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Food Allergy to Caper (*Capparis spinosa*)

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Palabras clave: *Capparis spinosa*. Alcaparra. Alergia a alimentos. Mostaza. Pro-heveina. Hev b 6.

Capparis spinosa is a bush that belongs to the Capparaceae family, member of the Brassicales order, as well as the mustard family (Brassicaceae), whose ability to induce allergic symptoms has been well documented, with several allergens described [1,2]. *C spinosa* is typical of the Mediterranean region. Its buds and fruits are eaten pickled, as a snack, seasoning, or as part of sauces. Moreover, members of the Capparaceae family have numerous medical applications thanks to their antimicrobial, antioxidative, anti-inflammatory, immunomodulatory, and antiviral properties [3-5]. All the species of this family contain *iso*thiocyanate (mustard oil), and are therefore expected to irritate the skin [6]. There have been reports of contact dermatitis due to *C spinosa* [7], but to the best of our knowledge, the present study is the first to report allergy following the ingestion of caper fruit.

We describe a 24-year-old man who reported allergic symptoms after the ingestion of shellfish paella and caper fruit. He presented at the emergency department of Complejo Hospitalario in Jaén, Spain, with redness, angioedema of the face and hands, and aphonia. The patient was treated with methylprednisolone and dexchlorpheniramine. After several hours under observation, he was discharged with antihistamine and oral corticosteroid treatment.

The patient had previously been diagnosed as allergic to *Olea europaea* and grasses, and experienced rhinoconjunctivitis and moderate, persistent asthma in the spring. However, he had not previously presented allergic reactions to food.

The patient was skin-prick tested with a battery of standardized aeroallergens including grasses, *Salsola kali, Chenopodium album, Cupressus arizonica, Parietaria judaica, Ambrosia elatior, O europaea*, and *Platanus hybrida*, mites (*Dermatophagoides*), epithelia (cat and dog), mold (*Alternaria alternata*), *Anisakis simplex*, and latex (Bial-Aristegui). The results were positive for *Lolium perenne* (wheal diameter, 8 mm), *Cynodon dactylon* (4 mm), and *O europaea* (9 mm), and negative in all other cases. We also performed prick to prick tests with mustard, clam, shrimp, squid, caper bud, and caper fruit.

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A Novel Small Compound SH-2251 Suppresses Th2 Cell-Dependent Airway Inflammation through Selective Modulation of Chromatin Status at the *Il5* Gene Locus

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Abstract

IL-5 is a key cytokine that plays an important role in the development of pathological conditions in allergic inflammation. Identifying strategies to inhibit IL-5 production is important in order to establish new therapies for treating allergic inflammation. We found that SH-2251, a novel thioamide-related small compound, selectively inhibits the differentiation of IL-5-producing Th2 cells. SH-2251 inhibited the induction of active histone marks at the *Il5* gene locus during Th2 cell differentiation. The recruitment of RNA polymerase II, and following expression of the Th2 cell-specific intergenic transcripts around the *Il5* gene locus was also inhibited. Furthermore, Th2 cell-dependent airway inflammation in mice was suppressed by the oral administration of SH-2251. Gfi1, a transcriptional repressor, was identified as a downstream target molecule of SH-2251 using a DNA microarray analysis. The Gfi1 expression dramatically decreased in SH-2251-treated Th2 cells, and the SH-2251-mediated inhibition of IL-5-producing Th2 cell differentiation was restored by transduction of *Gfi1*. Therefore, our study unearthed SH-2251 as a novel therapeutic candidate for allergic inflammation that selectively inhibits active histone marks at the *Il5* gene locus.

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Introduction

Asthma is a complex chronic inflammatory disease characterized by airway inflammation and hyperresponsiveness obstruction that affects approximately 300 million individuals worldwide [1]. A large number of clinical studies and animal experimental models support a central role of antigen-specific Th2 cells in the pathological responses of atopic asthma [2,3]. In particular, antigen-specific effector and memory Th2 cells appear to play an important role in initiating allergic inflammatory status in the early stage of atopic asthma. Although eliminating Th2 cells and/or inhibiting Th2 cell functions at the early stage of atopic asthma may lead to complete remission, strategies for modulating Th2 cell numbers and/or functions have not been established.

IL-5 is a hematopoietic cytokine that exerts important effects on eosinophils and basophils. IL-5 induces differentiation and maturation of eosinophils in bone marrow, migration to tissue sites and prevention of eosinophil apoptosis [4] [5]. IL-5 also plays a role in the development, metabolism, and function of basophils [6]. Eosinophilic inflammation is a hallmark of asthma that correlates with bronchial hyperresponsiveness and disease severity. In an asthma model, IL-5-deficient mice did not display eosinophilia, airway hyperreactivity or pulmonary injury, in contrast to that observed in control mice [7]. Treatment of mice with anti-IL-5 mAb also results in decreases in eosinophilic inflammation that are associated with reduced reactivity of methacholine. Therefore, IL-5 is a therapeutic target for allergic inflammation as well as hypereosinophilic syndrome.

Th2 cells produce IL-4, IL-5 and IL-13, and have been shown to play a crucial role in IgE production and eosinophil recruitment. Th2 cells are involved in clearance of extracellular parasites and also promote pathogenic responses associated with allergic inflammation. In peripheral CD4 T cells, IL-4-mediated activation of the transcription factor STAT6 induces the expression of *Gata3* mRNA, which drives Th2 cell differentiation [8]. GATA-3 binds to various regulatory regions on the Th2 cytokine gene loci and induces chromatin remodeling [9,10,11]. In addition, GATA-3 binds to the *Il5* promoter and acts as a transcriptional factor for IL-5 [12].

In addition to Th2 cells, a large number of cell types produce IL-5, including eosinophils [5] [4], natural killer (NK)T cells [13], nuocytes [14], natural helper (NH) cells [15] and IL-5-producing

innate cells [16]. Recently, the IL-33-induced production of IL-5 from innate cells was reported. IL-33-mediated production of IL-5 plays critical roles in lung eosinophil regulation [16], lung inflammation [17] and protease allergen-induced airway inflammation [18]. In addition, the IL-33/IL-5 signaling pathway plays a crucial role in the disease pathogenesis of severe asthma that is resistant to high doses of inhaled corticosteroids but responsive to systemic corticosteroids and anti-IL-5 therapy [19].

Gfi1 is a DNA binding transcriptional repressor that plays important roles in several hematopoietic cells [20]. Gfi1 exerts its role as a transcriptional repressor by interacting with a number of histone modification enzyme including LSD-1/CoRest, G9a and HDACs [21,22,23]. It is well established that Gfi1 regulates the development of Th cell subsets. Zu et al. demonstrated that Gfi1 regulates Th2 cell expansion via enhancement of Stat5 activity [24]. However, the forced expression of constitutively active Stat5 fails to restore Th2 cell development in *Gfi1*-dificient CD4 T cells, possibly because Gfi1 might also play additional roles in Th2 cell development that are independent of Stat5. We previously reported that the expression level of Gata3 proteins and generation of IL-5-producing Th2 cells are severely impaired in *Gfi1*-deficient CD4 T cells [25]. The transduction of Gata3 into *Gfi1*-deficient Th2 cells partially restores the development of IL-5-producing Th2 cells, thus indicating that Gfi1 controls IL-5-producing Th2 cell generation in part through regulation of the Gata3 protein expression.

SH-2251, a thioamide-related compound, was originally synthesized as an inhibitor of IL-5 production. However, the molecular mechanisms by which SH-2251 inhibits IL-5 production and the effects of SH-2251 on Th2 cell differentiation remain to be elucidated. We herein investigated the effects of SH-2251 on Th2 cell differentiation and demonstrated that SH-2251 negatively regulates IL-5-producing Th2 cell differentiation and chromatin remodeling at the *Il5* gene locus. Furthermore, we demonstrated that Th2 cell-dependent allergic airway inflammation is suppressed by oral administration of SH-2251. A DNA microarray analysis revealed that SH-2251 inhibits the differentiation of IL-5-producing Th2 cells via repression of the Gfi1 expression. Therefore, SH-2251 belongs to a unique class of inhibitors of Th2-dependent immune responses that modulate chromatin remodeling at the *Il5* gene locus and the subsequent the differentiation of IL-5 producing Th2 cells.

Results

SH-2251 selectively inhibits the generation of IL-5-producing Th2 cells

SH-2251 (**Fig. 1A**), a novel thioamide-related compound, was originally synthesized as an inhibitor of IL-5 production. However, the effects of SH-2251 on Th2 cell differentiation were not determined. To assess the effects of SH-2251 on Th2 cell differentiation, naïve CD4 T cells were purified and cultured under Th2-conditions in the presence or absence of SH-2251 for five days, and the ability to produce Th2 cytokines was determined using intracellular staining. As shown in **Fig. 1B**, the generation of IL-5-producing Th2 cells decreased in the SH-2251-treated cultures, whereas the number of IL-4- and IL-13-producing cells slightly increased. The selective reduction of IL-5 production was also confirmed on ELISA (**Fig. 1C**). The generation of IFN-γ-producing Th1 cells and IL-17A-producing Th17 cells was moderately decreased, while development of IL-9-producing Th9 cells was augmented by treatment with SH-2251 (**Fig. S1A–C in File S1**). To determine the optimal concentration for inhibition of IL-5-producing Th2 cell differentiation, naïve CD4 T cells were cultured under Th2-conditions in the presence of the indicated concentrations of SH-2251. Inhibitory effects were observed at the 10 nM concentration of SH-2251 and peaked at 100 nM (**Fig. 1D**). Dose-dependent effects of SH-2251 on the inhibition of IL-5 induction were also confirmed using ELISA (**Fig. 1E**). The production of IL-4 and IL-13 was not impaired (**Fig. 1E**). These results indicate that SH-2251 inhibits IL-5-producing Th2 cell differentiation without inhibiting the generation of IL-4- or IL-13-producing Th2 cells.

