (a) The ear skin thickness of Ni-sensitized GATA-3 Tg and WT mice was measured at the indicated times after the subcutaneous injection of 10 μL of a Ni solution (1000 ppm) into the left ear and 10 μL of a saline solution into the right ear. The differences in the thicknesses between the right and left ears after the challenge are shown. The data are the mean ± SD of 5 animals per group and are representative of 3 independent experiments. (b) The area under the curve (AUC) for ear swelling in the GATA-3 Tg and WT mice at 30 days was calculated and compared statistically.

are found in humans but not in mice, whereas group 2 (CD1d) is found in both species (Brossay et al., 1998). CD1d-restricted T cell populations have been described by many authors as being rather heterogeneous. The most characterized is the invariant NKT cell (iNKT), which refers to the use of the invariant TCR α-chain (Vα14-Jα18 in mice and Vα24-Jα18 in humans) and the coexpression of NK markers, specifically NK1.1, on its surface. These cells have also been named type I NKTs (Godfrey et al., 2004).

Type I NKTs can be further subdivided into $CD4^+$ and $CD4^-CD8^-$ categories in mice, and in humans, a population of $CD8^+$ NKTs has been identified (Gumperz et al., 2002; Hammond et al., 1999; Hammond et al., 2001). The exact effect of CD4 on non-MHC class II-restricted cells is unclear, but different subpopulations seem to have different cytokine-secreting patterns post-stimulation. By investigating $CD4^+$ T cells in MHC class II-deficient mice, it was found that CD1d-dependent cells are thymically derived. They exhibit an activated, memory phenotype and add diverse TCR-bearing T cells to the CD1d-restricted population (Cardell et al., 1995). This NKT cell population with a variant TCR has since been referred to as type II CD1d-restricted NKTs (Godfrey et al., 2004). These type I NKTs respond strongly to stimulation with α-GalCer via CD1d, in contrast to type II NKTs (Makowska et al., 2000). This phenomenon has hindered research on type II NKTs; most of our present knowledge regarding the biological function of CD1d-restricted T cells originates from experiments on type I invariant NKT cell functions. The development of CD1d-restricted NKTs has been shown to be dependent on the expression of CD1d in the thymus; hence, this population is missing in targeted mutant mice lacking the expression of CD1d (Chen et al., 1997; Gapin et al., 2001; Adachi et al., 1995; Smiley et al., 1997). CD1d-restricted NKTs have been described as fast, potent cytokine producers; when stimulated in vitro, they rapidly release large quantities of cytokines, including IL-4 and IFN-γ (Kronenberg and Gapin, 2002; Sko ¨ld et al., 1999; Stetson et al., 2003; Yoshimoto and Paul, 1994).

NKT cells are a unique T cell subset expressing both TCR and NK cell receptors. Most NKT cells are restricted by the MHC class I-like molecule CD1d. These CD1d-restricted NKT cells are known to include two subsets: the better characterized, more widely studied subset expressing the canonical TCR, Vα14Jα18 (type I) NKT cells, and a less well-characterized subset of non-Vα14Jα18 (type II) NKT cells (Behar et al., 1999). In mice, most CD1d-restricted NKT cells are $CD4^+$, and the rest are $CD4^-$ $CD8^-$ (Arase et al., 1992). Because we could not rule out the possible contribution of NKT cells to the finding obtained using the GATA-3 Tg mice, we examined the contribution of NKT cells to the Ni allergy response using $CD1d^{-/-}$ mice and NKT cell-activated mice treated with α-GalCer. Thus, in the present study, we further examined the role of Th2-type immune-deviation in a Ni allergic contact dermatitis model, focusing on the T-cell subsets and invariant NKT cells.

2. Materials and methods

2.1. Mice

Male and female 8-week-old C57BL/6 mice (B6; Clea Japan Inc., Tokyo Japan), B6.$CD1d^{-/-}$ (NKT-deficient) mice (Mendiratta et al., 1997), and GATA-3 transgenic (GATA-3 Tg) mice (Tamauchi et al., 2004) were used. The mice were maintained on food and water *ad libitum* until they reached the desired weight (20–24 g) or age (8 weeks) under specific pathogen-free conditions. We used C57BL/6 mice (wild type; WT) as a control for the GATA-3 Tg and B6.$CD1d^{-/-}$ mice. All the experiments were approved by the Committee for Animal Experimentation at Kitasato University.

2.2. Contact sensitizing agent

The test wire was composed of a Ni-Ti alloy (Ni-49.2at%Ti; ACT-MENT Co., Ltd., Japan) that was processed to a wire with a length of 7 mm and a diameter of 1 mm. The Ni-Ti wire was polished to a washed surface using acetone and ethanol.