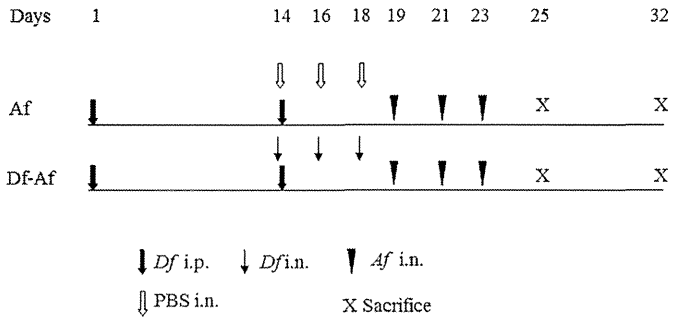

Figure 1. Experimental protocol. BALB/c mice were immunized intraperitoneally (i.p.) on days 1 and 14 with 0.5 mg of *Dermatophagoides farinae* (*Df*) precipitated in aluminum hydroxide. *Aspergillus fumigatus* (*Af*) group: Mice were sham-sensitized intranasally (i.n.) with phosphate buffered saline (PBS) on days 14, 16, and 18 and infected intranasally with *Af* on days 19, 21, and 23. *Df-Af* group: After immunization with *Df*, mice were challenged intranasally with *Df* allergen on days 14, 16, and 18. Subsequently, mice were infected intranasally with *Af* on days 19, 21, and 23. On days 25 and 32, all mice were sacrificed (n = 6 for each group).

authors hypothesized that the T_H2-skewed immunity in a murine model of asthma could contribute to impairment of the T_H1 and T_H17 response against *Af*. In the present study, these issues were addressed by comparing fungal burden between *Af*-infected control mice (*Af* mice) and *Af*-infected *Dermatophagoides farinae* (*Df*)–sensitized mice (*Df-Af* mice) in a murine model of asthma, and then, in a different set of experiments, by comparing the production of some cytokines, including IL-12, IL-4, IL-23, interferon-γ (IFN-γ), IL-5, and IL-17, between *Af* mice and *Df-Af* mice. In addition, the phagocytotic activity against *Af* and the expression of pathogen recognition receptors on AMs were compared in AMs isolated from untreated naive mice and mite allergen-sensitized mice.

Methods

Preparation of Af Conidia

The *Af* MF-13 isolated from the sputum of a patient with pulmonary aspergilloma was prepared for intranasal infection, as described previously.[10] The *Af* MF-13 was subcultured on Sabouraud dextrose agar (Becton Dickinson, Cockeysville, Maryland) at 30°C for 7 days. Then, conidia were harvested with sterile saline containing 0.02% Tween-80 (Wako Pure Chemical Industries, Tokyo, Japan). The suspension was filtered through a 40-μm cell strainer (Falcon, Tokyo, Japan) to separate the conidia from the contaminating mycelia and was verified microscopically (100% resting conidia). The suspension was counted in a hemocytometer and diluted with sterile saline.

Experimental Protocol

Female BALB/c mice (5 and 10 weeks old) were purchased from Charles River (Yokohama, Japan) and housed in a specific pathogen-free facility. As illustrated in Figure 1, mice were immunized twice intraperitoneally on days 1 and 14 with 0.5 mg of *Df* (crude extract of mite: LG-5339; Cosmo Bio, Tokyo, Japan) precipitated in aluminum hydroxide. Then, mice were challenged intranasally with 50 μg/50 μL of *Df* allergen (*Df-Af* group) or phosphate buffered saline (*Af* group) on days 14, 16, and 18. The 2 groups of mice were intranasally infected with 1×10^5 *Af* conidia on days 19, 21, and 23. Two days (day 25) or 9 days (day 32) after infection, the 2 groups of mice were sacrificed to obtain bronchoalveolar lavage (BAL) fluid and lung tissues. The procedures were reviewed and approved by the Nagasaki University School of Medicine committee on animal research. All experiments were repeated at least 3 times.

Bronchoalveolar Lavage and Lung Pathology

The BAL was conducted with 1 mL of phosphate buffered saline in the immediate postmortem period. Obtained BAL samples were

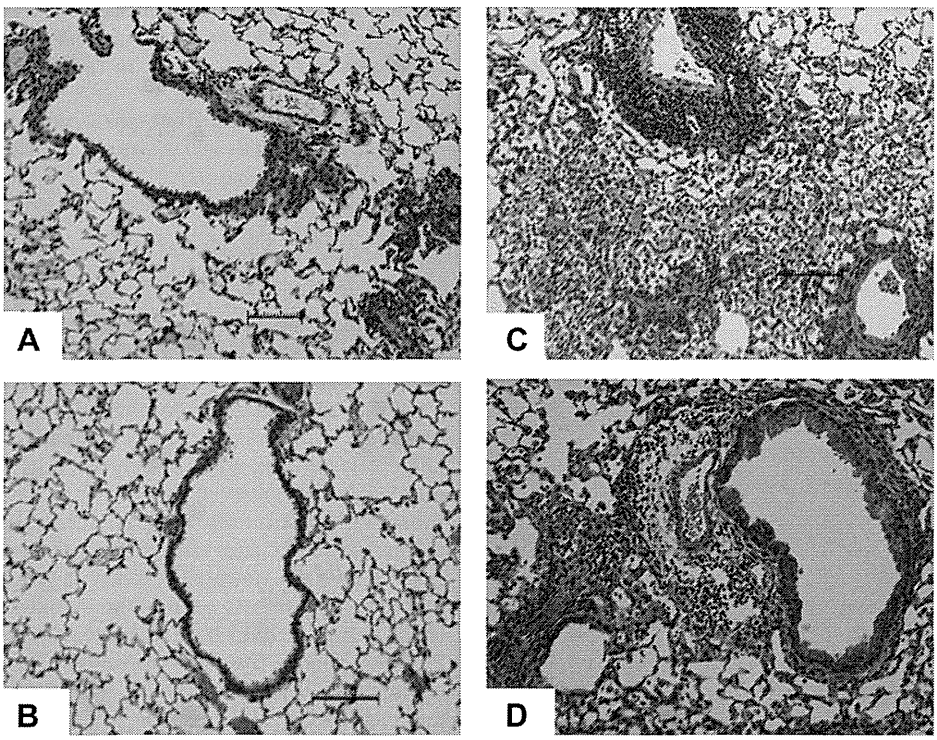

Figure 2. Pulmonary pathology (hematoxylin and eosin stain). Lung tissues were obtained from each group. Representative photomicrographs display lung tissues from each group (n = 6 for each group). *Aspergillus fumigatus*–infected mice at *(A)* day 25 and *(B)* day 32. *Aspergillus fumigatus*–infected *Dermatophagoides farinae*–sensitized mice at *(C)* day 25 and *(D)* day 32.

Table 1
Differential cell counts in bronchoalveolar lavage fluid[a]

	Macrophages ($\times 10^5$ cells)	Neutrophils ($\times 10^5$ cells)	Lymphocytes ($\times 10^5$ cells)	Eosinophils ($\times 10^5$ cells)
Af at day 25	13.8 ± 3.6	12.6 ± 7.8	1.7 ± 0.2	1.1 ± 0.7
Af at day 32	6.8 ± 2.5[b]	0.4 ± 0.8[b]	1.0 ± 0.5[b]	0.5 ± 0.2[b]
Df-Af at day 25	12.6 ± 4.1	9.8 ± 3.2	4.4 ± 5.1[b]	6.2 ± 1.7[b]
Df-Af at day 32	11.3 ± 3.9[b,c]	6.8 ± 2.9[b,c]	3.4 ± 1.9[b,c]	4.1 ± 2.0[b,c]

Abbreviations: *Af, Aspergillus fumigatus*–infected mice; *Df-Af, Aspergillus fumigatus*–infected *Dermatophagoides farinae*–sensitized mice.
[a]Results are expressed as mean ± SEM (n = 6 for each group).
[b]$P < .01$ vs *Af* at day 25.
[c]$P < .01$ vs *Af* at day 32.

centrifuged. Differential cell counts were performed using cytocentrifuged BAL samples stained with May-Grünwald-Giemsa stain. Formaldehyde fixative was gently infused through the lavage catheter set in the trachea. Resected lungs were fixed for an additional 24 hours and embedded in paraffin. Sections (4 μm) were stained with hematoxylin and eosin. After BAL, paraffin-embedded lung tissues were prepared for hematoxylin and eosin and Gomori methenamine-silver staining. For fungal-burden examination, numbers of colony-forming units per lung tissue were calculated as described elsewhere.[10]

Analysis of Cytokine Concentrations in Homogenized Lung

Lung homogenates were prepared by homogenizing a freshly excised lung. Concentrations of IL-12, IL-4, IL-23, IFN-γ, IL-5, and IL-17 in homogenized lung samples were measured by enzyme-linked immunosorbent assay, in accordance with the manufacturer's directions (Endogen, Wobum, Massachusetts).

Phagocytic Function of Alveolar Macrophages

In a different set of experiments, AMs were prepared from naive mice without any treatment and a murine model of asthma, which were prepared as described earlier. Lung tissues were chopped with sterile scissors, digested in a 37°C water bath for 2 hours in digestion buffer containing 1.5 mg/mL of collagenase A (type 1A; Boehringer Mannheim, Mannheim, Germany), and filtered with a metal mesh. After washing 3 times with RPMI-1640 medium (Gibco-BRL Life Technology, Grand Island, New York) containing 10% heat-inactivated fetal bovine serum, 100 U/mL of penicillin, and 100 μg/mL of streptomycin, cells were resuspended in the medium. Mononuclear cells were isolated using a density gradient method with Ficoll (Amersham Pharmacia Biotech, Piscataway, New Jersey). Fetal bovine serum was put into a dish, which was incubated at 37°C for 15 minutes. After the fetal bovine serum was discarded, suspended cells were placed in this dish and incubated overnight at 37°C. Thereafter, cells in the dish were collected using phosphate buffered saline containing ethylenediaminetetraacetic acid. Aliquots (1 mL) of cell suspension (10^6 cells/mL) were mixed with 1 mL of *Af* suspension (10^6 cells/mL) opsonized with 100 μL of normal serum and incubated for 60 minutes at 33°C. Ten minutes before completion of incubation, methylene blue (0.01%) was added. Then, 200 conidia were examined and the number of phagocytosed conidia was counted in 3 representative regions. Results are expressed as an index representing the percentage of phagocytosed *Af* conidia.

Analysis of Expression of TLR4 and Dectin-1 on AMs

To determine the effects of pathogen recognition receptor expression on AMs on phagocytotic activity, the expression of TLR4 and dectin-1 on AMs was determined by real-time polymerase chain reaction (PCR). In a different set of experiments, AMs were prepared from *Af* mice and *Df-Af* mice on days 25 and 32, as mentioned earlier. Total RNA also was isolated from each group of AM with TRIzol (Life Technologies, Gaithersburg, Maryland) using the method recommended by the supplier. A High-Capacity cDNA Archive Kit (Applied Biosystems, Tokyo, Japan) was used to synthesize cDNA from 2 μg of total RNA and 200 ng of cDNA was

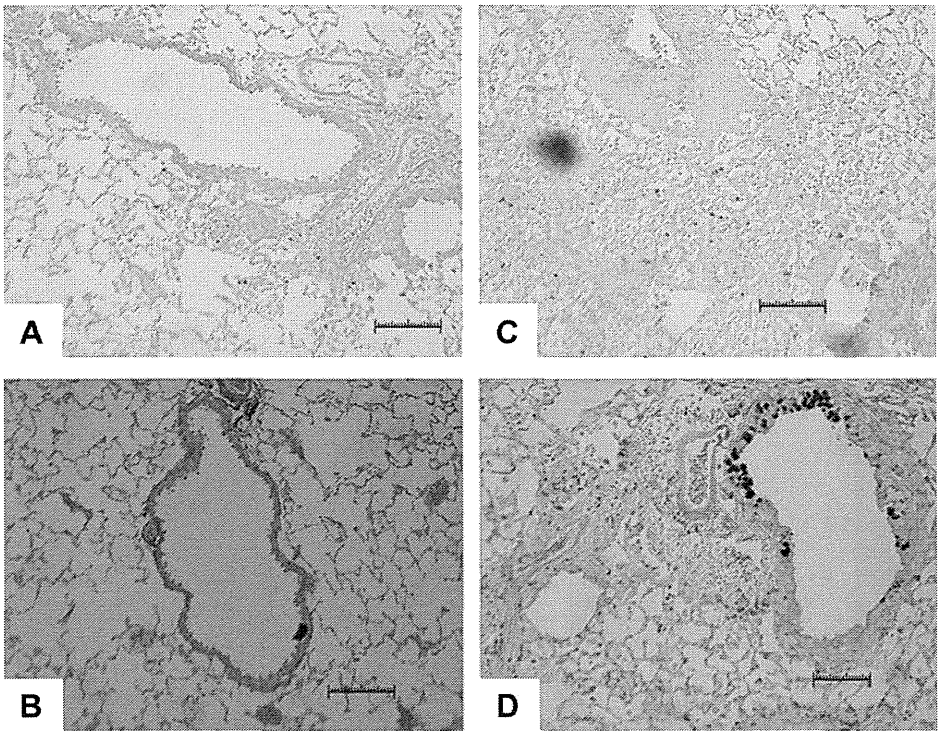

Figure 3. Pulmonary pathology (Gomori methenamine-silver stain). Representative photomicrographs display lung tissues from each group (n = 6 for each group). *Aspergillus fumigatus*–infected mice at *(A)* day 25 and *(B)* day 32. *Aspergillus fumigatus*–infected *Dermatophagoides farinae*–sensitized mice at *(C)* day 25 and *(D)* day 32.