SH-2251 selectively inhibits induction of active histone modifications at the *Il5* gene locus during Th2 cell differentiation

Changes in histone modification are a marker of chromatin remodeling [26,27]. During Th2 cell differentiation, active histone modifications including histones H3K4me2/3, H3K9ac and H3K27ac, are induced at Th2 cytokine gene loci [9] [11]. We examined the effect of SH-2251 on the induction of active histone modifications during Th2 cell differentiation. As shown in **Fig. 2A**, the levels of active histone modifications such as those of H3K4me3, H3K9ac and H3K27ac at the *Il5* promoter were reduced by treatment with SH-2251 in a dose-dependent manner. The levels of H3K9ac and H3K27ac, but not H3K4me3, at the Rad50 promoter decreased (**Fig. 2A**). In sharp contrast, the active histone modifications at the *Il4* and *Il13* promoters were unaffected by SH-2251 treatment (**Fig. 2A**). To confirm the selective effects of SH-2251 on the levels of active histone modifications around the *Il5* gene locus, we performed ChIP-sequencing with anti-histone H3K4me3 pAb and H3K27ac pAb. Decreased levels of H3K4me3 and H3K27ac were detected from the 5′ region of the *Rad50* gene to the *Il5* gene, while reduced levels were spread over the down stream region of the *Il5* gene locus in the SH2251-treated Th2 cells (**Fig. 2B and Fig. S2 in File S1**). Reduction in the levels of H3K4me3 and H3K27ac around the *Il5* gene locus in the SH-2251-treated Th2 cells were confirmed using a manual ChIP analysis (**Fig. 2C**). Changes in other histone modifications, including H3K4me2, H3K9me2, H3K36me3 and H3K9ac, around the *Il5* gene locus were also determined with a manual ChIP analysis. The levels of active histone marks such as those of H3K4me2, H3K36me3 and H3K9ac around the *Il5* gene locus were decreased in the SH-2251-treated Th2 cells (**Fig. 2C**). The level of H3K9me2 was not affected by treatment with SH-2251 (**Fig. 2C**). No obvious signals were detected with an anti-H3K27me3 pAb (data not shown). Finally, we assessed the effects of SH-2251 treatment on the recruitment of RNA polymerase II (PolII) and subsequent intergenic transcription around the *Il5* gene locus. SH-2251 reduced the recruitment of polII (**Fig. 2D upper panel**) and the level of transcription (**Fig. 2D lower panel**) in the Th2 cells. These results suggest that SH-2251 blocks the generation of IL-5-producing Th2 cells, presumably by inhibiting chromatin remodeling at the *Il5* gene locus.

Th2-dependent airway inflammation is attenuated by the administration of SH-2251

We next investigated the effects of the oral administration of SH-2251 (10 mg/kg) in mice model of airway inflammation. BALB/c mice were immunized with OVA absorbed by alum, then challenged with OVA intranasally. We observed decreases in the infiltration of inflammatory cells, including eosinophils, in the bronchoalveolar lavage (BAL) fluid of the OVA-immunized SH-2251-treated mice in comparison to that observed in the vehicle-administrated control group (**Fig. 3A**). The expressions of *Il4*, *Il5*

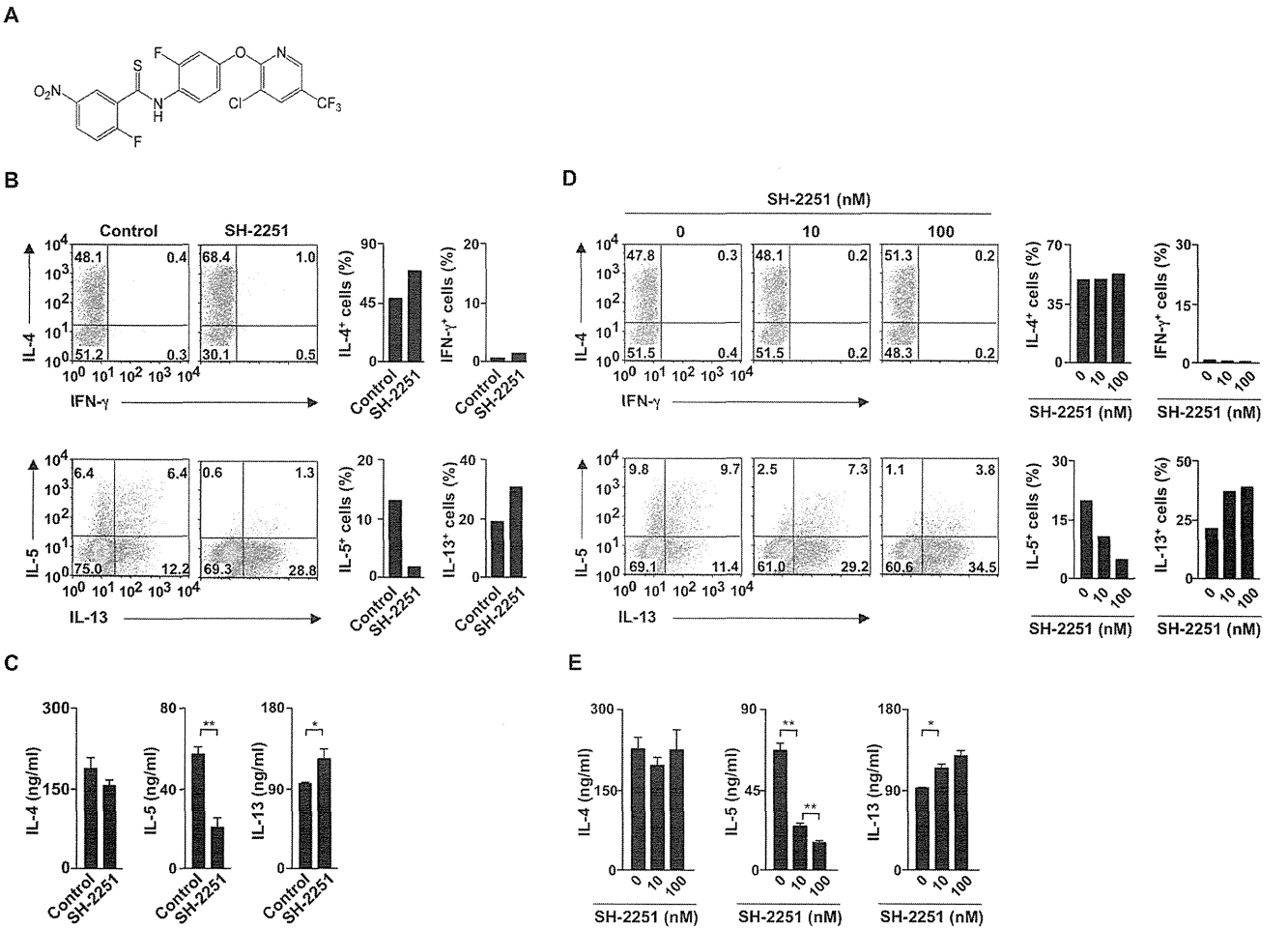

Figure 1. SH-2251 inhibits the generation of IL-5-producing Th2 cells. (A), The chemical structure of SH-2251. **(B)**, Naïve CD4 T cells were cultured under Th2-conditions in the presence or absence of SH-2251 (100 nM) for five days. The cells were restimulated with an immobilized anti-TCR-β mAb for six hours, and the intracellular staining profiles of IL-4/IFN-γ (upper panel) and IL-5/IL-13 (lower panel) were determined using intracellular staining, respectively. The percentages of each quadrant are indicated. The average percentages of the generated cytokine-producing cells of three independent experiments are also shown with the standard deviation (right). **(C)**, Cytokine production induced by the SH-2251-treated Th2 cells shown in panel (B) was determined with ELISA. **(D)**, Naïve CD4 T cells were cultured under Th2-conditions in the presence of the indicated concentrations of SH-2251 for five days. The intracellular profiles were determined as described (B). The average percentage of three independent experiments of the generated cytokine-producing cells are also shown with the standard deviation **(E)**, Cytokine production by the SH-2251-treated Th2 cells shown in panel (D) was determined with ELISA. $^{*}P<0.05$ and $^{**}P<0.01$ (Student's *t*-test). Three independent experiments (C and E) were performed with similar results.
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and *Il13* mRNA in the BAL fluid cells were also very low, whereas the reduction of *Ifnγ* was marginal in the SH-2251-administered group (**Fig. 3B**). A reduced expression of *eosinophil peroxidase* (*Epo*) mRNA in the BAL fluid cells of the SH-2251-administered mice supported decreased infiltration of eosinophils (**Fig. S3A in File S1**). We prepared CD4 T cells from the lungs of OVA-challenged mice to confirm the effects of SH-2251 administration. The expressions of mRNA for Th2 cytokines in the CD4 T cells purified from the lung tissue were reduced in the SH-2251-treated mice (**Fig. 3C**). The purified CD4 T cells were further stimulated with immobilized anti-TCR-β mAb for 48 hours *in vitro*, and the production of cytokines was determined using ELISA. The level of IL-5 production was low in the CD4 T cells obtained from the SH-2251-administered mice in comparison to that observed in the CD4 T cells obtained from the vehicle-treated mice (**Fig. 3D**). In addition, the productions of IL-4 and IL-13 were also significantly decreased in the lung CD4 T cells obtained from the SH-2251-treated mice, whereas the IFN-γ production was increased (**Fig. 3D**). The number of mononuclear cells infiltrating the peribronchiolar regions of the lungs was reduced by SH-2251 administration (**Fig. 3E**). Both mucus hyper-production and goblet cell metaplasia, as assessed with PAS staining, were lower in the bronchioles of the SH-2251-administrered mice compared to that observed in bronchioles of the vehicle-treated control mice (**Fig. 3F**). The serum levels of anti-OVA immunoglobulin were unaffected by the administration of SH-2251 (**Fig. S3B in File S1**). These results indicate that the oral administration of SH-2251 can suppress Th2 cell-mediated allergic airway inflammation.

The expression and functions of Gata3 are not influenced by SH-2251 treatment

Gata3 plays an essential role in the induction of chromatin remodeling at the Th2 cytokine gene locus following Th2 cell differentiation [9] [10] [11]. In addition, Gata3 induces the transcriptional activation of the *Il5* gene [12]. In this study,

