Flagellin suppresses experimental asthma by generating regulatory dendritic cells and T cells

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Background: Although the hygiene hypothesis suggests that microbial infections could subvert asthma and thus a microbial product might serve as a therapeutic adjuvant for asthma, the relationship between bacterial components and asthma is complex. Recently, low levels of flagellin, the Toll-like receptor (TLR) 5 ligand, have been reported to promote asthma. Objective: We show that a therapeutic dose of flagellin suppresses asthma and that the effect occurs through generating regulatory dendritic cells (rDCs) and regulatory T (Treg) cells. Methods: Ovalbumin (OVA)-induced wild-type and TLR5 knockout asthmatic mice were treated intranasally with a mixture of OVA and 10 μg of a flagellin B (FlaB; of Vibrio vulnificus). OVA/FlaB-treated rDCs were adoptively transferred to mice with OVA-induced asthma. Anti-CD25 mAb was used to deplete Treg cells. A mixture of house dust mite (HDM) and FlaB was used to treat mice with HDM-induced asthma. Blood CD14+ monocyte-derived dendritic cells from HDM-sensitive asthmatic patients were treated with FlaB and incubated with autologous CD4+ T cells.

Results: An OVA/FlaB mixture ameliorated OVA-induced asthma by inhibiting T H1 /T H2 /T H17 responses in a TLR5-dependent manner through generating rDCs and Treg cells. The adoptive transfer of OVA/FlaB-treated dendritic cells inhibited OVA-induced asthma, whereas the depletion of CD25+ cells eliminated the inhibitory effect. A similar effect of FlaB was observed in mice with HDM-induced asthma. In patients with HDM-sensitive asthma, FlaB-treated rDCs inhibited HDM-stimulated T H1 /T H2 responses while enhancing Treg cells in an IL-10–dependent manner.

Conclusion: These findings collectively suggest that flagellin could be used as a tolerogenic adjuvant to treat allergic asthma. (J Allergy Clin Immunol 2015; nn:n.nn–nn.nn.)

Key words: Asthma, Toll-like receptor 5, flagellin, dendritic cells, regulatory T cells

Asthma is an allergen-derived immunologic disorder in the airways characterized by airway hyperresponsiveness (AHR), chronic airway inflammation, a preferential T H2 immune profile, and enhanced allergen-specific IgE production. T H1 and T H17 cytokine responses can be involved in the pathogenesis of asthma.1 Asthma has been controlled with allergen avoidance, pharmacotherapy, and allergen-specific immunotherapy (SIT). However, complete allergen avoidance is not possible. Current antiasthma medications remain palliative. SIT is not so effective and requires too long of a duration.

Over the past several decades, the incidence of asthma has increased.2 The hygiene hypothesis is one of the major attempts to explain this phenomenon. The reduction in microorganism load in the environment might increase the chance for asthma development.3 Conversely, early childhood exposure to environmental microorganisms can shift the immune response toward a T H1 -type response, induce regulatory T (Treg) cells, or suppress aberrant T H2 immune responses and allergic diseases.4,5 Bacterial infections have been reported to inhibit asthma development.6,7,8 The hygiene hypothesis might offer new therapeutic approaches for asthma and allergic diseases by using immunomodulatory bacterial components.

Bacterial flagellin, also known as Toll-like receptor (TLR) 5 ligand, has been reported to have diverse immunoregulatory activities. The roles of flagellin and TLR5 signaling in patients with allergic diseases are sometimes contradictory. Similar to other TLR ligands, a small amount of flagellin associated with indoor allergens was reported to promote asthma by priming T H2 responses.10 However, multiple reports have addressed the antiallergic activities of flagellins. We have reported previously that flagellin B (FlaB) from Vibrio vulnificus induces antigen-specific IgA production11 and that the intranasal administration of a mixture of ovalbumin (OVA) and FlaB inhibits AHR and
A fusion protein of OV A and flagellin inhibited IgE-mediated intestinal allergy in mice.13 TLR5 expression and function have been impaired in asthmatic patients.14 TLR5 expression in human umbilical cord blood was inversely associated with the development of atopic dermatitis.15 We hypothesize that the contradictory effects of flagellin might be due to the different doses given to mice. Different doses of TLR ligands can act in opposition, depending on the activation status of target cells.16-18 Low and high doses of LPS, the TLR4 ligand, mediated through stimulation of TLR4 on dendritic cells (DCs).21 In the present study we show that a therapeutic dose of FlaB functioned as a tolerogenic adjuvant to generate regulatory dendritic cells (rDCs) and Treg cells and ameliorated asthma in 2 experimental models. Similar rDC and Treg cell inductions have been noted in peripheral blood from patients with house dust mite (HDM)-induced allergic asthma.