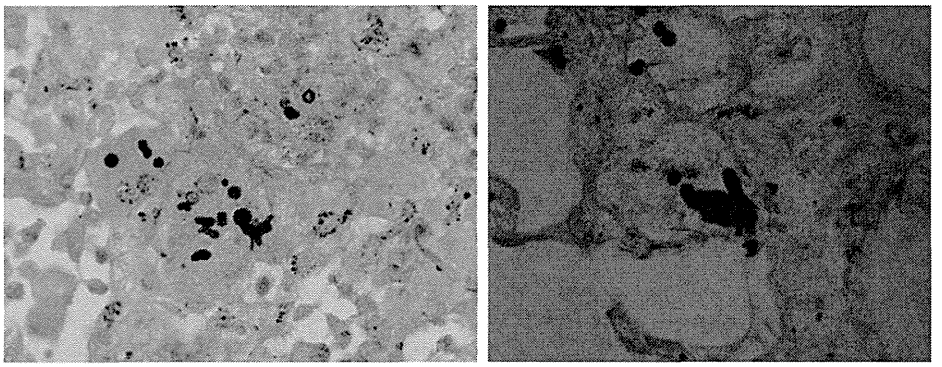

Figure 4. Form of *Aspergillus fumigatus* conidia found in lung tissue from *Aspergillus fumigatus*–infected *Dermatophagoides farinae*–sensitized mice on day 32 (Gomori methenamine-silver-stain). Representative high-resolution photomicrographs of *Aspergillus fumigatus* conidia in lung tissue from *Aspergillus fumigatus*–infected *Dermatophagoides farinae*–sensitized mice on day 32 display conidia germination.

amplified by primers complementary to the published sequences of murine TLR4, dectin-1, and control glyceraldehyde 3-phosphate dehydrogenase. Quantitative real-time PCR was performed on an ABI 7500 (Applied Biosystems) using TaqMan Universal PCR Master Mix (Applied Biosystems). Probes (IDT, Coralville, Iowa) labeled with 5′ FAM and 3′ TAMRA modifications were used at a final concentration of 0.9 mmol/L, and primers were used at 0.2 mmol/L (Gibco-BRL). The PCR program was as follows: 50°C for 2 minutes and 95°C for 10 minutes, 95°C for 15 seconds, and 60°C for 1 minute for 40 cycles. Specific signals were normalized against the signals from constitutively expressed glyceraldehyde 3-phosphate dehydrogenase. Data are presented as relative mRNA units and represent the average of at least 3 values ± SEM.

Statistical Analysis

Results are expressed as mean ± SEM. Differences between groups were examined for statistical significance using repeated-measures analysis of variance with a Bonferroni multiple comparison test. *P* values less than .05 were considered significant.

Results

Pulmonary Inflammation

Representative pulmonary pathologies of the 2 groups of mice sacrificed on days 25 and 32 after *Af* infection are shown in Figure 2. Neutrophilic inflammation was observed only on day 25 and disappeared by day 32 in *Af* mice. The airways of *Df-Af* mice exhibited neutrophilic and eosinophilic inflammation on day 25, which persisted to day 32. Pathologic changes were confirmed in a quantitative manner by BAL (Table 1). In *Df-Af* mice, total cell counts were significantly elevated compared with *Af* mice on day 32. Irrespective of sacrifice day, airway eosinophils were significantly elevated in *Df-Af* mice compared with *Af* mice. Airway neutrophils were significantly fewer in *Df-Af* mice compared with *Af* mice on day 25, although they were significantly more numerous in the former compared with the latter on day 32.

Aspergillus fumigatus Pathology, *Fungal Burden, and Phagocytosis*

Representative pulmonary pathologies (Gomori methenamine-silver stain) of the 2 groups of mice sacrificed on days 25 and 32 after *Af* infection are shown in Figure 3. *Aspergillus fumigatus* conidia were found only on day 25 and disappeared by day 32 in *Af* mice. However, *Af* conidia persisted in the airway of *Df-Af* mice on day 32, and some of these conidia had germinated (Fig 4). Quantitative evaluation of fungal burden in lung tissue showed that a significantly larger number of *Af* conidia was present on days 25 and 32 in *Df-Af* mice compared with *Af* mice (Fig 5). To determine the mechanisms of increased fungal number in *Df-Af* mice, phagocytosis of *Af* conidia by AMs isolated from naive mice without any treatment and AMs isolated from the murine model of asthma was compared. Compared with AMs isolated from naive mice, those from the murine model of asthma showed a significant decrease in phagocytosis (Fig 6). Compared with AMs isolated from *Af* mice on day 25, TLR4 and dectin-1 expression on AMs isolated from *Df-Af*

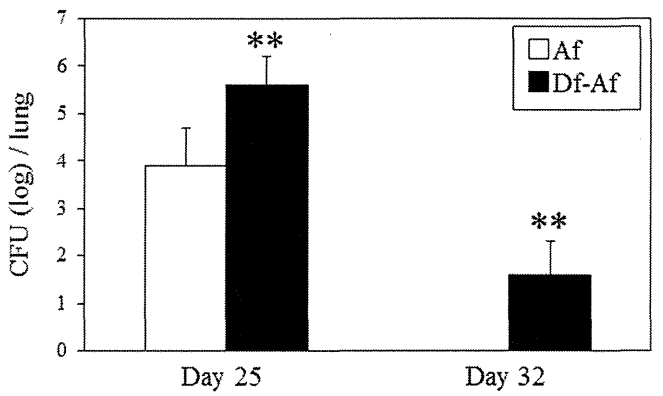

Figure 5. *Aspergillus fumigatus* fungal burden. Fungal burden in lung tissue from the 2 groups was quantitatively evaluated. Results are expressed as mean ± SEM (n = 6 for each group). $^{**}P < .01$ vs *Af*. *Af*, *Aspergillus fumigatus*–infected mice; *Df-Af*, *Aspergillus fumigatus*–infected *Dermatophagoides farinae*–sensitized mice.

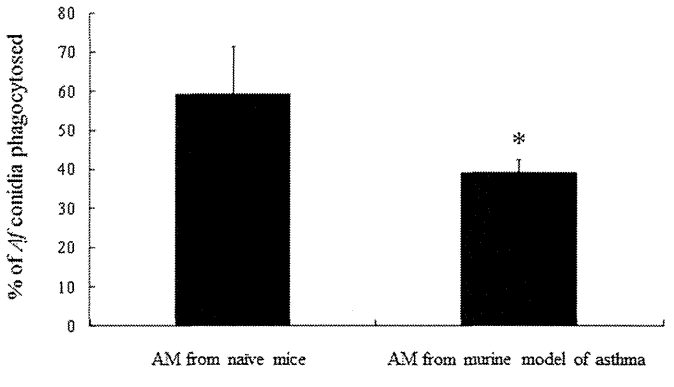

Figure 6. Alveolar macrophage (AM) phagocytotic activity against *Aspergillus fumigatus* (*Af*) conidia. The AMs isolated from naive mice and a murine model of asthma were cultured with *Af* conidia. The number of phagocytosed conidia in each mouse was counted. Results are expressed as an index representing the percentage of phagocytosed *Af* conidia. *Bars* represent mean ± SEM (n = 6). $^{*}P < .01$ vs naive mice.

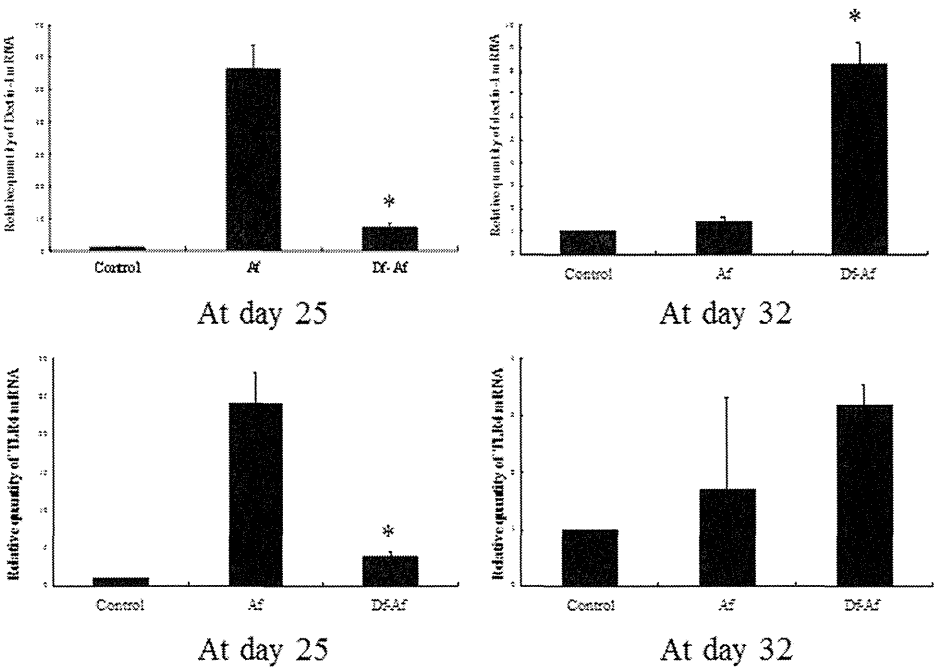

Figure 7. Quantitative analysis of TLR4 and dectin-1 mRNA expression in alveolar macrophages. Expression of TLR4 and dectin-1 mRNA of alveolar macrophages isolated from *Af* mice and *Df-Af* mice on days 25 and 32 was determined by quantitative real-time polymerase chain reaction and is depicted as the number of transcripts per 10^3 copies of the housekeeping gene glyceraldehyde 3-phosphate dehydrogenase. Data from experiments with cells from each group are summarized and presented as mean ± SEM (n = 8 for each group). *$P < .01$ vs *Af* mice. *Af, Aspergillus fumigatus*–infected mice; *Df-Af, Aspergillus fumigatus*–infected *Dermatophagoides farinae*–sensitized mice; TLR4, Toll-like receptor-4.

mice on day 25 showed a significant decrease. Compared with AMs isolated from *Af* mice on day 32, dectin-1 expression on AMs isolated from *Df-Af* mice at day 32 showed a significant increase (Fig 7).

Cytokine Profile in Lung Homogenate

Analysis of cytokine concentrations in lung homogenates (Fig 8) showed that, compared with *Af* mice sacrificed on day 25, *Df-Af* mice sacrificed on day 25 showed significant increases in T_H2-like cytokines (IL-4 and IL-5) and significant decreases in T_H1-like (IFN-γ and IL-12) and IL-17 production. In *Af* mice, T_H1-like and T_H17-like (IL-23 and IL-17) cytokines significantly decreased on day 32 compared with those on day 25. T_H2-like cytokines in *Df-Af* mice significantly decreased on day 32 compared with day 25 but were still significantly higher than in *Af* mice. In marked contrast, IL-17 levels in *Df-Af* mice increased significantly on day 32 compared with those on day 25, and T_H17-like cytokines in *Df-Af* mice increased significantly compared with those in *Af* mice.