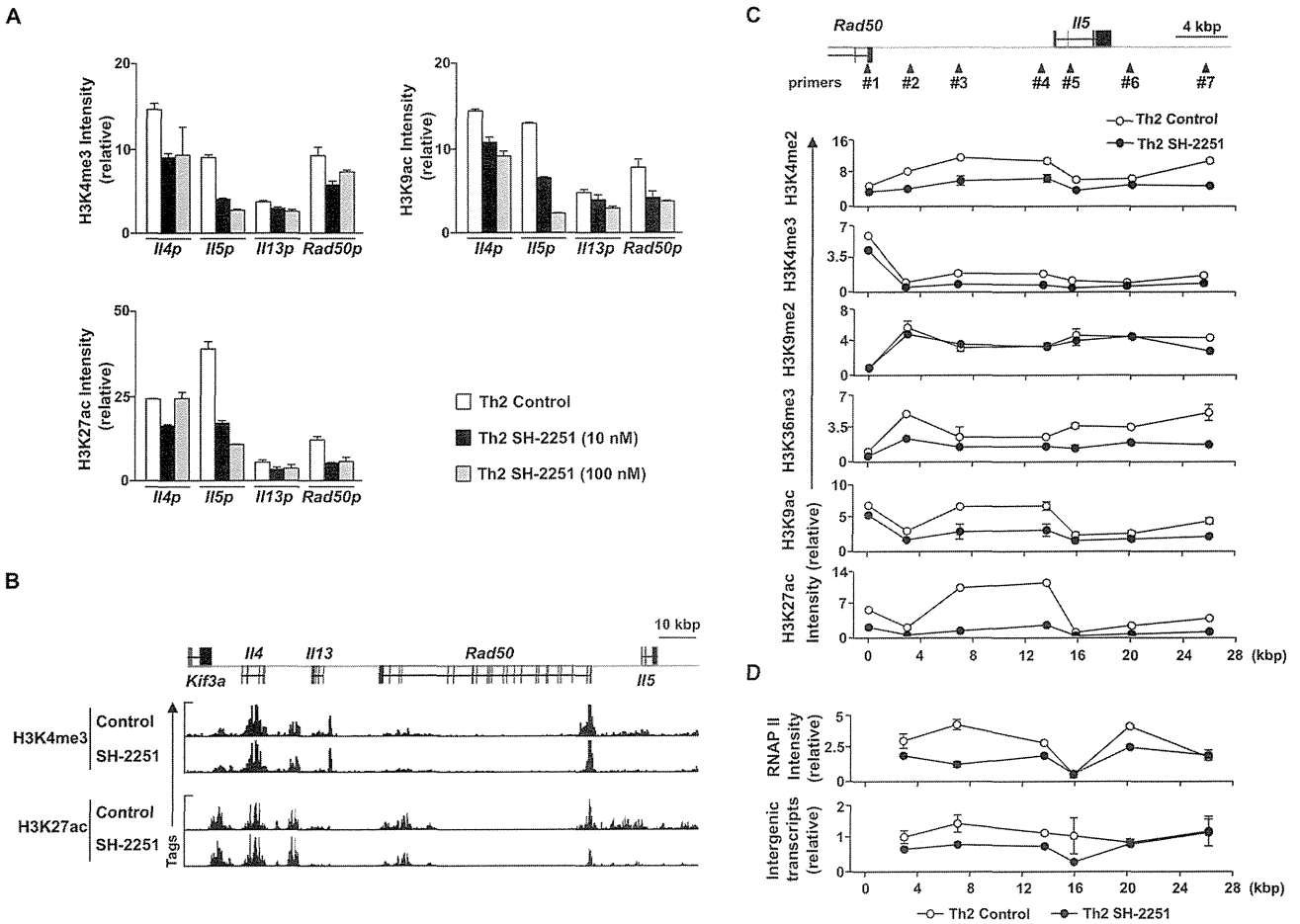

Figure 2. The induction of active histone marks at the *Il5* gene locus is inhibited by SH-2251. (A), Naïve CD4 T cells were cultured under Th2-conditions for five days in the presence of the indicated concentration of SH-2251, and a ChIP assay was performed with the indicated antibodies. The relative intensity (/Input) is shown with the standard deviation. **(B)**, The global patterns of histones H3K4me3 and H3K27ac at the Th2 cytokine gene loci were determined using ChIP-sequencing. **(C)**, The indicated histone modification status around the *Il5* gene locus in the SH-2251-treated Th2 cells was determined using a manual ChIP assay. The relative intensity (/Input) is shown with the standard deviation. **(D)**, Recruitment of RNA polymerase II around the *Il5* gene locus (upper panel) was determined using a manual ChIP assay. The relative intensity (/Input) is shown with the standard deviation. The transcripts around the *Il5* gene in the SH-2251-treated Th2 cells (lower panel) were determined using quantitative RT-PCR. The relative intensity (/*Hprt*) is shown with the standard deviation. Four independent experiments (A, C and D) were performed with similar results.
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treatment with SH-2251 showed no effects on the Gata3 mRNA (**Fig. 4A**) or protein (**Fig. 4B**) expressions in the Th2 cells. Next, we wanted to determine the effects of SH-2251 on binding of Gata3 at the Th2 cytokine gene locus. The binding of Gata3 throughout the Th2 cytokine gene locus was determined comprehensively using ChIP-sequencing with an anti-Gata3 pAb. Gata3 has been reported to bind to the V_A enhancer [28], *Il4* intron2 [29], CGRE [30], Th2 LCR [31,32] and *Il5* promoter regions [33] in Th2 cells. The binding of Gata3 at these regions was confirmed with ChIP-sequencing (**Fig. 4C upper panel**). In addition, we newly identified several Gata3 binding genomic regions around the *Il5* gene locus (**Fig 4C lower panel**: #1~#7). The binding of Gata3 at these regions in the Th2 cells was not inhibited by SH-2251 treatment (**Fig. 4D**). Finally, we examined whether SH-2251 can inhibit the Gata3-induced transcriptional activation of the *Il5* promoter using a reporter gene analysis. As indicated in **Fig. 4E**, SH-2251 showed only marginal effects on the Gata3-dependent activation of the *Il5* promoter. These data suggest that Gata3 is unlikely to be a target of SH-2251 in the inhibition of IL-5-producing Th2 cell development.

A decreased expression of Gfi1 is involved in the SH-2251-mediated inhibition of IL-5-producing Th2 cell generation

We conducted a DNA microarray analysis to identify the target gene(s) that are involved in the SH-2251-mediated inhibition of IL-5-producing Th2 cell generation. We found that the expression of *Gfi1* mRNA was dramatically decreased in the SH-2251-treated Th2 cells (**Fig. 5A**). A reduction in the Gfi1 protein expression was also induced by SH-2251-treatment (**Fig. 5B**). To assess the molecular mechanisms by which SH-2251 inhibits the *Gfi1* expression, the histone modifications present at the *Gfi1* gene locus were determined using ChIP-sequencing with an anti-histone H3K27ac pAb or an H3K4me3 pAb, respectively. As shown in **Fig. 5C lower panel**, a striking reduction in the histone H3K27ac level at the *Gfi1* gene locus in the SH-2251-treated Th2 cells was detected. The level of H3K4me3 at the *Gfi1* gene locus was moderately decreased (**Fig. 5C upper panel**). A

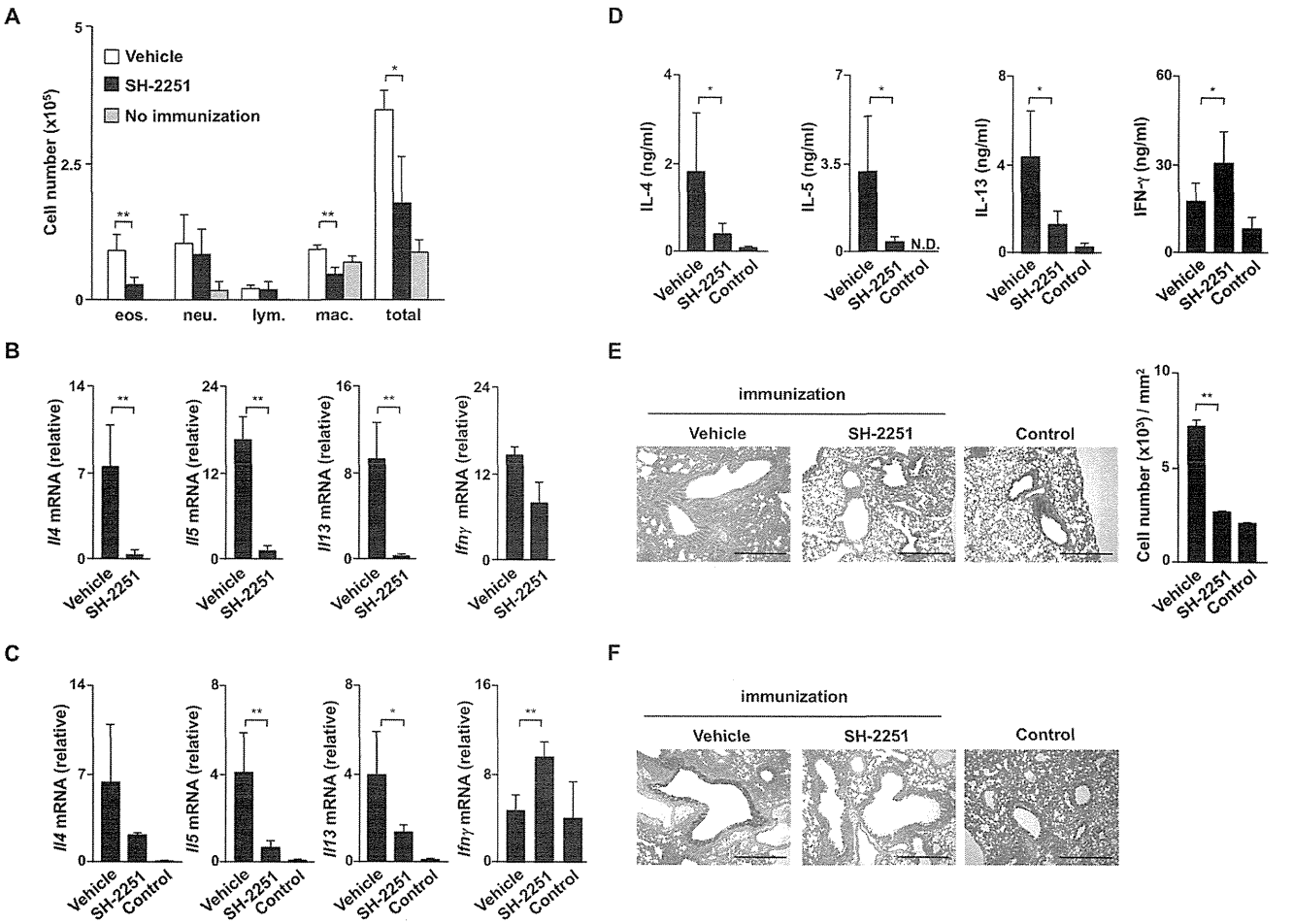

Figure 3. OVA-induced airway inflammation is attenuated by oral administration of SH-2251. (A), Decreased infiltration of eosinophils in the BAL fluid of asthmatic SH-2251-administered mice. The absolute numbers of eosinophils (Eos.), neutrophils (Neu.), lymphocytes (Lym.) and macrophages (Mac.) in the BAL fluid are shown with standard deviations (n = 5 per group). *$P<0.01$ and **$P<0.001$ by ANOVA and the Bonferroni-test. (B), Quantitative RT-PCR of *Il4*, *Il5* and *Il13* mRNA in the BAL fluid cells of vehicle and SH-2251-administered mice. (C), Quantitative RT-PCR of *Il4*, *Il5*, *Il13* and *Ifnγ* mRNA in the lung CD4 T cells of vehicle and SH-2251-administered mice. (n = 5 per group). (D), Cytokine production from lung CD4 T cells of vehicle and SH-2251-administered mice stimulated *in vitro*. The lung CD4 T cells were stimulated with immobilized anti-TCR-β mAb for 48 hours and the concentrations of cytokines in the culture supernatants were determined using ELISA. The lungs were fixed and stained with hematoxylin and eosin (E, left) or periodic acid-Schiff reagent (F). The scale bars represent 500 μm. The numbers of infiltrated leukocytes in the peribronchiolar regions are shown (mean cell numbers/mm^2) (E, right). Three independent experiments were performed with similar results. Student's *t*-test was used for the statistical analyses. *$P<0.05$ and **$P<0.01$ (B, C, D and E)
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dose-dependent inhibition of the H3K4me3 and H3K27ac levels at the *Gfi1* gene locus induced by SH-2251 was confirmed using a manual ChIP assay (**Fig. 5D**). The level of H3K9ac at the *Gfi1* gene locus was also inhibited by SH-2251 treatment (**Fig. 5D**). As indicated in **Fig. S4 in File S1**, the levels of histone H3K27ac and H3K4me3 at the *Gfi1* gene locus were higher in the Th2 cells than that in the naïve CD4 T cells. The levels of histone H3K27ac and H3K4me3 modifications in the SH-2251-treated Th2 cells were almost comparable to those in the naïve CD4 T cells (**Fig. S4 in File S1**), thus indicating that SH-2251 inhibits the induction of the histone H3K27ac at the *Gfi1* locus during Th2 cell differentiation.