**METHODS**

Further information can be found in the Methods section in this article's Online Repository at www.jacionline.org.

**Mice**

Female, 6-week-old BALB/c mice were purchased from Orient Bio Company (Seongnam, Korea). C57BL/6 TLR5 knockout mice obtained from the Research Institute for Microbial Diseases of Osaka University were backcrossed more than 10 times with wild-type (WT) BALB/c mice to generate BALB/c TLR5 knockout mice. The deletion of TLR5 in BALB/c mice was confirmed by using genome PCR and Western blotting. The mouse study protocol was approved by the Committee on Animal Welfare at Chonnam National University Medical School.

**Asthmatic patients and healthy subjects**

HDM-sensitive asthmatic patients (see Table E1 in this article’s Online Repository at www.jacionline.org) and healthy subjects were included in the study. Asthma was diagnosed based on typical symptoms and AHR to methacholine or the presence of a significant bronchodilator response. Sensitization to HDM was documented by using a positive skin prick test response, ImmunoCAP result (Pharmacia Diagnostics, Uppsala, Sweden), or both. Three healthy control subjects who had no allergic diseases, such as asthma, allergic rhinitis, and atopic dermatitis, were enrolled. Human study was approved by the Institutional Review Board at Chonnam National University Hospital. Written informed consent was received from 13 patients and 3 healthy subjects.

**Statistical analysis**

Comparisons were performed with the Mann-Whitney U test, Wilcoxon signed-rank test (SPSS Version 17; IBM, Huntsville, Ala), unpaired t test, or nonlinear regression analysis (Prism 5; GraphPad Software, La Jolla, Calif). A P value of less than .05 was considered statistically significant.

**RESULTS**

**OVA/FlaB mixture ameliorates OVA-induced asthma**

In our mouse model of asthma (Fig 1, A), 3 intranasal administrations of a mixture of 20 μg of OVA and 10 μg of FlaB decreased OVA-induced AHR (Fig 1, B); eosinophilic and neutrophilic airway inflammation (Fig 1, C); peribronchial and perivascular inflammation in lung tissues (Fig 1, D); IL-4, IL-5, and IL-13 levels in bronchoalveolar lavage fluid (BALF; Fig 1, E); OVA-stimulated IL-4, IL-5, and IL-13 production in bronchial lymph node (LN) cultures (Fig 1, F); and OVA-specific serum IgE levels (Fig 1, G). At the same time, IFN-γ production in both BALF (Fig 1, E) and LN cultures (Fig 1, F) was reduced. In contrast, levels of IL-10 and TGF-β, typical Treg cytokines, were increased in both BALF (Fig 1, E) and LN (Fig 1, F) cultures. We also determined the IL-17 level known to play a role in the pathogenesis of neutrophilic asthma.22 Treatment with the OVA/FlaB mixture decreased IL-17 production in both BALF and LN cultures (see Fig E1 in this article’s Online Repository at www.jacionline.org).

In contrast to our results, Wilson et al10 reported that flagellin promoted asthma. They used a very low dose (0.025 μg per dose) of flagellin compared with ours (10.0 μg per dose). To address these contradictory results, we tested the effects of different doses of FlaB. Treatment with 0.025 μg of FlaB, but not 10 μg of FlaB, increased AHR (see Fig E2, A, in this article’s Online Repository at www.jacionline.org), airway inflammation (see Fig E2, B), and IL-13 levels in BALF (see Fig E2, C). Treatment with 0.025 μg of FlaB, but not 10 μg of FlaB, increased AHR (see Fig E2, A), airway inflammation (see Fig E2, B), and IL-13 levels in BALF (see Fig E2, C). However, treatment with 0.025 μg of FlaB had no effect on IL-10 and TGF-β levels in LN cultures (see Fig E2, D). We checked the effects of 0.25 μg and 1 μg of FlaB. The 0.25-μg dose induced neither a Th2 nor a Treg cell response. A Treg cell response was noted with 1 μg of FlaB, whereas AHR inhibition was observed only at 10 μg of FlaB (see Fig E3 in this article’s Online Repository at www.jacionline.org). These results suggest that the differential effect of FlaB in the modulation of asthma progression and the Th2 response should depend on the dose: a lower dose (0.025 μg) exacerbates the disease, whereas a higher dose (10 μg) suppresses it. Based on this experiment, we determined 10 μg of FlaB to be the therapeutic dose.