Discussion

In this study, after *Af* infection, the production of cytokines involved in protective immunity against *Af* and the phagocytotic activity of AMs decreased in a murine model of allergic asthma. Experimental studies have indicated a critical role for macrophages in conidial defense.[11,12] It also has been reported that TLR4 on macrophages is required for an optimal immune response to *Af* in vivo.[5] In contrast, neutrophils play a predominant role in killing hyphae.[13,14] In addition, other innate immune cell subsets contribute to antifungal defense. For example, pulmonary dendritic cells transport conidia to draining mediastinal lymph nodes to activate fungus-specific T cells, and when *Af* arrives in the airways, *Af*-specific T cells are rapidly primed and fully differentiated into IFN-γ–producing T_H1 $CD4^+$ T cells in immune-competent mice. Thereafter, inhaled conidia are rapidly cleared from the airway.[15] In the present study, after infection with *Af* conidia, IL-12 and IFN-γ production in the airway and expression of TLR4 on AMs were decreased in a murine model of asthma compared with control mice. In addition, the phagocytosis of *Af* conidia by AMs isolated from the murine model of asthma was impaired compared with AMs isolated from untreated naive mice. This may be due to the pre-existing T_H2-skewed immunity in asthmatic airways, which inhibits T_H1 cytokine production against *Af* infection and decreases IFN-γ, leading to decreased phagocytosis of *Af* conidia by the low expression of TLR4 on AMs.

In addition to pre-existing T_H2-skewed immunity before *Af* infection, IL-17 levels increased after *Af* infection in *Df-Af* mice. Recently, it has been reported that excess T_H17 immunity attenuates antifungal immune defense.[16,17] It also has been reported that the T_H17 response is initiated thorough the recognition of β-glucan, which increases on the surface of fungi during their growth from conidia to hyphae.[18] In addition, the authors previously reported that high levels of ligand for dectin-1 receptors induce upregulation of these receptors on antigen-presenting cells and enhance signaling.[19] It is likely that pre-existing T_H2 immunity attenuated T_H1 immunity, which permitted colonization of conidia in the asthmatic airway in the present study. Subsequently, the growth of conidia to hyphae could further stimulate dectin-1 in the host, thus resulting in higher levels of IL-17 and IL-23 production. Persistent colonization of *Af* may maintain significantly higher levels of IL-17 and IL-23 in the asthmatic airway compared with those in controls by a continuous stimulation of dectin-1 signaling. It also has been reported that T_H17 immunity not only attenuates T_H1 immunity but also upregulates T_H2 immunity.[16,20] It also has been reported that protease secreted from *Af* and IL-17 induce enhanced MUC5AC gene expression in airway epithelial cells.[21,22] Collectively, the present study suggests that pre-existing T_H2-skewed immunity in asthma permits *Af* to colonize in the airway by inhibiting innate antifungal defense. Once colonized in the airway, *Af* stimulates excess

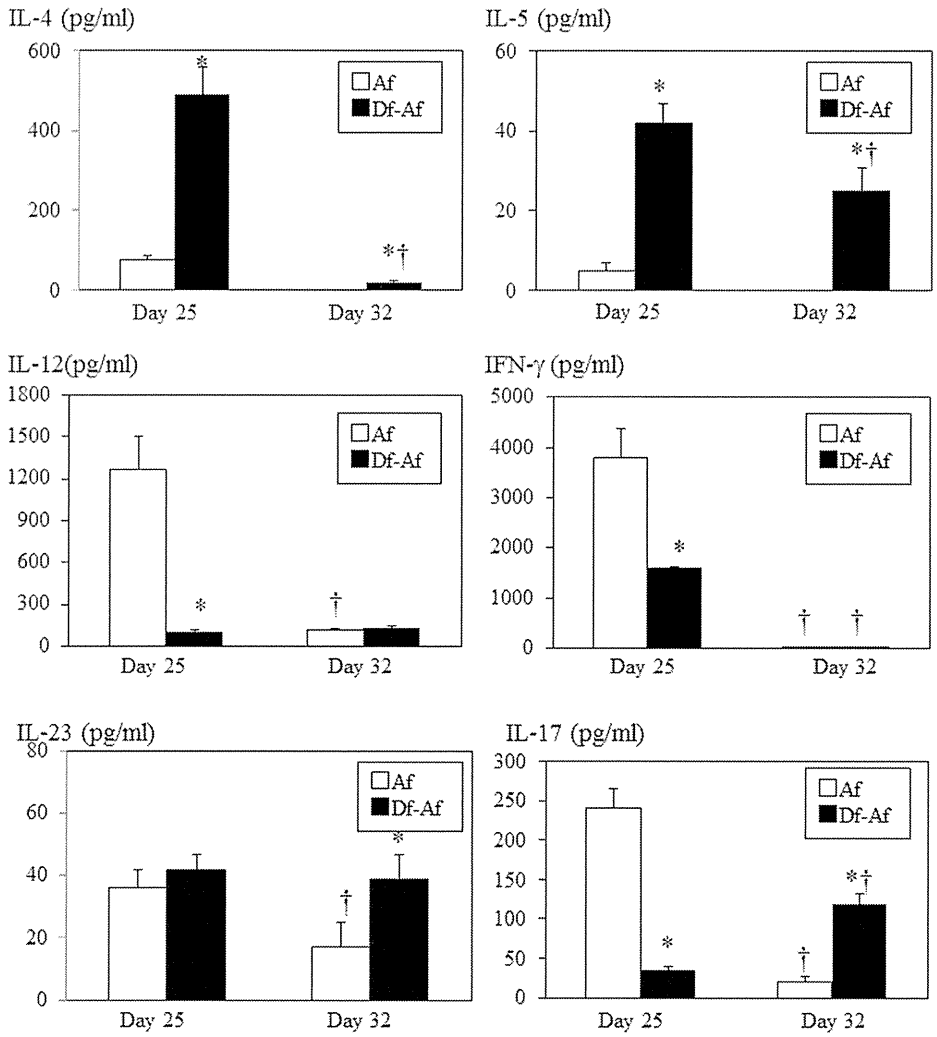

Figure 8. Cytokine profile in lung homogenates. Cytokine concentrations in lung homogenates in each mouse were determined by enzyme-linked immunosorbent assay. *Bars* represent mean ± SEM (n = 6). *$P < .01$ vs *Af*, †$P < .01$ vs day. *Af*, *Aspergillus fumigatus*—infected mice; *Df-Af*, *Aspergillus fumigatus*—infected *Dermatophagoides farinae*—sensitized mice; IL, interleukin; IFN, interferon.

expression of dectin-1 and T_H17 immunity, which further enhances *Af* colonization by upregulating T_H2 immunity and overproduction of mucus in a vicious circle.

In contrast, other investigators have reported that in the murine model of asthma, the ingestion potential of conducting airway neutrophils is enhanced compared with control mice.[23] Interestingly, in the present study, although colonization of *Af* in the airway of the murine model of asthma was seen, penetration of *Af* into the airway epithelial barrier and dissemination of *Af* into the airway was not seen. The reason for this may be that the enhanced phagocytotic activity of neutrophils in the murine model of asthma controlled the development of colonization of *Af* to dissemination of *Af* in the airway.

A distinct characteristic feature of *Df-Af* mice in the present study included neutrophilic airway inflammation. In this regard, several studies have indicated that IL-17 is important for neutrophilic inflammation in patients with acute airway inflammation.[24–26] Airway neutrophils also were associated with IL-17 in the lung tissues in the present study. A key characteristic of fungal-associated asthma is the increased severity of asthma. Neutrophilic airway inflammation caused by *Af* may explain at least in part the increased severity of fungus-associated asthma. Indeed, current anti-inflammatory therapies for asthma, including inhaled corticosteroids, are effective in managing eosinophilic airway inflammation but have little or no impact on neutrophilic airway inflammation.[27,28] Accordingly, additive treatment, which has an impact on neutrophilic inflammation, is required for fungus-associated asthma. Thus, the development of a therapeutic modality targeting IL-17 for the treatment of fungus-associated asthma is a critical issue for the future.

However, this study has several limitations. Only results for mice are described, and it is uncertain whether these results can be applied to humans. In addition, although the authors hypothesized that T_H2-dominant immune response could contribute to the impairment of T_H1 response against an *Af* challenge, they did not directly show whether a specific T_H2 response inhibition in the murine model of asthma improves the T_H1 response against the *Af* challenge.

In conclusion, these results support the mechanism of *Af* colonization in the asthmatic airway. Mite allergen sensitization concomitant with *Af* infection enhanced the T_H2-dominant immune response in the airway, in which the T_H1 response against *Af* conidia infection was attenuated and the T_H17 response against *Af* was promoted, which impair antifungal defense and permit further colonization of *Af* in the asthmatic airway. T_H17-associated neutrophilic airway inflammation may be involved in the pathogenesis of steroid-resistant severe asthma with fungal sensitization.[29]

References

[1] Latge JP. *Aspergillus fumigatus* and aspergillosis. *Clin Microbiol.* 1999;12:310–350.

[2] Garlanda C, Hirsch E, Bozza S, et al. Non-redundant role of the long pentraxin PTX3 in anti-fungal innate immune response. *Nature.* 2002;420:182–186.

[3] Madan T, Kishore U, Singh M, et al. Protective role of lung surfactant protein D in a murine model of invasive pulmonary aspergillosis. *Infect Immun.* 2001;69:2728–2731.

[4] Luther K, Torosantucci A, Brakhage AA, Heesemann J, Ebel F. Phagocytosis of *Aspergillus fumigatus* conidia by murine macrophages involves recognition by the dectin-1 beta-glucan receptor and Toll-like receptor 2. *Cell Microbiol.* 2007;9:368–381.

[5] Meier A, Kirschning CJ, Nikolaus T, Wagner H, Heesemann J, Ebel F. Toll-like receptor (TLR) 2 and TLR4 are essential for *Aspergillus*-induced activation of murine macrophages. *Cell Microbiol.* 2003;5:561–570.

[6] Gersuk GM, Underhill DM, Zhu L, Marr KA. Dectin-1 and TLRs permit macrophages to distinguish between different *Aspergillus fumigatus* cellular states. *J Immunol.* 2006;176:3717–3724.

[7] LeibundGut-Landmann S, Gross O, Robinson MJ, et al. Syk- and CARD9-dependent coupling of innate immunity to the induction of T helper cells that produce interleukin 17. *Nat Immunol.* 2007;8:630–638.

[8] Nakayama T, Yamashita M. Initiation and maintenance of Th2 cell identity. *Curr Opin Immunol.* 2008;20:265–271.

[9] Harrington LE, Hatton RD, Mangan PR, et al. Interleukin 17–producing $CD4^+$ effector T cells develop via a lineage distinct from the T helper type 1 and 2 lineages. *Nat Immunol.* 2005;6:1123–1132.

[10] Takazono T, Izumikawa K, Mihara T, et al. Efficacy of combination antifungal therapy with intraperitoneally administered micafungin and aerosolized liposomal amphotericin B against murine invasive pulmonary aspergillosis. *Antimicrob Agents Chemother.* 2009;53:3508–3510.

[11] Park SJ, Mehrad B. Innate immunity to Aspergillus species. *Clin Microbiol Rev.* 2009;22:535–551.

[12] Philippe B, Ibrahim-Granet O, Prévost MC, et al. Killing of Aspergillus fumigatus by alveolar macrophages is mediated by reactive oxidant intermediates. *Infect Immun.* 2003;71:3034–3042.

[13] Schaffner A, Douglas H, Braude A. Selective protection against conidia by mononuclear and against mycelia by polymorphonuclear phagocytes in resistance to Aspergillus. Observations on these two lines of defense in vivo and in vitro with human and mouse phagocytes. *J Clin Invest.* 1982;69:617–631.

[14] Mircescu MM, Lipuma L, van Rooijen N, Pamer EG, Hohl TM. Essential role for neutrophils but not alveolar macrophages at early time points following Aspergillus fumigatus infection. *J Infect Dis.* 2009;200:647–656.

[15] Bozza S, Gaziano R, Spreca A, et al. Dendritic cells transport conidia and hyphae of *Aspergillus fumigatus* from the airways to the draining lymph nodes and initiate disparate Th responses to the fungus. *J Immunol.* 2002;168:1362–1371.

[16] Zelante T, Bozza S, De Luca A, et al. Th17 cells in the setting of *Aspergillus* infection and pathology. *Med Mycol.* 2009;47(suppl 1):S162–S169.

[17] Zelante T, De Luca A, Bonifazi P, et al. IL-23 and the Th17 pathway promote inflammation and impair antifungal immune resistance. *Eur J Immunol.* 2007;37:2695–2706.

[18] Chamilos G, Ganguly D, Lande R, et al. Generation of IL-23 producing dendritic cells (DCs) by airborne fungi regulates fungal pathogenicity via the induction of T(H)-17 responses. *PLoS One.* 2010;5:e12955.

[19] Fukahori S, Matsuse H, Tsuchida T, et al. *Aspergillus fumigatus* regulates mite allergen-pulsed dendritic cells in the development of asthma. *Clin Exp Allergy.* 2010;40:1507–1515.

[20] Wakashin H, Hirose K, Maezawa Y, et al. IL-23 and Th17 cells enhance Th2-cell–mediated eosinophilic airway inflammation in mice. *Am J Respir Crit Care Med.* 2008;178:1023–1032.