Transduction of *Gfi1* into SH-2251-treated Th2 cells restores IL-5 production

To elucidate the role of Gfi1 reduction in the SH-2251-mediated inhibition of IL-5-producing Th2 cell differentiation, we transduced *Gfi1* into SH-2251-treated Th2 cells using retrovirus vectors and measured the IL-5 production ability. As shown in **Fig. 6A**, the transduction of *Gfi1* into the SH-2251-treated Th2 cells partially restored the generation of IL-5 producing Th2 cells. The production levels of IL-5 in the SH-2251-treated Th2 cells were completely restored by the transduction of *Gfi1* (**Fig. 6B**). The production of IL-4 and IL-13 in the *Gfi1*-transduced Th2 cells was not altered in comparison to that observed in the *Mock*-transduced SH-2251-treated Th2 cells (**Fig. 6B**). The levels of histone H3K4me3 and H3K27ac around the *Il5* gene locus were also ameliorated in the *Gfi1*-transduced SH-2251-treated Th2 cells (**Fig. 6C**). Histones H3K4me3, H3K9ac and H3K27ac at the *Il4* and *Il13* promoters were not influenced by the transduction of Gfi1 (**Fig. S5 in File S1**). To examine the molecular mechanisms by which Gfi1 controls the histone modification status at the *Il5* gene locus, the binding of Gfi1 around the *Il5* gene locus was determined using a ChIP-sequence analysis with an anti-Gfi1 pAb. Low, but reproducible binding of Gfi1 was detected around the *Il5* gene locus in the Th2 cells (**Fig. 6D**). The binding of Gfi1 around

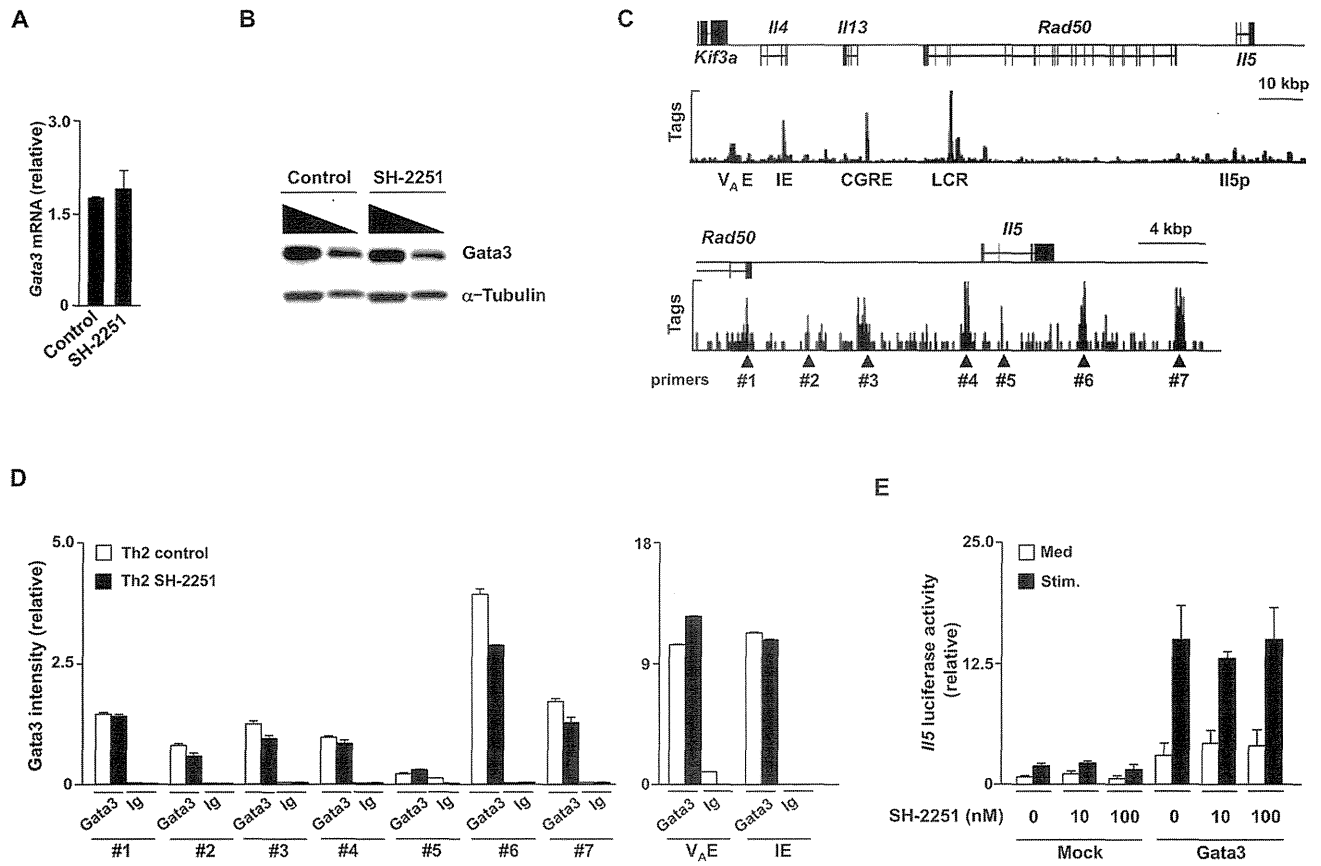

Figure 4. The expression and functions of Gata3 are not impaired by treatment with SH-2251. (A), The mRNA expression of Gata3 in the SH-2251-trearted Th2 cells was determined using quantitative RT-PCR. The relative intensity (/*Hprt*) is shown with the standard deviation. **(B)**, The protein expression level of Gata3 was determined with immunoblotting. The nuclear (Gata3) and cytoplasmic (α-Tubulin) lysates with a three fold serial dilution were used. Three independent experiments (A and B) were performed with similar results. **(C)**, The global patterns of Gata3 binding at the Th2 cytokine gene loci (upper panel) and the *Il5* gene locus (lower panel) were determined using ChIP-sequencing with an anti-Gata3 pAb. The locations of PCR primer pairs (triangle) used in a manual ChIP assay are also listed. **(D)**, The binding of Gata3 around the *Il5* gene locus (left) panel and the V_A enhancer (V_A E) and intronic enhancer (IE) regions of the *Il4* gene locus (right panel) in the SH-2251-treated Th2 cells was determined using a manual ChIP assay. The relative intensity (/Input) is shown with the standard deviation. Three independent experiments were performed with similar results. **(E)**, The effects of SH-2251 on the Gata3-dependent transcriptional activation of the *Il5* promoter were determined using a Dual luciferase assay. The mean and standard deviation of the relative luciferase activity of three different experiments are shown. Stim: PMA (30 ng/ml)+dbcAMP (100 μM). Four independent experiments (A, B, D and E) were performed with similar results.
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the *Il5* gene locus in the Th2 cells decreased by the treatment with SH-2251 (**Fig. 6E**). These results suggest that SH-2251 inhibits the chromatin remodeling at the *Il5* gene locus and subsequent IL-5-producing Th2 cell differentiation in part by attenuating the Gfi1 expression.

Discussion

We herein demonstrated that a thioamide-related small chemical compound, SH-2251, inhibits the differentiation of IL-5-producing Th2 cells by attenuating the Gfi1 expression. Treatment of developing Th2 cells with SH-2251 reduced the generation of IL-5-producing Th2 cells and the expression of Gfi1. SH-2251 also inhibited induction of active histone modifications at the *Il5* gene locus as well as the *Gfi1* locus in developing Th2 cells. We found that Gfi1 binds to several genomic regions around the *Il5* gene locus in Th2 cells, which was reduced by treatment with SH-2251. We previously reported that the induction of histones H3K4me3 and H3K9/14ac at the *Il5* gene locus and subsequent IL-5-producing Th2 cell differentiation are impaired in *Gfi1*-deficient CD4 T cells [25]. In addition, in this study, retrovirus vector-mediated transduction of *Gfi1* into SH-2251-treated developing Th2 cells restored the levels of active histone modifications at the *Il5* gene locus, and subsequent generation of IL-5-producing Th2 cells. Therefore, the *Gfi1-Il5* axis is a target for SH2251-mediated inhibition of IL-5-producing Th2 cell differentiation.

Clinical trials of the anti-IL-5 mAb have demonstrated therapeutic benefits across a spectrum of eosinophil-related disorders [34]. Recently, a result of a clinical trial of a humanized anti-IL-5 mAb (Mepolizumab) was reported [35]. Although no changes in airway hyperresponsiveness were noted, reductions in blood/sputum eosinophilia and the number of asthma exacerbations occurring during the year were reported. In addition, reductions in airway wall thickness were also observed. These results indicate that the neutralization of IL-5 might have a positive impact on airway remodeling. We demonstrated the inhibitory effects of SH-2251 on the generation of IL-5-producing Th2 cells and IL-5 production. Furthermore, the oral administration of SH-2251 was found to suppress OVA-induced allergic airway inflammation in a mice model. These data suggest that SH-