The therapeutic effect of the OVA/FlaB mixture appeared to be mediated through the systemic immune response (see the Results section and Fig E4 in this article’s Online Repository at www.jacionline.org). The small amount of LPS contained in the OVA preparations did not affect experimental results (see the Results section and Fig E5 in this article’s Online Repository...
FIG 1. Suppressive effect of the OVA/FlaB mixture on asthma. A, Experimental protocol. B, Noninvasive (n = 5) and invasive (n = 5) AHR. *P < .001 versus OVA + OVA by using nonlinear regression analysis. C, Inflammatory cells in BALF (n = 5). D, Photomicrographs of lung tissues. Scale bars = 200 μm. E, Cytokines in BALF (n = 5). F, Cytokines in LN cell cultures (performed in triplicate in supernatants pooled from 3 independent experiments). G, OVA-specific IgE in serum (n = 5). Fig 1, C and E-G: *P < .05 and **P < .01, Mann-Whitney U test.
TLR5 is responsible for the suppressive effect of the OVA/FlaB mixture on asthma

To further confirm whether the inhibitory effect of the OVA/FlaB mixture on asthma was specifically TLR5 dependent, TLR5 knockout mice were used. In TLR5 knockout mice the OVA/FlaB mixture did not reduce OVA-induced AHR (Fig E7, A, and Fig E8, A, in this article’s Online Repository at www.jacionline.org); eosinophilic and neutrophilic airway inflammation (Fig E7, B, and Fig E8, B); peribronchial and perivascular inflammation (Fig E7, C); IL-4, IL-13, and IFN-γ levels in BALF (Fig E7, D, and Fig E8, C) and LN (see Fig E7, E, and Fig E8, D) cell cultures; or the concentration of OVA-specific serum IgE (see Fig E7, F, and Fig E8, E). IL-10 and TGF-β levels in BALF (Fig E7, D, and Fig E8, C) and LN cultures (see Fig E7, E, and Fig E8, D) did not increase. These findings corroborate that the inhibitory effect of the OVA/FlaB mixture on asthma would be mediated by suppression of the Th1/Th2 responses and the induction of Treg cells through TLR5.

OVA/FlaB mixture generates rDCs

Because DCs express high TLR levels, we hypothesized that the OVA/FlaB-induced immune regulation would be primarily related to DCs. We tested whether in vivo treatment with the OVA/FlaB mixture would induce rDCs. CD11c+ DCs were isolated from the bronchial LNs, and the expression levels of rDC marker molecules were analyzed. The OVA/FlaB mixture–treated mice showed a significant increase in mRNA expression of IL-10, indolamine 2,3 dioxygenase (IDO), and COX-2 (Fig 2, A). Indolamine 2,3 dioxygenase (IDO) and COX-2 are representative markers of rDCs. Bronchial LN DCs from mice treated with 10 μg of FlaB expressed higher CD80 and MHC class II levels but not CD86 levels (Fig E9, A, in this article’s Online Repository at www.jacionline.org). IL-10 and TGF-β production significantly increased, whereas IL-12 and TNF-α production decreased (Fig E9, B). In the same animals we also assayed cytokine production from splenic DCs. Similar to LN DCs, IL-10 and TGF-β production increased, whereas IL-12, TNF-α, and IL-1β production decreased (Fig E9, C). On the other hand, 0.025 μg of FlaB did not enhance rDC marker levels but rather enhanced production of proinflammatory cytokines, such as IL-12 and TNF-α (see Fig E9).