[21] Oguma T, Asano K, Tomomatsu K, et al. Induction of mucin and MUC5AC expression by the protease activity of *Aspergillus fumigatus* in airway epithelial cells. *J Immunol.* 2011;187:999–1005.

[22] Chen Y, Thai P, Zhao YH, Ho YS, DeSouza MM, Wu R. Stimulation of airway mucin gene expression by interleukin (IL)-17 through IL-6 paracrine/autocrine loop. *J Biol Chem.* 2003;278:17036–17043.

[23] Shevchenko MA, Bolkhovitina EL, Servuli EA, Sapozhnikov AM. Elimination of *Aspergillus fumigatus* conidia from the airways of mice with allergic airway inflammation. *Respir Res.* 2013;14:14–78.

[24] Iwakura Y, Ishigame H, Saijo S, Nakae S. Functional specialization of interleukin-17 family members. *Immunity.* 2011;34:149–162.

[25] Nakae S, Saijo S, Horai R, Sudo K, Mori S, Iwakura Y. IL-17 production from activated T cells is required for the spontaneous development of destructive arthritis in mice deficient in IL-1 receptor antagonist. *Proc Natl Acad Sci U S A.* 2003;100:5986–5990.

[26] Hellings PW, Kasran A, Liu Z, et al. Interleukin-17 orchestrates the granulocyte influx into airways after allergen inhalation in a mouse model of allergic asthma. *Am J Respir Cell Mol Biol.* 2003;28:42–50.

[27] Wenzel SE, Schwartz LB, Langmack EL, et al. Evidence that severe asthma can be divided pathologically into two inflammatory subtypes with distinct physiologic and clinical characteristics. *Am J Respir Crit Care Med.* 1999;160:1001–1008.

[28] Berry M, Morgan A, Shaw DE, et al. Pathological features and inhaled corticosteroid response of eosinophilic and non-eosinophilic asthma. *Thorax.* 2007;62:1043–1049.

[29] Denning DW, O'Driscoll BR, Hogaboam CM, Bowyer P, Niven RM. The link between fungi and severe asthma: a summary of the evidence. *Eur Respir J.* 2006;27:615–626.

解　説　臨　床

アレルギー性気管支肺真菌症(ABPM)の現状と問題点

小熊　剛

要　旨　アレルギー性気管支肺真菌症(ABPM)は気道内に腐生する真菌(主にアスペルギルス)によって惹起されるアレルギー性気道・肺疾患であるが，その病態の詳細はいまだ不明であり，診断・治療は十分確立していない。現在のABPM臨床における問題点として，①病因論では，なぜ他の真菌に比しアスペルギルスがABPMを惹起しやすいのか，なぜ基礎疾患としての気管支喘息・嚢胞性線維症が多いのか不明であること，②診断においては，血清総IgE値の適切なcut-off値，中枢性気管支拡張・mucoid impactionの診断的意義，適切な診断基準が不明であること，③治療においてはステロイド剤・抗真菌剤投与の最適な投与法が不明であること，などが挙げられる。また，昨年行われた本邦初のABPMに関する全国調査では従来の海外の報告に比し，高齢発症が多く，スエヒロタケによるABPMの頻度が高いことなどが判明した。

小熊 剛：アレルギー性気管支肺真菌症(ABPM)の現状と問題点，呼吸34(2)：149—154，2015
キーワード：アレルギー性気管支肺真菌症　アレルギー性気管支肺アスペルギルス症　ABPM　ABPA

はじめに

アレルギー性気管支肺アスペルギルス症(allergic bronchopulmonary aspergillosis：ABPA)はHinsonら[1]によってはじめて報告された，気道内に腐生するアスペルギルスによって引き起こされるアレルギー性気道・肺疾患である。同様の病態は他の真菌によっても惹起され，総称してアレルギー性気管支肺真菌症(allergic bronchopulmonary mycosis：ABPM)と呼称される。ABPAは再発例も多く，放置すれば肺の線維化・呼吸不全にいたる症例も存在する慢性疾患である。しかし，ABPAの病態はまだ十分に解明されておらず，診断・治療法も十分に確立していない。さらにアスペルギルス以外の真菌によるABPMは血清学的マーカーもないことからさらに不明な点が多い。本稿では昨年行われた本邦初のABPMに関する全国調査の検討の結果も踏まえ，ABPM臨床の現状・問題点を明らかにしたい。

Current issues and perspectives in allergic bronchopulmonary mycosis
東海大学医学部内科学系呼吸器内科学
Tsuyoshi Oguma
Division of Pulmonary Medicine, Department of Medicine, Tokai University School of Medicine, Kanagawa 259-1193, Japan

Ⅰ．病因・病態における問題点

ABPMは特定の真菌が気道内に頻回に侵入または腐生することにより，Ⅰ型アレルギーのみならず，IgG抗体も産生され，Ⅲ型アレルギーが引き起こされ，発症するとされている。本症の原因真菌としては*Aspergillus fumigatus*(*A. fumigatus*)の頻度が最も高いが，アスペルギルス属では*A. fumigatus*以外にも*A. niger*なども原因となることが知られている。その他，後述のように本邦ではスエヒロタケ(*Schizophyllum commune*)によるABPMの頻度が高い。その発症の要因としては真菌側の要因と宿主側の要因が挙げられる。

表1 各種病態における真菌感作の頻度

atopic patients(n=1,311)		SAFS(n=58)	
fungus	%	fungus	%
Alternaria spp.	14	*Aspergillus*	45
Cladosporium spp.	7	*Candida*	36
Aspergillus spp.	5	*Penicillum*	29
Fusarium spp.	3	*Cladosporium*	24
Rhizopus spp.	3	*Alternaria*	22
		Botrytis	18

(O'Driscoll BR, *et al*[10]. *Clin Exp Allergy* 39：2009, Gioulekas D, *et al*[20]. *J Invest Allergol Clin Immunol* 14：2004 より引用，改変)

1．真菌側の要因—なぜアスペルギルスはABPMを起こしやすいのか—

菌側の要因としてはアスペルギルス属がABPMの原因真菌としては圧倒的に頻度が高いことが挙げられる。興味深いことに表1[10][20]に示すように各種病態により，頻度の高い真菌は異なる。アトピー患者の吸入アレルゲンとしての真菌は，*Alternaria*，*Cladosporium* などに比し，必ずしもアスペルギルス属の頻度が突出して高いわけではない。近年，話題となっている真菌感作重症喘息(severe asthma with fungal sensitization：SAFS)の真菌感作の頻度はABPMと同様にアスペルギルスが高いが，他の真菌との差がABPMほど大きいわけではない。では，なぜ，ABPMの原因真菌として *A. fumigatus* の頻度が圧倒的に高いのだろうか。1つの可能性としては *A. fumigatus* は他の真菌に比し分生子のサイズが小さいこと，ヒト気道のような高温環境で発芽・成長し得るということがある[2]。また，筆者らは *A. fumigatus* は他の真菌に比し高いセリンプロテアーゼ活性を有し，その高いセリンプロテアーゼ活性依存性にムチン遺伝子(MUC5AC)を気道上皮細胞で強く誘導することを報告した[3]。気道粘液分泌過多はABPMの1つの特徴であり，*A. fumigatus* は高いセリンプロテアーゼ活性を有することで他の真菌に比し，ABPMの発症を惹起しやすい可能性が示唆された。

2．宿主側の要因—なぜ気管支喘息・嚢胞性線維症でABPMを起こしやすいのか—

一方，宿主側の要因として基礎疾患として気管支喘息，嚢胞性肺線維症を有する症例が圧倒的に多いことが挙げられる。気管支喘息・嚢胞性線維症は後述の診断基準で必須条件に挙げているものもある。気管支喘息はアレルギー性疾患であり，嚢胞性線維症は遺伝性疾患・感染性疾患であるが，両者の共通点として，いずれも気道の疾患であり，粘液分泌亢進を伴うことが挙げられる。分泌粘液の主成分であるムチン存在下で *A. fumigatus* が増殖しやすいとの報告もあり，気管支喘息・嚢胞性線維症の気道が *A. fumigatus* の増殖に好ましい環境である可能性がある。一方，基礎疾患以外にも遺伝学的解析もなされ，mannose-binding lectin，IL-4Rα，CFTR などの遺伝子変異との関連性が報告されている[4]。

Ⅱ．診断における問題点

以下の診断・治療における問題点に関してはABPAに絞って述べ，ABPMに関しては別途述べる。

1．検査所見における問題点

血清総IgEは診断，その後の経過観察時のいずれにも有用である。全身的ステロイド剤の投与なしで血清総IgE値が正常値以下の際は活動性のABPAは否定的であるとされ，診断基準にも盛り込まれている。しかし，ABPA診断のための至適なcut-off値に関しては417 IU/m*l*，1,000 IU/m*l* など意見が分かれる。Agarwalらの最近のreviewでは500 IU/m*l* ではover-diagnosisであるとし，1,000 IU/m*l* を推奨しているが，今後の検討が必要である[4]。なお，血清総IgE値は疾患活動性の評価に有用であることが知られており，25～50％の減少が治療の目安とされているが，こちらも明確な基準値はない。また，アスペルギルス特異的IgE抗体の至適cut off値にも異論が多い。Agarwalらは暫定的に>0.35 kUA/*l* をcut off値として挙げている[4]。

喀痰検査は診断に必須とはされず，あくまで補助的な検査とされている。陽性率は39～60％といわれている。近年，potato dextrose agar培地の有用性も報告され，今後，検出真菌の薬剤耐性の情報を知り得る喀痰培養の有用性はさらに高く評価されるであろう。

2．画像検査における問題点

以前のような画像検査が胸部X線であった時代に比し，HRCTが気軽に施行できる現在では画像での所見の解釈が異なってきている。胸部X線が主であった時代にはABPAで最も頻度の高い所見はconsolidationであった。しかし，CTでの検討ではmucoid impactionの頻度が多いことが報告されている。その他HRCTの所見で頻度が高いのはbronchiectasis，mosaic attenuation，centrilobular nodules，tree-in-bud opacities，pleuropulmonary fibrosisである。

ABPAで当初より画像上での特徴として挙げられ，診断にも重要とされてきた所見に中枢性気管支拡張がある。ABPAで特徴的な気管支拡張は中枢側で拡張し末梢側でtaperingする中枢性気管支拡張であり，当初は肺門部か

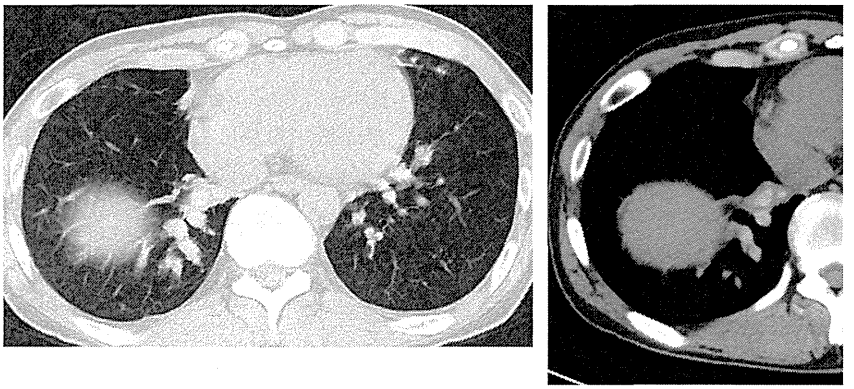

図1　ABPM の胸部 CT 像
高吸収を呈する mucoid impaction〔high-attenuation mucus(HAM)〕を認める。

ら胸壁の 2/3～1/2 の範囲での気管支拡張がその定義として用いられてきた。しかし，胸部 CT での検討では 30～40 %程度がその基準では末梢まで気管支拡張が広がっているといわれている。当初より気管支拡張は診断基準に挙げられていたが，その頻度は 40 %程度に留まっており，現在，気管支拡張はむしろ ABPA の合併症の 1 つと考えられている。

Mucoid impaction は一般的には低吸収であるが，ABPA の約 20 %の症例で高吸収を呈する mucoid impaction が存在し，high-attenuation mucus(HAM)と呼称される(**図1**)。カルシウム濃度の高い粘液栓であることがその画像的特徴の原因とされ，ABPA の重要な画像サインとして知られている。一方，HAM を有する ABPA は再発の頻度が高いことも知られている。

3．診断基準における問題点

本邦では長く 1977 年に Rosenberg らが提唱した基準が用いられてきた(**表2**)[5]。しかし，早期例，典型例では必ずしも項目を満たさないこと，日常的に皮内反応は施行されていない施設も多いこと，ABPA の重要な側面と認識されている粘液栓はあくまで 2 次基準とされ，必須とはされていないこと，など問題は多い。