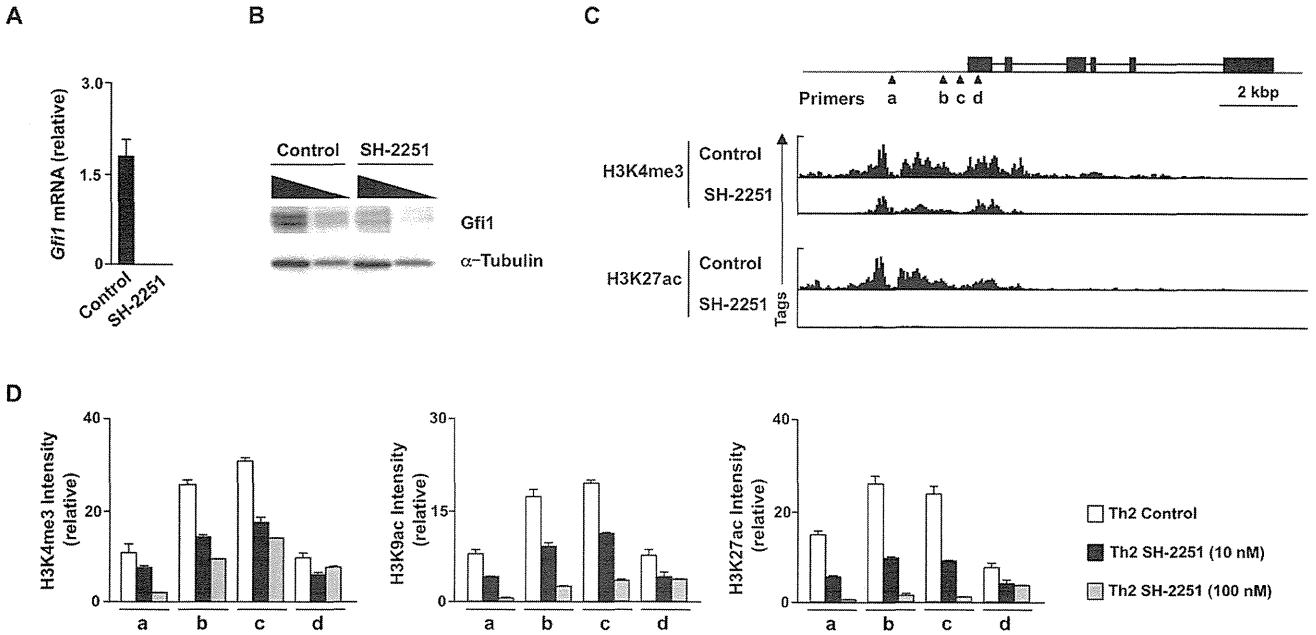

Figure 5. Gfi1 is a downstream target of SH-2251. (A), A decreased *Gfi1* mRNA expression in the SH-2251 treated Th2 cells. The expression of *Gfi1* was determined with quantitative RT-PCR. The relative intensity (/*Hprt*) is shown with the standard deviation. **(B)**, A decreased level of Gfi1 proteins was detected with immunoblotting. The nuclear (Gfi1) and cytoplasmic (α-Tubulin) lysates with a three fold serial dilution were used. **(C)**, The global patterns of the histone H3K4me3 and H3K27ac levels at the *Gfi1* gene locus were determined using ChIP-sequencing. The locations of PCR primer pairs (triangle) used in a manual ChIP assay are also listed. **(D)**, Manual ChIP assays were performed with anti-histone H3K4me3, H3K9ac or H3K27ac pAb. The relative intensity (/Input) is shown with the standard deviation. Four independent experiments (A, B and D) were performed with similar results.

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2251 is a novel therapeutic candidate for diseases involving allergic inflammation, including asthma.

The generation of IL-13-producing cells and IL-13 production were augmented by SH-2251 treatment *in vitro*. The IL-13 production moderately increased in the *Gfi1*-deficient CD4 T cells (M.Y. unpublished observation), suggesting that Gfi1 may inhibit IL-13 production in CD4 T cells. However, our *in vivo* experimental results demonstrated a reduction in IL-13 production induced by the administration of SH-2251. In addition, the production of IL-4 was also moderately decreased *in vivo*. A DNA microarray analysis indicated a reduced expression of *Ccr3* mRNA in the SH-2251-treated Th2 cells. Therefore, it is likely that SH-2251 exerts some effects on the expressions of chemokine receptors in Th2 cells and that recruitment of Th2 cells to the inflamed sites is inhibited. In addition, it is possible that SH-2251 also affects the function of antigen-presenting cells. Taken together, although an SH-2251-mediated increase in IL-13 production was detected in the *in vitro* experiments, the administration of SH-2251 provides beneficial effects in the treatment of asthmatic patients.

Lung epithelial cells can produce multiple cytokines, including IL-25 and IL-33, in response to various stressors. The intranasal administration of IL-25 induces asthmatic symptoms [36], and anti-IL-25 antibody treatment suppresses OVA-induced allergic inflammation [37]. It is thought that IL-25 acts on NKT cells and promotes Th2 cytokine production [38]. Recently, the IL-33-mediated production of IL-5 has been reported to play a critical role in lung eosinophil regulation [16], lung inflammation [17] and protease allergen-induced airway inflammation [18]. Gfi1, a downstream target of SH-2251, is broadly expressed in hematopoietic lineage cells, and *Gfi1* knockout animals display many abnormalities, including neutropenia, T cell development defects, hematopoietic stem cell defects and defects in dendritic cell development and functions [20]. It is likely that Gfi1 is also expressed in NKT cells, NH cells, neuocytes and IL-5-producing innate cells. Therefore, it is interesting to examine whether the treatment of SH-2251 can inhibit both the IL-25- and IL-33-induced production from these cell populations.

SH-2251 inhibits the generation of IL-5-producing Th2 cells, in part by repressing Gfi1 induction. Gfi1 is induced by the TCR-mediated activation of the ERK MAPK cascade [25]. In this study, although SH-2251 inhibited the Gfi1 expression, the inhibitory activity for ERK MAPK was very weak (IC_{50}>1 μM; M.I. and F.K. personal communication). The activation of the Ras-ERK MAPK cascade also prevents the ubiquitin/proteasome-dependent degradation of Gata3 [39]. The treatment of developing Th2 cells with SH-2251 failed to inhibit the Gata3 protein expression. Therefore, it is unlikely that SH-2251 inhibits IL-5-producing Th2 cell differentiation by suppressing Ras-ERK MAPK cascade activation.

Gfi1 is a DNA binding transcriptional repressor that interacts with a number of histone modification enzymes, including LSD-1/CoRest [22], G9a [23] and HDACs [21]. However, these histone modification enzymes introduce repressive marks on the histones. We previously demonstrated that Gfi1 is required for induction of active histone marks on the *Il5* gene locus [25]. In addition, the transduction of Gfi1 into SH-2251-treated Th2 cells restored active histone modifications (H3K4me3, H3K9ac and H3K27ac) at the *Il5* gene locus. Although precious molecular mechanisms remain to be elucidated, our data indicate the possible role of Gfi1 in the formation of the active chromatin status.

An increased activity of histone acetyltransferases (HATs) and concomitant reductions in histone deacetylase (HDAC) activity have been reported in asthmatic patients [40] [41]. Changes in these histone modification enzymes result in hyperacetylations of

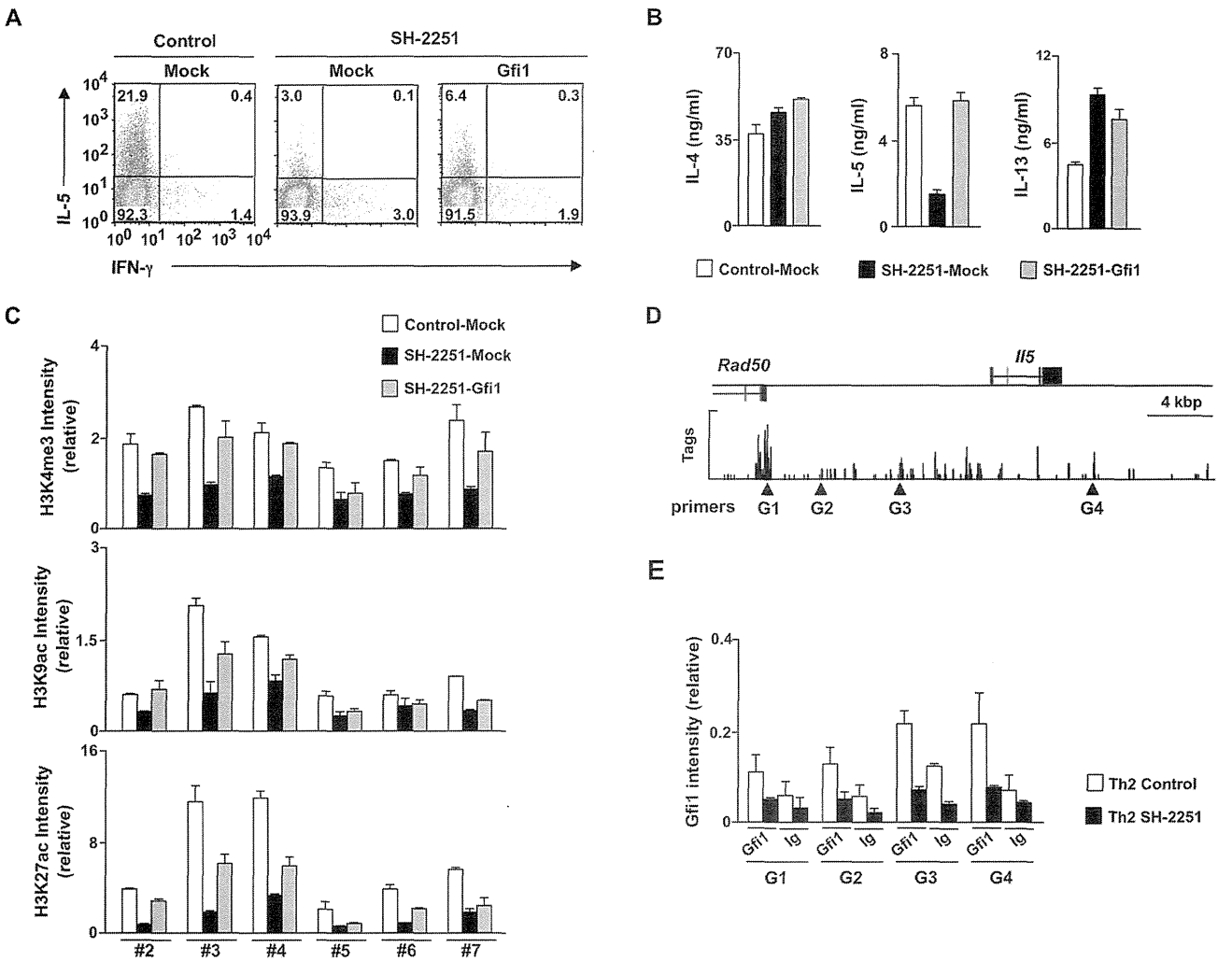

Figure 6. Transduction of *Gfi1* into SH-2251-treated Th2 cells restores the differentiation of IL-5-producing Th2 cells. (A), CD4 T cells were cultured under Th2-conditions in the presence or absence of SH-2251 (100 nM) for two days, then the cells were transduced with Mock- or *Gfi1*-IRES-hNGFR-containing retrovirus vectors. Three days after transduction, the IL-5/IFN-γ staining profiles of the transduced cells (hNGFR-positive cells) were determined with intracellular staining. The percentages of cells in each quadrant are indicated. **(B)**, The cytokine production from SH-2251-treated Th2 cells transduced with *Gfi1* was determined. **(C)**, Histones H3K4me3, H3K9ac and H3K27ac at the *Il5* gene locus in hNGFR-positive *Gfi1*-transduced SH-2251-treated Th2 cells. The relative intensity (/Input) is shown with the standard deviation. Three (A, B and C) independent experiments were performed with similar results. **(D)**, The global pattern of Gfi1 binding around the *Il5* gene locus was determined using ChIP-sequencing with an anti-Gfi1 mAb. The locations of the PCR primer pairs (triangle) used in a manual ChIP assay are also listed. **(E)**, The binding of Gfi1 around the *Il5* gene locus in SH-2251-treated Th2 cells was determined using a manual ChIP assay. The relative intensity (/Input) is shown with the standard deviation. Two independent experiments were performed with similar results.
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histone, opening up the chromatin structure and increasing recruitment of RNA polymerase II [42]. Although the gene locus specific inhibitor for histone acetylation is expected to appear, such molecules have not yet been identified. We demonstrated that SH-2251 selectively inhibits induction of active histone marks, in particular H3K27ac at the *Il5* gene locus and the *Gfi1* gene locus. The transduction of *Gfi1* into SH-2251 treated Th2 cells restores the IL-5 production and active histone modifications at the *Il5* gene locus. These results indicate that SH-2251 belongs to a novel class of inhibitors that modulate histone modification status in a gene locus-specific manner.