一方，Greenberger らは ABPA を，中枢性気管支拡張を伴う ABPA-bronchiectasis とそれを伴わず血清学的見地から診断される ABPA-seropositive に分け，より早期の診断に適した基準を提唱した[6]。Rosenberg らの基準からのその他の変更点は沈降抗体の代わりに IgG 抗体とした点，IgE または IgG 抗体のいずれかが陽性であればよいこととした点，好酸球増多をその基準から外した点である。

さらに 2013 年には ISHAM(the inter national society for human animal mycology)が新しい基準を提唱した(**表3**)[4]。この基準ではいままで必須とされていた気管支

表2　Rosenberg らの診断基準

1 次基準
気管支喘息
末梢血好酸球増多
アスペルギルス抗原の即時型皮内反応陽性
血清総 IgE 値の上昇
アスペルギルスに対する沈降抗体陽性
肺浸潤影の既往(固定性または移動性)
中枢性気管支拡張
2 次基準
繰り返し喀痰からアスペルギルスが検出される
褐色の粘液栓を喀出した既往
アスペルギルス抗原へのアルサス型(遅延型)皮膚反応陽性
1 次基準をすべて満たした場合，ABPA 確実例
1 次基準のうち 6 項目を満たした場合，ABPA 疑い例と判断する
2 次基準は診断の参考

(Rosenberg M, *et al*[5]. *Ann Intern Med*　86：1977 より引用，改変)

喘息は発症しやすい状態(predisposing conditions)として嚢胞性線維症と併記され，必須項目としてはアスペルギルスに対する即時型皮膚反応または *A. fumigatus* 特異的 IgE 抗体陽性，血清総 IgE＞1,000 IU/m*l* が挙げられ，それに加え，その他 *A. fumigatus* に対する沈降抗体または IgG 抗体陽性など 3 項目中 2 つを満たすことで診断可能としている。さらに検証の余地はあるが，現在最も実践的な診断基準であろう。また，近年，さらに ABPA の亜分類として，Kumar らは ABPA-S，ABPA-CB，ABPA-CB-ORF(ABPA-CB with other radiological findings)と分けることを提唱した(ORF：線維化，ブラ，ブレブ，気胸，気腫性病変，線維空洞性病変，アスペルギローマ，胸水，胸膜肥厚など)[7]。また，Agarwal らは近年 ABPA-S，ABPA-CB，ABPA-CB-HAM(ABPA-CB with HAM)

表3 ISHAMの診断基準

Predisposing Conditions(発症しやすい状態)
気管支喘息，嚢胞性線維症
Obligatory Criteria(必須項目)
アスペルギルスの即時型皮内反応陽性または*A. fumigatus*の特異的IgEが上昇
血清総IgEが上昇(>1,000 IU/m*l*)*
Other Criteria(その他の項目　3項目中最低2項目)
*A. fumigatus*に対する沈降抗体陽性またはアスペルギルス特異的IgG抗体陽性
ABPAに合致する肺陰影**
ステロイド非投与下で総好酸球数>500/μl(その病歴において)

*もしすべてのOther Criteriaが合致すれば，血清総IgE<1,000 IU/m*l*でも可

**ABPAに合致する肺陰影 一過性のもの：コンソリデーション，結節，tram-track opacities，練り歯磨き/finger-globe opacities，永続的なもの：parallel line，リング状陰影，気管支拡張，肺・胸膜の線維化

(Agarwal R, *et al*[4]. *Clin Exp Allergy* 43：2013より引用，改変)

に分類し，これらが各々免疫学的重症度で各々mild, moderate, severeにあてはまることを報告し，画像的にはさらにABPA-CB-ORFを加えた4群の分類を提唱した[8]。

4．SAFSとの相違

近年，SAFS(severe asthma with fungal sensitization)という疾患概念が提唱されている。SAFSは真菌感作を伴った重症喘息でABPAが除外された群として定義され，抗真菌剤の有用性が報告された。その際，アスペルギルスに対する沈降抗体陰性，著明なIgEの上昇(>1,000 IU/m*l*)を伴わない症例として定義された[9][10]。気管支拡張，粘液栓を伴う症例はABPAの除外は容易であるが，SAFSと鑑別を要するのは前述のABPA-Sであろう。ステロイド剤の全身投与が行われた症例では沈降抗体の陰性化，IgEの低下を認める症例があり，その際の鑑別は困難である。現在，SAFSはABPA-Sの前段階ではなく，異なる疾患として扱われている。同じ真菌に感作を伴う気道アレルギー性疾患である両者の病態の差の解明はABPAの病態の本態に迫ることになる可能性もあり，興味深い。

Ⅲ．治療における問題点

現在のABPA治療の目標は喘息症状のコントロール，急性増悪の予防・治療，気管支拡張・線維化の予防である。これまで抗炎症療法として全身性ステロイド剤投与が，気道の真菌量の減少を目指して抗真菌剤が使用されてきた。全身性ステロイド剤の投与量・投与法は十分なevidenceに基づくものではなく，至適投与量・投与期間は不明であり，様々なレジメンが使用されてきた。当初プレドニゾロン0.5 mg/kg/日を1～2週間投与した後，隔日に変更し，6～8週継続した後，5～10 mg/日ずつ減量するレジメンが用いられたがこの方法では45％が再燃した[11]。その後，0.75 mg/kg/日で6週間継続した後，0.5 mg/kg/日で6週間，5～10 mgずつ6週間ごとで減量し，6～12カ月継続する方法が行われ，高率の寛解(13.5％)と低い再発率が得られた[12]。現在，ステロイド剤の至適投与量に関する臨床試験が終了しており，その結果が待たれる(NCT00974766)。

抗真菌剤に関してはステロイド剤の減量・中止に伴う再燃を来した難治症例に対し，抗真菌剤イトラコナゾールの有用性が2000年にStevensら[13]，2003年にWarksら[14]により報告された。これらの報告を元にイトラコナゾール200(-400) mg/日の16週間の投与が行われている。抗真菌剤を単剤でABPAの急性期に使用した報告も散在し，現在，抗真菌剤単剤療法とステロイド療法のランダム化試験も行われ，結果が待たれる(NCT01321827)。

近年，米国感染症学会のガイドラインでは早期の抗真菌剤の投与が勧められている[15]。しかし，早期導入はevidenceに乏しく，また菌耐性化などの問題を含んでいる。本邦で本年度に感染症学会から発表されたガイドラインでは抗真菌剤の導入時期は明記されていない。その他，ボリコナゾール，ポサコナゾールなど新規抗真菌剤の効果はさらなる検証が待たれる。

また，ABPA治療における吸入ステロイド剤の役割には議論がある。AgarwalらはABPA-S症例にブデソニド/ホルモテロールを投与された症例を検討し，症状に変

化なく，血清総IgEはむしろ治療導入後に増加したことを報告している。ABPAに対する初期の吸入ステロイド療法は慎重に適応を検討する必要がある[16]。

今後，期待すべき治療としては抗IgE抗体療法(オマリズマブ)がある。少数例の報告ながら増悪，経口ステロイド剤減量効果を示した報告もあり，さらなる検討が望まれる[17]。

Ⅳ．ABPMに関する問題点

上記は主にABPAに関して述べてきた。ABPA以外のABPMに関しては多数例の報告に乏しく，多くはcase report，少数例の報告に留まる。Chowdharyらは近年，2012年までにABPMとして報告された159例をまとめて報告した[18]。報告されたABPMの原因真菌を**表4**にまとめた。この報告によると最も検出頻度が高かったのは*C. albicans*であり，多くはインドからの報告であった。*S. commune*は*Bipolaris* spp.についで報告されていたが，多くは日本からの報告であった。

現在，アスペルギルス以外の真菌の血清学的な診断は一般診療では行えず，また指標となる診断基準がないことから，臨床像，画像所見がABPAと類似し，血清学的にアスペルギルスへのアレルギー検査で陽性とならない症例をABPMとしているのが現状である。喀痰検査で他の真菌が検出された際には，検出真菌をアレルゲンと想定し診断しているケースも多い。一般臨床の範囲内で提出できるアスペルギルスの沈降抗体/IgG(保険適応外)が陽性で検出された場合にはABPAと診断されていることが多いが，一方で真菌は高率に他の真菌と交差反応を呈することも知られている。ABPAと診断された症例のなかにはアスペルギルス以外の真菌によるABPMが混在している可能性を常に念頭におく必要がある。MatsuseらのABPA症例において喀痰培養で検出された真菌と感作真菌とで乖離があることを示した論文はそのことを裏づけている[19]。

Ⅴ．本邦での現状

本症は環境によりその頻度・原因真菌が異なることが推測され，その地域差の存在が以前より指摘されていた。そこで本邦での実情を検討するため，昨年，厚生労働省のアレルギー性気管支肺真菌症の診断・治療指針確立のための調査研究班(研究代表者：東海大学 浅野浩一郎)で本邦初のABPMに関する全国調査が施行された。

調査は日本呼吸器学会認定施設・関連施設，日本アレルギー学会認定教育施設(内科系)の計906施設を対象に行われ，その中間報告を筆者らはアレルギー学会総会で報告した。以下，その概要を記す。

表4　非アスペルギルスABPMの原因真菌

Chowdharyら[18]		本邦での検討	
fungus	n	fungus	n
C. albicans	94	*S. commune*	16
Bipolaris spp.	20	*C. albicans*	15
S. commune	17	*Penicillium* spp.	6
Curvularia spp.	6	*Mucor.* spp.	3
P. boydii species complex	6	*C. glabrata*	2
Penicillum spp.	3	*C. tropicalis*	2
A. alternaria	2		
F. vansinfectum	2		
Cladosporium spp.	2		

Chowdharyらの検討は現在までの非アスペルギルスABPMの報告をレビューしたもの。
本邦での検討は，本邦におけるABPM症例(479症例)の喀痰培養で検出されたアスペルギルス以外の主な真菌。

各施設でのABPM症例の経験症例数を，専門医数で修正し，地域差を検討するとわずかに北海道，東北地方で少ない印象であった。ケースカードが得られたABPM 479例(アスペルギルス以外の真菌によるABPMは59例を含む)のうち，アスペルギルスに対するⅠまたはⅢ型アレルギー検査陽性で，かつ喘息合併，IgE高値，気管支拡張または粘液栓，肺浸潤影の4項目中3項目を満たした346症例をABPA症例として解析すると，男女比は女性が55％を占め女性優位であり，従来の海外の報告に類似していたが，発症年齢の中央値は50歳以上の高齢発症が70％近くを占め従来の報告より高齢発症であった。また，従来診断の必須項目とされていた気管支喘息は本検討では83％であった。また，免疫学的検査では，本邦では即時型反応の検索は即時型皮膚反応で施行された症例は少なく，多くは特異的IgEの計測でなされていた。また，Ⅲ型アレルギーの検索はアスペルギルス特異的IgG検査の施行例は少数であり，多くはアスペルギルス抗原に対する沈降抗体測定でなされていた。また，本邦の医療事情を反映してか胸部CTはほぼ全例に施行されていた。そのうち気管支拡張，粘液栓は80％以上で認められた。HAMは約40％で認め，これは従来報告(20％前後)より高頻度であった。また，進行例で認める嚢胞化・線維化も10％強で認め，重症例が一定の割合で存在していることが示唆された。また，治療は経口ステロイド剤が70％以上で投与され(初期投与量はPSL換算で平均25 mg/日)，40％で1年以上継続して服用をしていた。また，抗真菌剤は約半数で使用され，主にイトラコナゾールが，少数例でボリコナゾールが

投与されていた。

なお，今回集計されたABPM症例では喀痰検査は61％でなされ，陽性率は76％であった。特筆すべき点は喀痰検査でのスエヒロダケの検出頻度の高さであった(**表4**)。アスペルギルス属につぐ高頻度(喀痰施行例の9％)で検出され，本邦のABPMの地域性が示唆された。

おわりに

上述のようにABPM診療は多くの解明すべき問題を残している。また，全国調査の結果で本邦のABPM症例は海外の症例と発症年齢，検出真菌などが異なっていた。今後，本邦独自の診断・治療指針の作成が必要性と思われる。

文　献

1) Hinson K, *et al.* Broncho-pulmonary Aspergillosis；a review and a report of eight new cases. *Thorax* 7：317—333, 1952
2) Araujo R, *et al.* Variability of germinative potential among pathogenic species of Aspergillus. *J Clin Microbiol* 42：4335—4337, 2004
3) Oguma T, *et al.* Induction of mucin and MUC5AC expression by the protease activity of Aspergillus fumigatus in airway epithelial cells. *J Immunol* 187：999—1005, 2011
4) Agarwal R, *et al.* Allergic bronchopulmonary aspergillosis：review of literature and proposal of new diagnostic and classification criteria. *Clin Exp Allergy* 43：850—873, 2013
5) Rosenberg M, *et al.* Clinical and immunologic criteria for the diagnosis of allergic bronchopulmonary aspergillosis. *Ann Intern Med* 86：405—414, 1977
6) Patterson R, *et al.* Allergic bronchopulmonary aspergillosis. Natural history and classification of early disease by serologic and roentgenographic studies. *Arch Intern Med* 146：916—918, 1986
7) Kumar R. Mild, moderate, and severe forms of allergic bronchopulmonary aspergillosis：a clinical and serologic evaluation. *Chest* 124：890—892, 2003
8) Agarwal R. High attenuation mucoid impaction in allergic bronchopulmonary aspergillosis. *World J Radiol* 2：41, 2010
9) Denning DW, *et al.* Randomized Controlled Trial of Oral Antifungal Treatment for Severe Asthma with Fungal Sensitization. *Am J Respir Crit Care Med* 179：11—18, 2009
10) O' Driscoll BR, *et al.* Comparison of skin prick tests with specific serum immunoglobulin E in the diagnosis of fungal sensitization in patients with severe asthma. *Clin Exp Allergy* 39：1677—1683, 2009
11) Greenberger PA. Allergic bronchopulmonary aspergillosis. *J Allergy clin immunol* 110：685—692, 2002
12) Vlahakis NE, *et al.* Diagnosis and treatment of allergic bronchopulmonary aspergillosis. *Mayo Clin Proc* 76：930—938, 2001
13) Stevens DA, *et al.* A randomized trial of itraconazole in allergic bronchopulmonary aspergillosis. *N Engl J Med* 342：756—762, 2000
14) Wark PAB, *et al.* Anti-inflammatory effect of itraconazole in stable allergic bronchopulmonary aspergillosis：a randomized controlled trial. *J Allergy clin immunol* 111：952—957, 2003
15) Walsh TJ, *et al.* Treatment of aspergillosis：clinical practice guidelines of the Infectious Diseases Society of America. *Clin Infect Dis* 46：327—360, 2008
16) Agarwal R, *et al.* Role of inhaled corticosteroids in the management of serological allergic bronchopulmonary aspergillosis(ABPA). *Intern Med*, 2010
17) Tillie-Leblond I, *et al.* Allergic bronchopulmonary aspergillosis and omalizumab. *Allergy* 66：1254—1256, 2011
18) Chowdhary A, *et al.* Allergic bronchopulmonary mycosis due to fungi other than Aspergillus：a global overview. *Crit Rev Microbiol* 40：30—48, 2014
19) Matsuse H, *et al.* Dissociation between sensitizing and colonizing fungi in patients with allergic bronchopulmonary aspergillosis. *Ann Allergy Asthma Immunol* 111：190—193, 2013
20) Gioulekas D, *et al.* Allergenic fungi spore records(15 years) and sensitization in patients with respiratory allergy in Thessaloniki-Greece. *J Invest Allergol Clin Immunol* 14：225—231, 2004

Allergology International 64 (2015) 175–180

Contents lists available at ScienceDirect

Allergology International

journal homepage: http://www.elsevier.com/locate/alit

Original article

Phenotype of asthma related with high serum periostin levels

Masako Matsusaka [a], Hiroki Kabata [a, b], Koichi Fukunaga [a], Yusuke Suzuki [a], Katsunori Masaki [a], Takao Mochimaru [a], Fumio Sakamaki [c, d], Yoshitaka Oyamada [e], Takashi Inoue [f], Tsuyoshi Oguma [b, g], Koichi Sayama [b], Hidefumi Koh [h, i], Morio Nakamura [d, j], Akira Umeda [k], Junya Ono [l], Shoichiro Ohta [m], Kenji Izuhara [n], Koichiro Asano [a, g, *], Tomoko Betsuyaku [a]

[a] Division of Pulmonary Medicine, Department of Medicine, Keio University, School of Medicine, Tokyo, Japan
[b] Kawasaki Municipal Hospital, Kanagawa, Japan
[c] Division of Pulmonary Medicine, Department of Medicine, Tokai University Hachioji Hospital, Tokyo, Japan
[d] Department of Medicine, Tokyo Saiseikai Central Hospital, Tokyo, Japan
[e] Department of Medicine, National Tokyo Medical Center, Tokyo, Japan
[f] Department of Medicine, Sanokousei General Hospital, Tochigi, Japan
[g] Division of Pulmonary Medicine, Department of Medicine, Tokai University, School of Medicine, Kanagawa, Japan
[h] Department of Medicine, Saiseikai Utsunomiya Hospital, Tochigi, Japan
[i] Department of Medicine, Tachikawa Kyosai Hospital, Tokyo, Japan
[j] Department of Medicine, Eiju General Hospital, Tokyo, Japan
[k] Department of Medicine, Shioya Hospital, International University of Health and Welfare, Tochigi, Japan
[l] Shino-Test Corporation, Tokyo, Japan
[m] Department of Laboratory Medicine, Saga Medical School, Saga, Japan
[n] Division of Medical Biochemistry, Department of Biomolecular Sciences, Saga Medical School, Saga, Japan

ARTICLE INFO

Article history:
Received 30 June 2014
Received in revised form
14 November 2014
Accepted 22 November 2014
Available online 13 January 2015

Keywords:
Chronic rhinosinusitis
Eosinophils
Nasal polyp
Olfactory dysfunction
T_H2-high asthma

ABSTRACT

Background: Asthma is a heterogeneous disease composed of various phenotypes. Periostin, a molecule inducible with interleukin (IL)-4 or IL-13 in bronchial epithelial cells, is a biomarker of "TH2-high" asthma. The objective of this study is to examine whether the serum periostin concentrations are correlated with the severity, specific phenotype(s), or comorbidity of asthma.
Methods: Serum concentrations of periostin were measured in 190 Japanese asthmatic patients and 11 healthy controls. The protocol was registered under UMIN 000002980 in the clinical trial registry.
Results: The serum concentrations of periostin were significantly higher ($P = 0.014$) in asthmatics [70.0 (54.0–93.5) ng/ml] than in healthy subjects [57.0 (39.0–63.0) ng/ml], though we found no correlation between serum periostin concentrations and treatment steps required to control asthma. To characterize "high-periostin" phenotype(s), the patients with asthma were divided among tertiles based on the serum concentrations of periostin. The high-periostin group was older at onset of asthma ($P = 0.04$), had a higher prevalence of aspirin intolerance ($P = 0.04$) or concomitant nasal disorders ($P = 0.03$–0.001), higher peripheral eosinophil counts ($P < 0.001$), and lower pulmonary function ($P = 0.02$–0.07). The serum concentrations of periostin were particularly high in asthmatic patients complicated by chronic rhinosinusitis with nasal polyps and olfactory dysfunction. In contrast, neither atopic status, control status of asthma, nor quality of life were related with the "high-periostin" phenotype.
Conclusion: Elevated periostin concentrations in serum were correlated with a specific phenotype of eosinophilic asthma, late-onset and often complicated by obstructive pulmonary dysfunction and nasal disorders.

Introduction

Asthma is an inflammatory disease of the airways characterized by bronchial hyperresponsiveness and reversible airflow limitation, affecting about 300 million people in the world.[1] While

* Corresponding author. Division of Pulmonary Medicine, Department of Medicine, Tokai University School of Medicine, 143 Shimokasuya, Isehara, Kanagawa 259-1193, Japan.
E-mail address: ko-asano@tokai-u.jp (K. Asano).
Peer review under responsibility of Japanese Society of Allergology.

http://dx.doi.org/10.1016/j.alit.2014.07.003

airway inflammation and respiratory symptoms can be controlled with inhaled corticosteroids in most instances, they remain refractory to the highest tolerable doses of inhaled corticosteroids, long-acting bronchodilators, and leukotriene receptor antagonists in patients with severe asthma.[2] The frequent disease exacerbations suffered by these patients, and multiple emergency department visits and hospitalisations represent a heavy social and economic burden.[3] Furthermore, because the heterogeneous characteristics of severe asthma preclude its control by a single therapeutic agent, relevant phenotyping and individualized treatment are essential.[4]

Interleukin (IL)-13, a TH2 cytokine, plays an important role in the development and persistence of eosinophilic inflammation and hyperresponsiveness in the asthmatic airways.[5] Patients with "TH2-high" asthma have been identified by transcriptome analysis, whose bronchial epithelial cells express excessive amounts of IL-13-inducible genes, such as *Clca1* and *Postn*.[6] These patients present with increased eosinophilic inflammation and airway hyperresponsiveness, thickened basement membranes, and greater responsiveness to corticosteroids.[7] On the other hand, periostin, the product of IL-13-inducible *Postn*, is an extracellular matrix protein of the fasciclin family,[8] and can be measured in serum. Serum periostin concentrations are correlated with a sustained eosinophilic inflammation of the airways[9] and rapid decline of pulmonary function[10] despite treatment with inhaled corticosteroids. Another study has suggested that the concentrations of serum periostin can be used to predict the responsiveness to treatment with anti–IL-13 antibody.[11] Therefore, periostin might be a useful biomarker as a companion diagnostic for severe asthma.[12] However, clinical characteristics or phenotype of asthmatics with elevated serum periostin levels are not well studied. This study examined whether, in asthmatic Japanese, the serum concentrations of periostin are correlated with the disease severity, specific phenotype or comorbidity.

Methods

Patient populations

Between April 1, 2010 and December 31, 2012, we enrolled Japanese patients ≥20 years of age, who presented with difficult-to treat asthma at Keio University Hospital and affiliated hospitals. Asthma was diagnosed on the basis of the Japanese Society of Allergology guideline.[13] Asthma requiring step 4 or 5 treatment actions, defined in the updated version of the 2006 statement by the Global Initiative for Asthma (GINA) to achieve its optimum control was defined as severe asthma.[14] Healthy subjects with no history of allergic diseases and patients with mild to moderate asthma controlled with step 1 to 3 treatment actions of GINA, served as controls. Patients with uncontrolled malignant tumours or widespread lung disease that prominently impaired lung function were excluded from enrolment. The protocol (no 2009-9-5) initially approved by the Institutional Review Board of Keio University School of Medicine, was subsequently approved by the Review Board of each participating institution, and implemented in compliance with the Declaration of Helsinki. All participants granted their written informed consent.

Collection of clinical information

The study participants reported their clinical information at the time of enrolment by means of a self-completed questionnaire. Poor adherence to the treatment was defined as < 5 day-use of inhaled corticosteroids per week. Olfactory dysfunction was defined by the presence of hyposmia/anosmia. The control status of asthma and the disease-specific quality of life were ascertained, using the Japanese versions of the asthma control test[15] and the Juniper's asthma quality of life questionnaire,[16,17] respectively. Laboratory data and information pertaining to medications and disease exacerbations were collected from medical records.

Serum concentrations of periostin and cytokines

The serum periostin concentrations were measured by enzyme-linked immunosorbent assay, as previously reported.[18] The serum concentrations of IL-4, IL-5, and IL-13 were measured, using the Bio-Plex® Suspension Array System (Bio-Rad Laboratories, Hercules, CA, USA). Total and allergen-specific serum immunoglobulin (Ig)E concentrations for house-dust mites, cat dander, fungi, and insects were measured using a fluorescence-enzyme immunoassay (Mitsubishi Chemical Medience Corporation, Tokyo, Japan). Atopic asthma was defined as one or more allergen-specific IgE concentrations >0.70 UA/mL.

Pulmonary function tests

Pulmonary function during stable asthma was measured using a CHESTAC-9800 spirometer (Chest, Tokyo, Japan), which met the criteria of the American Thoracic Society. The predicted value of vital capacity (VC) and forced expiratory volume in 1 s (FEV_1) for a Japanese population was calculated using the formula proposed by the Japanese Respiratory Society. The fraction of exhaled nitric oxide was measured with a Sievers nitric oxide analyser (GE Healthcare Japan, Tokyo, Japan) in some participating institutions.

High-resolution computed tomography

Airway wall thickness was measured by high-resolution computed tomography scans, using an Aquilion™ (TOSHIBA Medical Systems Corporation, Tochigi, Japan) or LightSpeed® volume scanner (GE Healthcare). The wall area and % wall area of the apical bronchus of the right upper lobe (RB1) were measured using the AZE VirtualPlace Lexus64® software (AZE, Tokyo, Japan).