In summary, SH-2251 selectively inhibits chromatin remodeling at the *Il5* gene locus and subsequent generation of IL-5-producing Th2 cells via attenuation of the Gfi1 expression. In addition, the oral administration of SH-2251 showed inhibitory effects on OVA-induced airway allergic inflammation. Therefore, SH-2251 is a unique class of therapeutic candidate for allergic inflammation acting through the selective inhibition of IL-5 production.

Materials and Methods

SH-2251

SH-2251 (United States Patent No.: US 7632865 B2) was synthesized and provided by Ishihara Sangyo Kaisha, Ltd. The purity of the SH-2251 used in the experiments was 99.1%.

Mice

C57BL/6 and BALB/c mice were purchased from CLEA Japan. All mice were maintained under specific pathogen-free conditions and were used at 6–10 weeks of age. All experiments

using mice received approval from the Kazusa DNA Research Institute Administrative Panel for Animal Care. All animal care was conducted in accordance with the guidelines of the Kazusa DNA Research Institute.

CD4 T cells differentiation *in vitro*

Naïve CD4 T ($CD44^{lo}CD62L^{hi}$) cells were prepared using a $CD4^{+}CD62L^{+}$ T cell isolation kit II (Miltenyi Biotec). Naïve CD4 T cells (1.5×10^{6}) were stimulated with an immobilized anti-TCR-β mAb (3 μg/ml; H57-597; BioLegend) and an anti-CD28 mAb (1 μg/ml; 37.5; BioLegend) with or without SH-2251 (Ishihara Sangyo Kaisha, Ltd.) under the indicated culture conditions for two days. Next, the cells were transferred onto a new plate and cultured for an additional three days in the presence of cytokines with or without SH-2251. If not mentioned, 100 nM of SH-2251 was used in the experiments. The cytokine conditions for Th2 cell differentiation were as follows: IL-2 (2.5 ng/ml), IL-4 (10 ng/ml; PeproTech) and anti-IFN-γ mAb (5 μg/ml; R4-6A2; BioLegend).

Intracellular staining of cytokines

The *in vitro* differentiated Th cells were stimulated with an immobilized anti-TCR-β mAb (3 μg/ml; H57–597; BioLegend) for six hours in the presence of monensin (1 μM), and intracellular staining was performed as previously described [25]. The following antibodies were used for intracellular staining: anti-IL-4-hycoerythrin (PE) mAb (11B11; BD Bioscience), IFN-γ-FITC mAb (XMG1.2; BD Bioscience), IL-5-allophycocyanin (APC) (TRFK5; eBioscience), and IL-13-PE (eBio13A; eBioscience). A flow cytometric analysis was performed using a FACSCalibur instrument (BD biosciences), and the results were analyzed using the FlowJo software program (Tree Star).

ELISA

The cells were stimulated with an immobilized anti-TCR-β mAb for 16 hours, and the culture supernatants were recovered. The amount of cytokines in the recovered supernatants was determined with ELISA, as described previously [43].

Quantitative RT-PCR

Total RNA was isolated using a TRIZOL Reagent (GIBCO). cDNA was synthesized using the Superscript VILO cDNA synthesis kit (Invitrogen). Quantitative RT-PCR was performed as previously described [43], using StepOnePlus Real-Time PCR Systems (Applied Biosystems). The specific primers, and Roche Universal Probes used in the experiments were as follows:

Hprt: 5′ TCCTCCTCAGACCGCTTT 3′ (forward), 5′ CCTGTTCATCATCGTAATC 3′ (reverse), probe #95; *Gata3*: 5′ TTATCAAGCCCAAGCGAAG 3′ (forward), TGGTGGTGGTCTGACAGTT 3′ (reverse), probe #108; *Gfi1*: 5′ TCCGAGTTCGAGGACTTTG 3′ (forward), 5′ GAGCGGCACAGTGACTTCT 3′ (reverse), probe #7.

Microarray analysis

The gene expression profiles of the SH-2251-treated Th2 cells were analyzed using the Agilent Whole Mouse 44K Array. The raw data were subjected to log2 transformation and normalized using the Subio Platform (Subio). The gene expression data were deposited in the GSE42131.

Chromatin Immunoprecipitation (ChIP) assay and ChIP-sequencing

The Magna ChIP kit was used for the ChIP assay according to the manufacturer's protocol (MILLIPORE). The anti-histone H3K4me2 pAb (ab7766; Abcam), anti-histone H3K4me3 pAb (cat#39159; Activemotif), anti-histone H3K27me3 pAb (cat#39155; Activemotif), anti-histone H3K36me3 pAb (ab9050; Abcam), anti-histone H3K9ac pAb (cat#39137; ActiveMotif), anti-histone H3K27ac pAb (cat#39133; ActiveMotif), anti-Gata3 (cat# AF2605; R&D) pAb and anti-Gfi1 (M-19; Santa Cruz) were used for immunoprecipitation. The specific primers at the Th2 cytokine gene locus and the Roche Universal probes used in the experiments were as follows: #1: 5′ ACGCTTCCGGAACTAGGG 3′ (forward), 5′ CGCTCTGGCATCTCGTTC 3′ (reverse), probe #38; #2 (G2): 5′ CAGATGTGATATGCGTACATGTAATTC 3′ (forward), 5′ TGAACTCCTGACCCTGCTTT 3′ (reverse), probe #79; #3: 5′ AGTGTCTGTCCCCCAGATCA 3′ (forward), 5′ GCTGCCTGGAACTTGGTG 3′ (reverse), probe #64; #4: (Il5p), 5′ TCACTTTATCAGGAATTGAGTTTAACA 3′ (forward), 5′ GATCGGCTTTTCTTGAGCA 3′ (reverse), probe #43; #5: 5′ TGCCTCTCTTTGTTTTCCTTG 3′ (forward), 5′ GCAATTCAGTGGTAGAGT

GCTCA 3′ (reverse), probe #81; #6 (G4): 5′ AGTACAAGGGCCAAGTCACG 3′ (forward), 5′ GCCAGAGACTGGGGGTAAGT 3′ (reverse), probe #16; #7: 5′ GCTGGCCTTGAACTTACTACG 3′ (forward), 5′ GTGTGTACCCGTAATCCCA

AC 3′ (reverse), probe #10; G1: 5′ GGAAGTGGGAGTCCTAAGCA 3′ (forward), 5′ CTCCCTGCCCAACTTCTAAA 3′ (reverse), probe #15; G3: 5′ AAGGGGAGAACTGCCTCCTA 3′ (forward), 5′ TCATGCCATGGGATACAGG (reverse), prove #99; Il4p: 5′ TTGGTCTGATTTCACAGGAAAA 3′ (forward), 5′ GGCCAATCAGCACCTCTCT 3′ (reverse), probe #2; V_A site in the IL-4 enhancer: 5′ GCCTGTTTCCTCTCAGCATT 3′ (forward), 5′ TGATAAAAGTGACTTGAAGGTT

GG 3′ (reverse), probe #4; IL-4 intronic enhancer: 5′ CCCAAAGGAGGTGCTTTT

ATC 3′ (forward), 5′ AAATCCGAAACTGAGGAGTGC 3′ (reverse), probe #75; Il13p: 5′ CCAGGTTCTGGGTGGTTTATT 3′ (forward), 5′ GAATTACTGGGGCGGAAGTT 3′ (reverse), probe #105; Rad50p: 5′ GGAAGTGGGAGTCCTAAGCA 3′ (forward), 5′ CTCCCTGCCCAACTTCTAAA 3′ (reverse), probe #15. The specific primers at the Gfi1 gene locus and the Roche Universal probes used in the experiments were as follows:

a: 5′ TTTGCAGAAGAGTGAGGTTTGA 3′ (forward), 5′ TGGAGGCGTGGGATTAAC 3′ (reverse), probe #55; b: 5′ GACCAAGGCGTGTGA

CTATACA 3′ (forward), 5′ CACACCCTGTTGTACCCACTT 3′ (reverse), probe #48; c: 5′ GTGCCACACCACTATTCCAG 3′ (forward), 5′ AGTGGCAAAGGACCAAC

ACT 3′ (reverse), probe #2; d: 5′ TGGGGACAGGTTTTACCACT 3′ (forward), 5′ GACAGGTGGCACGAATCC 3′ (reverse), probe #70.

The samples for the ChIP-sequencing were prepared according to the manufacturer's protocol (Illumina), and the ChIP-sequence was performed using Genome Analyzer IIx (Illumina).

Immunoblot analysis

Cytoplasmic and nuclear extracts were prepared using NE-PER Nuclear and Cytoplasmic Extraction Regents (Thermo Fisher Scientific) as previously described [43]. Anti-Gata3 mAb (HG3-31; Santa Cruz), anti-Gfi1 pAb (M-19; Santa Cruz) and anti-α-Tubulin mAb (DM1A; Lab Vision) were used for the immunoblot analysis.

Retrovirus-mediated gene transfer

The methods for generating retrovirus supernatant and infection were described previously [25]. Infected cells were detected using staining with anti-human NGFR-PE mAb (ME20.4-1.H4; Miltenyi Biotec) and anti-PE microbeads (#130-048-801; Miltenyi Biotec), and hNGFR-positive infected cells were purified using AutoMACS (Miltenyi Biotec).

Luciferase assay

The IL-5 promoter activity was determined as previously described [30]. In brief, M12 cells (B cell line) were cotransfected with a firefly luciferase reporter (pGL3-*Il5* promoter), a renila luciferase plasmid (pRL-TK; Promega) and an expression vector (pFlag-CMV2; Sigma) using Gene Pulser MXcell (BIO-RAD). Twenty-four hours after transfection, the cells were maintained in the presence or absence of SH-2251 for one hour, and then stimulated with PMA plus dibuteryl-cAMP for 12 hours. The luciferase activity was measured using a Dual-Luciferase Reporter Assay System (Promega).

OVA-induced allergic airway inflammation

BALB/c mice were immunized intraperitoneally with 100 μg OVA in 2 mg of aluminum hydroxide gel on day 0. Next, the mice were intranasally challenged with OVA in saline (100 μg/mouse) on days 8 and 10. SH-2251 (10 mg/kg) was orally administered every day from day 0 to day 11. Two days after the last OVA challenge, BAL fluid cells and lung samples were prepared for histological examination as previously described [44]. Lung mononuclear cells were also prepared two days after the last OVA challenge, as previously described [45]. CD4 T cells were purified from lung mononuclear cells using anti-mouse CD4 microbeads (Miltenyi Biotec).

Statistical analysis

Student's *t*-test was used for the statistical analyses. ANOVA and the Bonferroni-test were used in the *in vivo* experiments.