Statistical analysis

The data are expressed as means ± SD, median and interquartile range, or percentages. Categorical data were analysed with the chi-square test. Mann–Whitney test or Kruskal–Wallis test, as appropriate. Spearman's rank correlation coefficient was determined between serum levels of periostin, TH2 cytokines, and blood eosinophil counts. A regression analysis was performed to examine the correlations between pulmonary functions and age- and sex-adjusted or unadjusted, log-transformed serum periostin concentration, duration of asthma and smoking history. A statistically significant difference was defined as a two-tailed P value <0.05. All statistical analyses were performed with the SPSS statistical software package for Windows, version 20.0 (IBM Corporation, Armonk, NY, USA).

Results

Characteristics of the study groups

This study enrolled 11 healthy subjects (mean age 39.5 ± 12.1 years, 73% men) and 190 asthmatic patients (mean age 60.2 ± 14.5 years, 44% men), including 22 in the GINA steps 1 and 2, 20 in step 3, 83 in step 4 and 65 patients in step 5. In 58 patients in step 4 (70%) and 58 patients in step 5 (89%), the status corresponded to the definition of severe asthma by international ERS/ATS

Table 1
Characteristics of the study groups.

	GINA steps		P
	1–3 (n = 42)	4 & 5 (n = 148)	
Demographic and clinical observations			
Men	38	46	0.39
Age, y	60.6 ± 14.3	60.1 ± 14.7	0.82
Age at onset of asthma, y	36.1 ± 20.1	32.6 ± 22.8	0.32
Body mass index, kg/m^2	22.5 ± 3.2	24.2 ± 4.7	0.07
History of smoking	26.8	17.4	0.19
Aspirin intolerance	12.5	20.7	0.36
Atopic dermatitis	22.0	25.4	0.84
Allergic rhinitis	61.0	69.1	0.35
Chronic rhinosinusitis with nasal polyps	43.9	39.7	0.72
Olfactory dysfunction	36.6	28.3	0.34
Poor adherence to the treatment	15.4	5.1	0.04
Daily dose of inhaled corticosteroids, μg[†]	273 ± 195	744 ± 292	<0.001
Patients treated with daily oral corticosteroids	0	37.8	<0.001
Patients treated with omalizumab	0	12.8	0.009
Laboratory measurements			
Eosinophils/μl of blood	421 ± 667	465 ± 537	0.71
Total serum IgE, IU/ml, median (interquartile range)	160 (71–550)	330 (136–658)	0.04
Atopic type	53.7	59.5	0.59
Pulmonary function (n = 173)			
VC, % predicted	98 ± 13	92 ± 19	0.03
FEV_1, % predicted	91 ± 15	80 ± 23	<0.001
FEV_1/FVC, %	69 ± 10	64 ± 14	0.01
Fractional exhaled nitric oxide, ppb (n = 80)	29 ± 32	49 ± 51	0.03
Asthma severity and quality of life scores			
Asthma Control Test	22.5 ± 3.2	18.9 ± 5.0	<0.001
Asthma Quality of Life Questionnaire	5.5 ± 1.0	4.7 ± 1.2	<0.001

Values are proportions of patients in each study groups or means ± SD if not otherwise specified.
IgE, immunoglobulin E; VC, vital capacity; FEV_1, Forced expiratory volume in 1 s; FVC, forced vital capacity.
[†] Dose of inhaled corticosteroids are shown as fluticasone propionate equivalent.

guidelines.[2] Table 1 compares the characteristics of 42 patients included in the GINA treatment steps 1 to 3 with those of 148 patients included in steps 4 and 5. The serum concentration of total IgE and fractional exhaled nitric oxide in the latter group were significantly higher, while VC, FEV_1, and the asthma control test and asthma quality of life questionnaire scores were significantly lower. The rate of patients with poor adherence to the treatment was lower in severe asthma group, and there were no differences between the 2 groups in sex distributions, age, body mass index, peripheral blood eosinophil counts, atopic status, and prevalence of comorbidity, including aspirin intolerance, atopic dermatitis, allergic rhinitis and chronic rhinosinusitis with nasal polyps.

Relationship between serum periostin concentrations and severity of asthma

The median serum periostin concentrations in the 190 asthmatic patients was 70.0 (54.0–93.5) ng/ml, versus 57.0 (39.0–63.0) ng/ml in the 11 healthy subjects (P = 0.014). There were no significant differences, however, in the serum periostin concentrations measured among patients in GINA step 1-3 [66.5 (51.0–87.0) ng/mL], step 4; 70.0 (57.0–97.0) ng/mL, and step 5; 72.0 (54.0–103.0) ng/mL (Fig. 1). Periostin concentrations ≥90 ng/mL, observed in 33% of the step 4 & 5 group, was found in only in 14% of the step 1-3 group (P = 0.02 compared to step 4 & 5 group) and none of the healthy controls (P = 0.02 compared to step 4 & 5 group), suggesting that a specific asthmatic phenotype(s) characterized by elevated serum periostin concentrations ("periostin-high" asthma) is more prevalent among patients requiring the most intensive treatment.

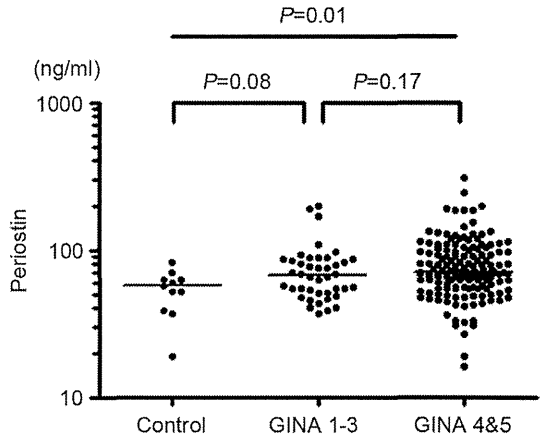

Fig. 1. Relationship between serum periostin concentrations and severity of asthma. The serum periostin concentrations in 11 healthy subjects 190 patients with asthma are shown. The patients with asthma were divided between GINA treatment steps 1–3 and step 4 & 5. Bars indicate median values.

High serum periostin concentrations indicate a specific asthmatic phenotype

To characterize the "periostin-high" phenotype of asthma, we divided the 190 asthmatic patients among tertiles according to the serum periostin concentrations (Table 2). The average age and age at onset of asthma were significantly older in the high-than in the low-periostin group, and the prevalence of late-onset asthma (age at onset ≥ 40 y) in high-periostin group (59.3%) was 1.7 times as high as that in low-periostin group (34.5%, P = 0.009). Furthermore, allergic rhinitis, olfactory dysfunction and aspirin-intolerance were more prevalent, and abnormalities of pulmonary function tests and peripheral eosinophil counts were significantly greater in the high-than the low-periostin group (Table 2). Finally, the serum concentrations of TH2 cytokines, IL-4 and IL-13, were higher in the high-periostin group, though the difference was of borderline statistical significance (Table 2). There was a weak to moderate correlation between serum periostin levels and peripheral blood eosinophilia (ρ = 0.38, p < 0.0001) and weak correlation with TH2 cytokine levels in serum (ρ = 0.18-0.21, p = 0.01-0.03, Supplementary Fig. 1). When the analysis was limited to the 148 patients included in the GINA step 4 and 5 group, the highest-periostin group was also older at the time of onset of asthma, was leaner, had a higher prevalence of nasal diseases, lower pulmonary functions, and higher serum TH2 cytokine concentrations and peripheral eosinophil counts (Supplementary Table 1).

Because high periostin concentrations were correlated with nasal disorders, such as allergic rhinitis, chronic rhinosinusitis with nasal polyps, and olfactory dysfunction (Supplementary Fig. 2), then we examined which component(s) of the disorders determined this relationship. Fig. 2 shows that the patients who suffered from both chronic rhinosinusitis with nasal polyps and olfactory dysfunction had the highest serum periostin concentrations (P = 0.001 compared with the patients without nasal disorder). FEV_1 and FEV_1/forced vital capacity (FVC) were lower among patients with high serum periostin concentrations (Table 2). We, therefore, performed single and multivariate analyses to examine whether serum periostin was correlated with obstructive pulmonary dysfunction, and found that log-transformed serum periostin concentrations were weakly correlated with FEV_1/FVC (P = 0.03; Table 3), but not with %predicted FEV_1 (P = 0.27). By multiple variable analysis, serum periostin concentration was marginally correlated with FEV_1/FVC independently of asthma duration or smoking history (Table 3).

Table 2
Characteristics of patients in the low, intermediate and high periostin groups.

	Periostin			$P^{\dagger}$
	Low ($n = 63$)	Intermediate ($n = 64$)	High ($n = 63$)	
Serum periostin, ng/ml, median (interquartile range)	49.0 (43.5–54.0)	70.0 (65.0–77.3)	109 (94.0–129.0)	
Demographic and clinical observations				
Men	44.4	39.1	49.2	0.72
Age, y	56.1 ± 15.0	60.6 ± 14.3	64.1 ± 13.4	0.002
Age at onset of asthma, y	29.4 ± 23.4	33.8 ± 24.0	36.9 ± 18.4	0.04
Early-onset asthma (age at onset < 16 y)	37.9	32.2	18.5	0.02
Late-onset asthma (age at onset ≥ 40 y)	34.5	47.5	59.3	0.009
Body mass index, kg/m^2	24.4 ± 5.3	24.0 ± 4.4	22.9 ± 3.2	0.23
History of smoking	16.4	19.0	23.7	0.59
Aspirin intolerance	11.5	18.3	27.1	0.04
Atopic dermatitis	16.4	3.3	24.1	0.36
Allergic rhinitis	57.4	68.3	76.3	0.03
Chronic rhinosinusitis with nasal polyps	31.7	42.4	48.3	0.09
Olfactory dysfunction	16.4	28.3	46.6	0.001
Poor adherence to the treatment	3.5	10.0	8.6	0.23
Daily dose of inhaled corticosteroids, μg‡	611 ± 348	638 ± 303	676 ± 356	0.31
Patients treated with daily oral corticosteroids	27.7	26.1	32.3	0.70
Patients treated with omalizumab	11.1	7.8	11.1	1.00
Laboratory measurements				
Eosinophils/μl of blood	319 ± 490	469 ± 629	584 ± 540	<0.001
Total serum IgE, IU/ml, median (interquartile range)	250 (73–654)	325 (98–636)	313 (134–643)	0.34
Atopic type	62.9	59.4	52.4	0.28
Interleukins (IL)				
Serum IL-4, pg/dl	11.7 ± 7.6	14.3 ± 8.9	15.3 ± 9.1	0.06
Serum IL-5, pg/dl	34.0 ± 18.9	40.1 ± 20.6	41.0 ± 22.8	0.22
Serum IL-13, pg/dl	22.0 ± 22.0	20.2 ± 7.5	30.4 ± 44.1	0.06
Pulmonary function ($n = 173$)				
VC, % predicted	92.8 ± 16.1	96.2 ± 20.2	90.5 ± 16.5	0.32
FEV_1, % predicted	83.3 ± 22.7	87.5 ± 22.9	76.1 ± 20.0	0.07
FEV_1/FVC, %	67.2 ± 14.2	67.5 ± 11.7	61.4 ± 13.4	0.02
Fractional exhaled nitric oxide, ppb ($n = 80$)	45.9 ± 61.7	40.6 ± 35.9	49.3 ± 46.9	0.50
Computed tomography (n = 55)				
Wall area, %	59.1 ± 9.7	57.7 ± 7.0	56.9 ± 6.8	0.51
Asthma severity and quality of life scores				
Asthma Control Test	19.7 ± 4.7	19.3 ± 5.3	20.2 ± 4.6	0.53
Asthma Quality of Life Questionnaire	4.9 ± 1.2	4.8 ± 1.3	4.8 ± 1.3	0.88

Values are proportions of patients in each study groups or means ± SD if not otherwise specified.
IgE, immunoglobulin E; VC, vital capacity; FEV_1, Forced expiratory volume in 1 s; FVC, forced vital capacity.
† Low versus High periostin group.
‡ Dose of inhaled corticosteroids are shown as fluticasone propionate equivalent.

Discussion

Since asthma is a heterogeneous disease, its treatment, especially when severe, should be based on the specific molecular mechanism identified in each individual patient. The serum periostin concentration was expected to be a reliable surrogate marker of IL-13 activity *in vivo* and of persistent eosinophilic inflammation in the airways. The present study adds further evidence that serum periostin is clinically useful, by showing that its concentration is correlated with a specific phenotype of asthma, instead of with its severity.

Periostin is localized with other matricellular proteins in the subepithelial layer of the airways. Its elevated expression in bronchial epithelial cells is related to subepithelial fibrosis of the airways.[6] Furthermore, the serum periostin concentrations correlate with an annual decline in FEV_1, independently of the severity of asthma or smoking history, in asthmatics treated with inhaled corticosteroids.[10] Therefore, periostin seems to contrast with other biomarkers of asthma, such as YKL-40 and C-reactive protein, which reflect airway inflammation, but not a specific phenotype such as a rapid deterioration of pulmonary function.[19] Our observation that patients with high serum periostin concentrations had low FEV_1 and FEV_1/FVC in spite of shorter duration of asthma confirms that periostin is a biomarker of rapid decline in pulmonary function. However, we could not show a relationship between serum periostin concentrations and airway remodelling assessed by the airway wall thickness on computed tomography morphometry. That relationship will require further studies.