Supporting Information

File S1 The effects of SH-2251 on Th1-, Th9-, and Th17-differentiation. Naïve CD4 T cells were cultured under Th1- (A), Th9- (B) or Th17- (C) conditions in the presence or absence of SH-2251 (100 nM) for five days. The cells were restimulated with an immobilized anti-TCR-β mAb for six hours, and the intracellular staining profiles were determined using intracellular staining (left). The following antibodies were used for intracellular staining: anti-IL-4-PE mAb (11B11; BD Bioscience), IFN-γ-FITC mAb (XMG1.2; BD Bioscience), anti-IL-9-PE mAb (RM9A4; BioLegend), anti-IL-17A-Alexa647 mAb (TC11-18H10.1; BioLegend) and IL-17F-Alexa488 mAb (9D3.1C8; BioLegend). The percentages of each quadrant are indicated. The cytokine production by the SH-2251-treated Th cells stimulated with an immobilized anti-TCR-β mAb for 16 hours was determined with ELISA. The culture conditions for each Th cell differentiations were as follows. Th1-conditions: IL-2 (2.5 ng/ml), IL-12 (1 ng/ml; PeproTech) and anti-IL-4 mAb (5 μg/ml; 11B11; BioLegend). Th9-conditions: IL-2 (2.5 ng/ml), IL-4 (10 ng/ml), TGF-β (10 ng/ml; PeproTech) and anti-IFN-γ mAb (5 μg/ml). The Th17-conditions were as follows: IL-6 (10 ng/ml; PeproTech), IL-1β (5 ng/ml; PeproTech), TGF-β (1 ng/ml), anti-IL-2 (5 μg/ml; BioLegend), anti-IL-4 mAb (5 μg/ml) and anti-IFN-γ mAb. Three independent experiments were performed with similar results. $*P<0.05$ and $**P<0.01$ (Student's *t*-test).
(DOCX)

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Author Contributions

Conceived and designed the experiments: JS MY. Performed the experiments: JS MK ST TN OO MY. Analyzed the data: JS MK. Contributed reagents/materials/analysis tools: MI FK. Wrote the paper: JS MY.

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シンポジウム

2. 幹細胞異常と内科系疾患，現況と展望
1）造血幹細胞の異常：先天性免疫不全症

原　寿郎

Key words：抗体不全，複合型免疫不全症，マススクリーニング，遺伝子治療

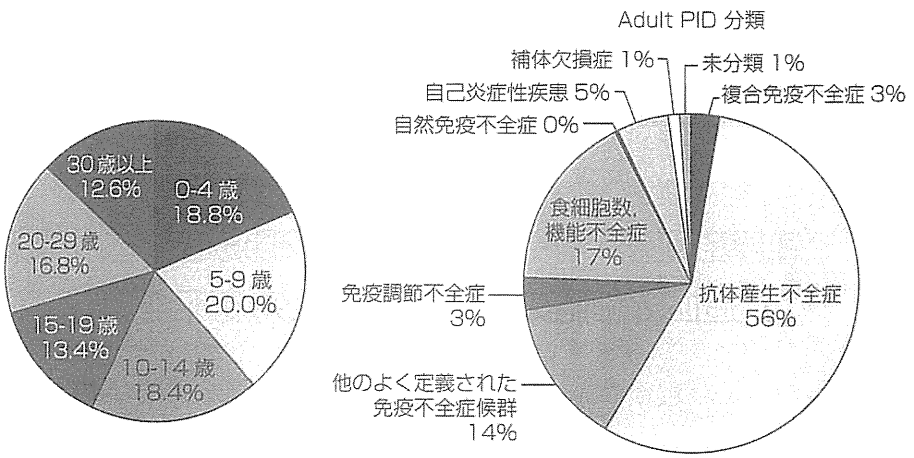
はじめに

先天性（原発性）免疫不全症（Primary Immunodeficiency Disease：PID）とは，自然免疫系，獲得免疫系の発達成熟過程のどこかに先天性の欠陥が生じた疾患群である．国際免疫学会連合会（International Union of Immunological Societies：IUIS）による先天性免疫不全症の分類では，①T細胞系とB細胞系双方の異常を示す複合免疫不全症，②主として抗体系の欠陥を示す免疫不全症，③特徴的な症状を呈する免疫不全症，④免疫系の調節異常による疾患，⑤貪食細胞の数，機能，あるいは両方の先天的欠陥を示す疾患，⑥自然免疫系の欠陥を示す疾患，⑦自己炎症性疾患，⑧補体系の異常を示す疾患，の8つに分類され[1]，現在150以上の遺伝子異常，200以上の異なった病型が知られている．

我が国の疾患分類別の頻度では，複合免疫不全症7.2%，抗体不全37.6%，食細胞異常17.5%で，内科報告例では小児科報告例に比して抗体不全の頻度が高い．最新の全国疫学調査のPID患者の年齢分布は図1左に示すように，15～19歳は13.4%，20～29歳は16.8%，30歳以上は12.6%と15歳以上の割合が計42.8%で[2]，1981年の初回の日本の全国疫学調査での16歳以上の割合10.5%[3]に比し著増している．ヨーロッパの2009年のPID疫学調査での16歳以上の割合は46.6%[4]とほぼ同等である．小児期に診断した患者を含む20歳以上の成人PIDは29.4%と約3分の1を占め，Common variable immunodeficiency（CVID），X-linked agammaglobulinemia（XLA），Chronic granulomatous disease（CGD），高IgE症候群，Wiskott-Aldrich syndrome（WAS）等が主な疾患である（図1右）．また，発症が15歳以上のPIDは今回の調査では全体の5.5%で，主な疾患としてCVID，選択的IgA欠損症，Good症候群等がある．成人のPIDを疑う10のシグナルがJeffrey Modell Foundationから提示されており[5]，様々な感染症，体重減少を伴う下痢，あるいはPIDの家族歴がある場合にはぜひ成人でもPIDを鑑別診断の1つに考えていただきたい．

九州大学大学院医学研究院成長発達医学分野

110th Scientific Meeting of the Japanese Society of Internal Medicine : Symposium : 2. Diseases originated from stem cell abnormalities ; 1）Abnormalities in hematopoietic stem cells : Congenital immunodeficiencies.
Toshiro Hara : Department of Pediatrics, Graduate School of Medical Sciences, Kyusyu University, Japan.
本講演は，平成25年4月13日（土）東京都・東京国際フォーラムにて行われた．



(Ishimura M et al. J Clin Immunol. 2011)

図 1. 日本における 2008 年先天性免疫不全症疫学調査
二次調査の年齢分布と成人PID（20 歳以上）の疾患分類

表 1. SCIDの病態による分類

Mechanisms/Disease
Increased lymphocyte apoptosis
Reticular dysgenesis, ADA-SCID, PNP-SCID
Impaired cytokine-mediated signaling
Common γ chain defect, JAK3 defect, IL-7Rα-chain defect
Defects of the pre-T cell receptor
Defects in V(D)J recombination
RAG1 defect, RAG2 defect, Artemis defect, DNA-PKcs defect, DNA ligase IV defect,
Cernunnos/XLF defect
Impaired signaling through the pre-T cell receptor
CD3δ defect, CD3ε defect, CD3ζ defect, CD3γ defect, CD45, ZAP-70 defect, p56lck defect
Defects in thymus embryogenesis
Nude/SCID Syndrome, DiGeorge Syndrome (del22q11.2), CHARGE, Diabetic mother embryopathy
Impaired calcium flux
ORAI1 defect, STIM1 defect
Cell movement defect
Coronin-1A defect, DOCK8 defect
Other mechanisms
MHC Class Ⅱ defect, DHH (Cartilage hair hypoplasia), Hereditary folate malabsorption (HFM)

1. 造血幹細胞の異常としての先天性免疫不全症

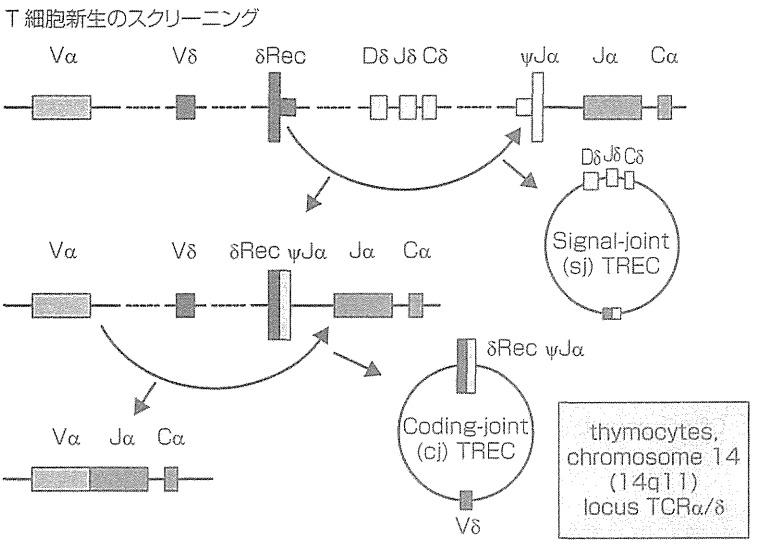
大部分のPIDは理論的には造血幹細胞移植により造血幹細胞を入れ替えると治癒すると考えられ，広義では造血幹細胞の異常による疾患である[6]．主な造血幹細胞疾患として，①T・Bリンパ系前駆細胞の異常による重症複合型免疫不全症(SCID)，②Bリンパ系前駆細胞の異常によるBリンパ球欠損，③単球・樹状細胞欠損を紹介する．

1）重症複合型免疫不全症（SCID）

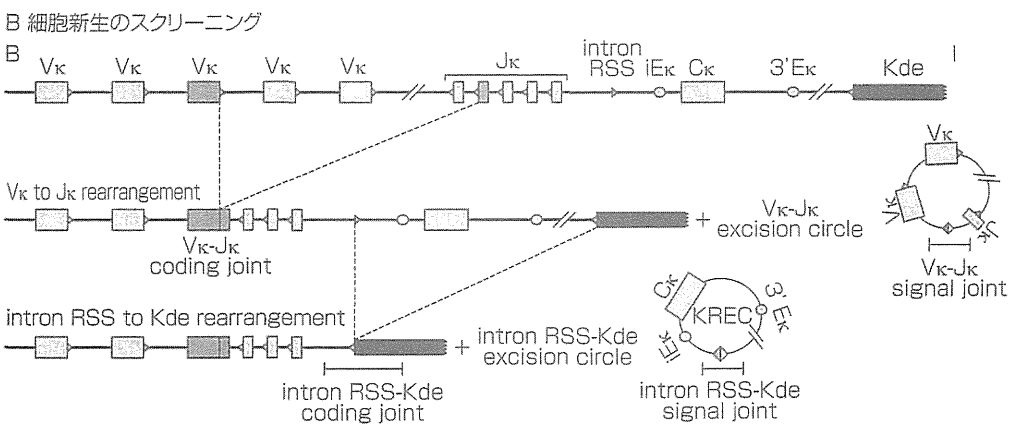
表1に示すように，SCIDの病態による分類では，a）リンパ球のアポトーシスの増加，b）サイトカインを介するシグナルの障害，c）Pre-T cell receptorの欠陥，d）胸腺の発生異常，などがある[7]．

a）リンパ球のアポトーシスの増加

細網異形成症（Reticular dysgenesis）はミトコンドリア膜間腔に存在するAdenylate kinase 2の異常によるSCIDの最重症型で，ミトコンドリアのエネルギー代謝やFADD/caspase 10を介



Cossu F. Italian J Pediatr 2010.
TREC（T cell receptor excision circles）の定量



van Zelm MC., et al. Frontiers in Immun. 2011.
KREC（Kappa-deleting recombination excision circles）の定量

図 2. T細胞欠損とB細胞欠損のマススクリーニング

してのアポトーシスの制御の異常によってミトコンドリア障害，あるいは小胞体ストレス応答に異常がおき，前駆細胞のアポトーシスがおきると考えられている．末梢血中の全ての有核細胞（好中球，リンパ球，単球，DC）が欠損するが，赤血球・血小板系は正常である[8,9]．

b）サイトカインを介するシグナルの障害

T-B+NK−重症複合免疫不全症としてCommon γ欠損によるX連鎖のタイプとJAK3欠損による常染色体劣性のものがある．Common γ鎖，JAK3はIL-2，IL-4，IL-7，IL-9，IL-15，IL-21のシグナルの伝達に関連し，それらの変異によりT細胞分化，NK細胞分化に異常が起こる[10]．また，T-B+NK+重症複合免疫不全症としてはIL-7受容体αの欠損症がある．IL-7シグナルの欠損によってT細胞の分化が障害されるが，IL-15シグナルの異常は起きないのでNK細胞は正常である．

表2. B細胞欠損と無ガンマグロブリン血症の病態による分類

Mechanisms/Disease
Increased lymphocyte apoptosis
Reticular dysgenesis, ADA-SCID
Defects of the pre-B cell receptor
Defects in V(D)J recombination
RAG1 defect, RAG2 defect
Impaired signaling through the pre-B cell receptor
BTK defect, μH defect, λ5 defect, Igα defect, Igβ defect, BLNK defect
Defects of the class switch recombination
AID defect, UNG defect, CD40 defect, CD40L defect
Other mechanisms
CVID

c) Pre-T cell receptorの欠陥

V(D)J recombinationの欠陥としてRAG1, RAG2, DNA-PKcs, Artemis, DNA ligase IV, Cernunnosの異常によるものが報告され，またPre-T cell receptorを介してのシグナルの障害としてはCD3γ, CD3δ, CD3ε, CD3ζ, Lck, ZAP-70, CD45などが知られている．

d) 胸腺発生異常

WHN(winged helix)遺伝子変異によるFOXN1異常により胸腺上皮細胞の分化とhair follicleの分化が障害され，ヌードマウスと同様にT-B+NK+SCID，脱毛，爪の異常が起こる[11]．DiGeorge症候群，CHARGE association，糖尿病の母親による胎児病も知られているので注意を要する．

TREC（T cell receptor excision circles）は，T細胞レセプターの組み替えの時に生じる環状DNAであり，T細胞新生の良いマーカーである（図2上）．これを定量することによりSCIDであるかどうか診断可能である．ガスリーカードからも診断可能で，米国ウィスコンシン州で最初に新生児スクリーニングが開始された．

2) B細胞の異常

表2に示すように，B細胞の欠損/無ガンマグロブリン血症の病態による分類では，a)リンパ球のアポトーシスの増加，b)pre-B cell receptorの欠陥(V(D)J recombinationの欠陥，あるいはpre-B cell receptorからシグナルの障害によるもの)，c)class switch recombinationの欠陥，などがある[12,13]．

B細胞レセプターの組み換えのときに生じる環状DNA，KREC(Kappa-deleting recombination excision circles)はB細胞新生のマーカーで（図2下)，B細胞がない患者の末梢血やガスリーで欠損している[14]．PID患者におけるTRECとKRECのコピー数の分布をみると，両者が正常なものはCVID，IgA欠損症，B細胞が欠損しているものがXLA，T細胞欠損がSCID，T細胞B細胞欠損がSCIDとATの患者であった[15]．しかしCVID患者多数例でTRECとKRECコピー数の分布をみると，CVIDの中に従来のCVID以外にB細胞が欠損しているものやT細胞が欠損しているもの，両者が欠損しているものが含まれることが明らかとなった[16](図3)．CVIDは成人で最も頻度が高い先天性（＋後天性）免疫不全症で，感染症，悪性腫瘍，自己免疫疾患を発症する．ほとんどが原因不明で，一部で原因遺伝子が明らかとなっている[17]．CVIDはTREC/KRECの測定から4グループに分類されることが明らかになり，今後CVIDの病因・病態の解明と，病態に応じた治療の開発が必要である．成人に多いGood症候群は胸腺腫を伴う低γグロブリン血症，B細胞数減少あるいは欠損，T細胞の機能異常が特徴であるが，2011年のN Engl J MedにIkaros-nullノックアウトマウスに類似した成人女性の症例が報告された[18]．その後，33週で生まれた羊水過多，胎児水腫，腹水を呈した新生児で，生下時より汎血球減少，T細胞数は正常でB細胞NK細胞欠損があり，IKAROSの遺伝子変異が見出された[19]．IKAROS欠損症はこのようにpancytopeniaを来すとともにB細胞NK細胞の分化障害を呈しT細胞は正常に分化する．我々が経験した4生月にニューモシスティス肺炎で発症した新規の先天性免疫不全症では，抗体産生不全，B細胞・NK

図3. CVIDの病態・病因

細胞欠損，pDC欠損を呈した．Pro-B cell，CLP（common lymphoid progenitors），Pro-pDCも欠損していた．NOD/SCID/IL2RγKOマウスに患者の骨髄CD34陽性細胞を移植したところ，CD19陽性細胞欠損，pDC欠損，NK欠損，B細胞欠損と同様の表現型が出現したので，外因性でなく内因性の異常と考えられる．想定される遺伝子に変異は見られず，エキソームシークエンシング等で解析中である．

3）単球・樹状細胞欠損症

樹状細胞はこのようにMyeloid DCとPlasmacytoid DCなどがあるが，GATA2異常症/DCML欠損症/MonoMAC症候群ではGATA2遺伝子変異により*Mycobacterium avium* complex感染や真菌・ウイルス感染等がおきやすく，検査では単球・DC欠損，B細胞・NK細胞欠損，免疫グロブリン正常，T細胞正常が明らかとなっている．IRF8異常症ではIRF8遺伝子変異により*Mycobacterium avium* complex感染，真菌，ウイルス感染に易感染性を呈し，B細胞・NK・T細胞・免疫グロブリン正常であるが，単球減少・DC欠損が見出されている[20]．

2. 遺伝子治療

1990年に行われたADA欠損症に対する遺伝子治療は末梢T細胞を標的としたもので十分な効果は得られなかった．2000年のX連鎖SCIDに対する遺伝子治療は極めて有効であったが，数年後に白血病が多発したため，10年近く遺伝子治療が停滞した．その後ベクターの改良も進み，X連鎖SCID，ADA欠損症，Wiskott-Aldrich症候群（WAS），X連鎖慢性肉芽腫症（効果は一過性）などで，CD34陽性細胞に遺伝子導入する遺伝子治療法は世界的に有効と認識されている．しかし遺伝子治療が行われたWAS 10例中4例に最近白血病が起こったので（2012 ASGCT）[21]，さらなるベクターの改良が必要である．またSCID（IL7Rα，RAG-1/RAG-2，JAK3，Artemis），SAP（signaling lymphocyte activation molecule-associated protein）欠損症，Perforin欠損症，CD40L欠損症，Btk欠損症，Blnk欠損症，IPEX（immunodysregulation polyendocrinopathy enteropathy X-linked syndrome）などの遺伝子治療研究も進められている[21]．日本ではADA欠損症の遺伝子治療が以前行われ，X連鎖慢性肉芽腫症の治療が近々始まる予定である．今後iPS細胞を用いた遺伝子治療も進んでいくと考えられる．

おわりに

今後日本でもT細胞・B細胞欠損の新生児スクリーニングを行い，早期診断・早期治療を行える体制を構築する必要がある．造血幹細胞から分化する免疫細胞の欠陥による先天性免疫不全症は遺伝子レベルで多くは解明されたが，未だ不明の疾患もあり今後病因の解明と新規治療法の開発を期待したい．

著者のCOI（conflicts of interest）開示：本論文発表内容に関連して特に申告なし

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第44回日本小児感染症学会会長講演

小児感染・免疫疾患の発症におけるヒト-環境相互作用

原　寿郎*

はじめに

ヒトの感染症は，ヒトと病原体の相互作用の総和として発症する．従来感染症においては，外部環境要因が大きな比重を占めていた．日本においては生活衛生環境が改善されるにつれ宿主側要因の比重が高くなり，環境要因では内部環境要因（ヒト微生物叢）の重要性が相対的に高くなってきている．

世界における5歳未満の小児死亡では41%が新生児死亡で，肺炎，敗血症，破傷風，下痢，麻疹，マラリア，エイズ，百日咳，髄膜炎など約2/3が感染症である（**図1**）[1]．先進国における5歳未満の小児死亡は日本20%，ドイツ16%，フランス28%，アメリカ20%が感染症によるもので，日本においても下痢，肺炎，新生児敗血症，その他などの感染症による死亡がある（**図2**）．

九州大学病院小児医療センター・NICUの10年間の死亡例の解析では，19%が感染症による死亡で，基礎疾患がないものが1%（乳児心筋炎）であった．残り18%が基礎疾患を有し，基礎疾患としては，6%（新生児），7%（悪性腫瘍・血液免疫疾患），5%（先天異常）であった．

先進国での感染症の死亡例の多くは新生児か基礎疾患を有する小児で，生理的な免疫不全状態，原発性免疫不全症候群，続発性免疫不全，解剖生理学的な異常，このようなものを十分理解してこれらに伴う死亡例を減らす必要がある．

Ⅰ．ヒト-微生物相互作用

1．小児における自然免疫の重要性

感染症は宿主と病原体の相互作用の総和として発症するので，病原体の理解とともに，病原体別の感染防御機構の理解，疾患あるいは治療別の感染防御機構の問題点の理解が必要になる．これらを理解すると，すべての基礎疾患に対応が可能となる（**図3**）．

自然免疫とは，その病原体に遭遇することなしに自然に備わっている免疫のことで，5歳未満の小児，あるいは乳幼児では自然免疫の役割が感染防御，疾患発症のうえで重要である．自然免疫疾患であるIRAK4/MyD88欠損症による重症感染症の発症は大部分乳幼児期に起こり（国際共同研究）[2]，TLR3/UNC93B1/TRAF3/TRIF/TBK1欠損症によるヘルペス脳炎発症も乳幼児期である[3]．5歳未満の小児では，自然免疫が感染防御に非常に重要な役割を果たし，成人では獲得免疫の発達とともに代償されるようである．

感染防御機構として，皮膚粘膜など物理的バリアや生物学的バリアとしての正常の常在菌に加え，化学的バリア，体液性バリア，細胞性バリアなどがある．自然免疫細胞としては顆粒球（好中球，好酸球，好塩基球），NK細胞，マクロファージ，樹状細胞，獲得免疫との中間としてγδT細胞やNKT細胞がある[4]．自然免疫系の細胞は微生物を構造パターン認識受容体（pattern recognition receptors：PRRs）で感知する．自然免疫受容体に

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