The "high-periostin" phenotype identified in our study, i.e. late-onset asthma with eosinophilia and concomitant nasal disorders, corresponds to the phenotypes/endotypes proposed by other researchers. A cluster analysis of severe asthma suggested the presence of an inflammation-predominant phenotype, characterized by a later onset of the disease with active eosinophilic inflammation.[20] Another study also described a population of patients with severe, adult-onset asthma, who were more likely to be non-atopic, associated with increased concentrations of exhaled nitric oxide and sputum eosinophils, and in whom nasal symptoms and polyposis were more prevalent.[21] This late-onset, hypereosinophilic asthma is also considered a specific endotype.[22]

We found that high concentrations of serum periostin were correlated with other nasal disorders, such as allergic and non-allergic rhinosinusitis. Among the nasal disease manifestations, the combination of chronic rhinosinusitis with nasal polyps and olfactory dysfunction was most prominently correlated with high concentrations of serum periostin. The combination of nasal polyps

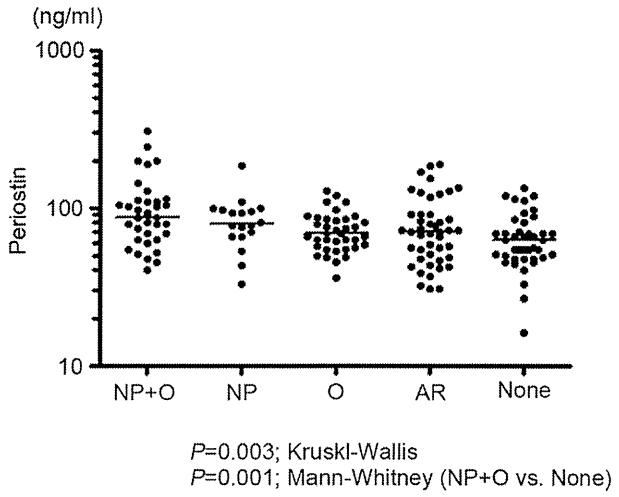

Fig. 2. Relationships between serum periostin concentrations and concomitant nasal disorders. The serum periostin concentrations in patients with versus without concomitant nasal disorders are compared. NP + O, chronic rhinosinusitis with nasal polyps + olfactory dysfunction; NP, chronic rhinosinusitis with nasal polyps without olfactory function; O, olfactory dysfunction without chronic rhinosinusitis with nasal polyps; AR, allergic rhinitis alone. Bars indicate median values. Kruskal–Wallis test and Mann–Whitney test were used to compare the groups. $P = 0.003$; Kruskal–Wallis, $P = 0.001$; Mann–Whitney (NP + O vs. none).

Table 3
Relationships between serum periostin concentrations and pulmonary function.

	FEV_1/FVC	
	β	P
Analysis		
Single variable		
Serum periostin concentration†	−0.16	0.03
Multiple variable		
Unadjusted		
Serum periostin concentration†	−0.15	0.05
Disease duration	−0.31	<0.001
History of smoking	−0.16	0.04
Adjusted for age and sex		
Serum periostin concentration†	−0.06	0.42
Disease duration	−0.27	<0.001
History of smoking	−0.03	0.70

FEV_1, Forced expiratory volume in 1 s; FVC, forced vital capacity.
† Log-transformed.

and hyposmia/anosmia is observed in patients with chronic eosinophilic rhinosinusitis, often complicated by peripheral blood eosinophilia and aspirin-intolerant asthma.[23] Therefore, a high proportion of our patients presenting with both chronic rhinosinusitis with nasal polyps and olfactory dysfunction might have suffered from chronic eosinophilic rhinosinusitis, though this was not confirmed by pathologic studies. Periostin mRNA is increasingly expressed in the nasal mucosa of patients presenting with chronic eosinophilic rhinosinusitis with nasal polyps.[24,25] Therefore, the concentrations of serum periostin may reflect an increased production of this molecule in both the upper and the lower airways.

It has been considered that early-onset atopic asthma is a TH2-related disease,[1] however, our study showed that the high serum periostin concentrations were not correlated with serum concentrations of IgE or atopic status, and rather associated with late-onset asthma. It has been reported that the expression of periostin is decreased by corticosteroids,[6,7] therefore, the periostin concentrations in early-onset atopic asthma might have been decreased by corticosteroid. The reason why the concentrations of periostin remained elevated despite corticosteroids in adult-onset eosinophilic asthma has not been clear yet. In *in vitro* experiments, corticosteroids completely inhibited the IL-4/13-induced periostin production in fibroblasts, and enhanced it in microvascular endothelial cells, however, the TGF-β-induced production of periostin in fibroblasts is resistant to corticosteroids,[26] suggesting that the site or microenvironment of periostin synthesis might be different in early-versus late-onset asthma. Serum periostin levels, therefore, can be a useful biomarker to identify specific phenotypes of severe asthma independently of atopic status.

Study limitations

The diagnoses of concomitant disorders, such as aspirin-intolerant asthma, atopic dermatitis, allergic rhinitis and chronic rhinosinusitis with nasal polyps were based on the patients' answers to the questionnaire, not on objective measurements from challenge tests, or radiographic and pathological examinations. Therefore, patients with asymptomatic concomitant diseases may have been missed in the analysis. Second, since this study was based on a cross-sectional analysis, we could not determine whether the concentrations of periostin in serum were stable through the course of the disease, or varied according to its control or therapeutic interventions. Third, we have no data whether serum periostin levels can be influenced by age or sex, therefore, the difference in serum periostin levels between healthy subjects and asthmatic patients may have been compromised by the difference in age and gender distribution.

In conclusion, periostin is a biomarker that reliably identifies a TH2-related asthma, late-onset, eosinophilic, and often complicated with declining pulmonary function and nasal diseases, chronic eosinophilic rhinosinusitis in particular.

Acknowledgements

We thank Ms. Chiyomi Uemura for her professional assistance.

This study was supported in part by a Grant-in-Aid for Scientific Research (C) (25461504) from the Japan Society for the Promotion of Science to Koichiro Asano, MD, a Grant in Aid for Research on Allergic disease and Immunology from the Ministry of Health, Labour, and Welfare to Koichiro Asano, MD, and a Grant-in-aid from Glaxo-Smith-Kline, PLC.

Yusuke Suzuki is affiliated with MSD Endowed Program for Allergy Research and has received funds for research.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.alit.2014.07.003.

Conflict of interest

KI received research funding from Shino-Test Corporation. KA received research funding from Astellas Pharma; honoraria as lecture fees from Astellas Pharma, GSK, and MSD. TB received research funding from GSK. The rest of the authors have no conflict of interest.

Authors' contributions

MM and HirK equally contributed to this work.

References

1. Wenzel SE. Asthma phenotypes: the evolution from clinical to molecular approaches. *Nat Med* 2012;**18**:716–25.
2. Chung KF, Wenzel SE, Brozek JL, Bush A, Castro M, Sterk PJ, et al. International ERS/ATS guidelines on definition, evaluation and treatment of severe asthma. *Eur Respir J* 2014;**43**:343–73.
3. McDonald VM, Gibson PG. Exacerbations of severe asthma. *Clin Exp Allergy* 2012;**42**:670–7.

4. Hashimoto S, Bel EH. Current treatment of severe asthma. *Clin Exp Allergy* 2012;**42**:693–705.
5. Brightling CE, Saha S, Hollins F. Interleukin-13: prospects for new treatments. *Clin Exp Allergy* 2010;**40**:42–9.
6. Woodruff PG, Modrek B, Choy DF, Jia G, Abbas AR, Ellwanger A, et al. T-helper type 2-driven inflammation defines major subphenotypes of asthma. *Am J Respir Crit Care Med* 2009;**180**:388–95.
7. Woodruff PG, Boushey HA, Dolganov GM, Barker CS, Yang YH, Donnelly S, et al. Genome-wide profiling identifies epithelial cell genes associated with asthma and with treatment response to corticosteroids. *Proc Natl Acad Sci U S A* 2007;**104**:15858–63.
8. Takayama G, Arima K, Kanaji T, Toda S, Tanaka H, Shoji S, et al. Periostin: a novel component of subepithelial fibrosis of bronchial asthma downstream of IL-4 and IL-13 signals. *J Allergy Clin Immunol* 2006;**118**:98–104.
9. Jia G, Erickson RW, Choy DF, Mosesova S, Wu LC, Solberg OD, et al. Periostin is a systemic biomarker of eosinophilic airway inflammation in asthmatic patients. *J Allergy Clin Immunol* 2012;**130**:647–54.e10.
10. Kanemitsu Y, Matsumoto H, Izuhara K, Tohda Y, Kita H, Horiguchi T, et al. Increased periostin associates with greater airflow limitation in patients receiving inhaled corticosteroids. *J Allergy Clin Immunol* 2013;**132**:305–12.e3.
11. Corren J, Lemanske RF, Hanania NA, Korenblat PE, Parsey MV, Arron JR, et al. Lebrikizumab treatment in adults with asthma. *N Engl J Med* 2011;**365**: 1088–98.
12. Matsumoto H. Serum periostin: a novel biomarker for asthma management. *Allergol Int* 2014;**63**:153–60.
13. Ohta K, Yamaguchi M, Akiyama K, Adachi M, Ichinose M, Takahashi K, et al. Japanese guideline for adult asthma. *Allergol Int* 2011;**60**:115–45.
14. Global Initiative for Asthma (GINA). *Global Strategy for Asthma Management and Prevention*. Updated 2006. Available from: www.ginasthma.org.
15. Hasegawa T, Koya T, Sakagami T, Kagamu H, Kawakami H, Hara K, et al. Efficacy of using the Japanese version of the asthma control test for determing the level of asthma control in clinical settings. *Allergol Int* 2012;**61**:609–17.
16. Apfelbacher CJ, Hankins M, Stenner P, Frew AJ, Smith HE. Measuring asthma-specific quality of life: structured review. *Allergy* 2011;**66**:439–57.
17. Juniper EF, Guyatt GH, Epstein RS, Ferrie PJ, Jaeschke R, Hiller TK. Evaluation of impairment of health related quality of life in asthma: development of a questionnaire for use in clinical trials. *Thorax* 1992;**47**:76–83.
18. Okamoto M, Hoshino T, Kitasato Y, Sakazaki Y, Kawayama T, Fujimoto K, et al. Periostin, a matrix protein, is a novel biomarker for idiopathic interstitial pneumonias. *Eur Respir J* 2011;**37**:1119–27.
19. Chupp GL, Lee CG, Jarjour N, Shim YM, Holm CT, He S, et al. A chitinase-like protein in the lung and circulation of patients with severe asthma. *N Engl J Med* 2007;**357**:2016–27.
20. Haldar P, Pavord ID, Shaw DE, Berry MA, Thomas M, Brightling CE, et al. Cluster analysis and clinical asthma phenotypes. *Am J Respir Crit Care Med* 2008;**178**: 218–24.
21. Amelink M, de Groot JC, de Nijs SB, Lutter R, Zwinderman AH, Sterk PJ, et al. Severe adult-onset asthma: a distinct phenotype. *J Allergy Clin Immunol* 2013;**132**:336–41.
22. Lotvall J, Akdis CA, Bacharier LB, Bjermer L, Casale TB, Custovic A, et al. Asthma endotypes: a new approach to classification of disease entities within the asthma syndrome. *J Allergy Clin Immunol* 2011;**127**:355–60.
23. Ishitoya J, Sakuma Y, Tsukuda M. Eosinophilic chronic rhinosinusitis in Japan. *Allergol Int* 2010;**59**:239–45.
24. Ishida A, Ohta N, Suzuki Y, Kakehata S, Okubo K, Ikeda H, et al. Expression of pendrin and periostin in allergic rhinitis and chronic rhinosinusitis. *Allergol Int* 2012;**61**:589–95.
25. Stankovic KM, Goldsztein H, Reh DD, Platt MP, Metson R. Gene expression profiling of nasal polyps associated with chronic sinusitis and aspirin-sensitive asthma. *Laryngoscope* 2008;**118**:881–9.
26. Shoda T, Futamura K, Kobayashi F, Saito H, Matsumoto K, Matsuda A. Cell type-dependent effects of corticosteroid on periostin production by primary human tissue cells. *Allergy* 2013;**68**:1467–70.