We also tested the regulatory property of rDCs induced by treatment with the OVA/FlaB mixture. Bronchial LN DCs from OVA/FlaB-treated mice were cultured with CD4+ T cells obtained from DO11.10 OVA transgenic mice in the presence of OVA peptides. The bronchial LN DCs significantly suppressed the proliferation of OVA peptide–stimulated CD4+ T cells compared with cells from mice treated only with OVA (Fig 2, B, and see Table E2). These findings suggest that FlaB treatment rendered LN DCs tolerogenic.

Adaptive transfer of OVA/FlaB-treated rDCs suppresses asthma

Spleen DCs from OVA-sensitized mice were treated with the OVA/FlaB mixture in vitro and adoptively transferred to other OVA-sensitized mice to determine whether DCs play a pivotal role in the asthma suppression induced by the OVA/FlaB mixture (Fig 3, A). In vitro the OVA/FlaB-treated splenic DCs from WT mice showed increased IL-10 production, suggesting rDC induction. However, DCs from TLR5 knockout mice did not increase IL-10 production on OVA/FlaB treatment (Fig 3, B). The transfer of OVA/FlaB-treated DCs from WT mice inhibited OVA-induced AHR (Fig 3, C) and eosinophilic and neutrophilic airway inflammation (Fig 3, D) in the recipients. A significant decrease in IL-4, IL-13, and IFN-γ production (Fig 3, E) and an increase in IL-10 and TGF-β production (Fig 3, F) in LN cultures were observed in the mice that received OVA/FlaB-treated DCs. Serum OVA–specific IgE levels were also significantly decreased in the adoptively transferred mice (Fig 3, G). These changes were not noted in recipients of DCs from TLR5 knockout mice treated with the OVA/FlaB mixture (Fig 3, C-G). These results demonstrate that the therapeutic effect of the OVA/FlaB mixture treatment in asthma is mediated by the generation of rDCs through TLR5.

Spleen DCs or bone marrow–derived dendritic cells (BMDCs) from OVA-sensitized mice were treated with different doses of
FlaB to determine the effect of FlaB doses on rDC generation. Compared with the low-dose FlaB (0.05 μg/mL), high-dose FlaB (0.5-10 μg/mL) significantly enhanced IL-10 and TGF-β production in splenic DCs, but no effect was observed in BMDCs (see Fig E10 in this article’s Online Repository at www.jacionline.org).

**Treg cells play a critical role in the suppressive effect of the OVA/FlaB mixture on asthma**

We examined whether treatment with the OVA/FlaB mixture induced forkhead box P3 (Foxp3) Treg cell responses. CD4+ T cells were isolated from bronchial LNs, and numbers of Foxp3+ Treg cells and levels of effector molecules were analyzed. CD4+Foxp3+ cell numbers increased in bronchial LNs of OVA/10 μg of FlaB mixture–treated WT mice but not in TLR5 knockout mice (Fig 4, A). The mean fluorescence intensity of positive Foxp3 signals was also significantly higher in the WT group treated with the therapeutic dose (10 μg) of FlaB and OVA, which was not noted in the knockout group (Fig 4, A). The mRNA expression levels of Treg cell–associated molecules, such as Foxp3, IL-10, TGF-β, cytotoxic T-lymphocyte antigen 4, and granzyme B, increased in CD4+ T cells from the bronchial LNs.
of OVA/FlaB mixture–treated mice (Fig 4, B). In addition, CD4+CD25+ Treg cells from OVA/FlaB-treated mice more effectively suppressed the proliferation of CD4+ effector T cells in coculture experiments (see Fig E11 in this article’s Online Repository at www.jacionline.org). Thus we inferred that intranasal treatment with the OVA/FlaB mixture inhibited asthma and TH1 and TH2 responses through enhancing CD4+CD25+Foxp3+ Treg cells.

We further determined whether Treg cells induced by OVA/FlaB mixture treatment mediate an immunomodulatory function by depleting Treg cells in OVA-sensitized mice with an anti-CD25 mAb (see Fig E12 in this article’s Online Repository at www.jacionline.org). The anti-CD25 mAb treatment abrogated the immunoregulatory effect of the OVA/FlaB mixture on OVA-induced AHR (see Fig E13, A, in this article’s Online Repository at www.jacionline.org), eosinophilic and neutrophilic airway inflammation (see Fig E13, B), peribronchial and perivascular inflammatory pathology in the lung (see Fig E13, C), cytokine responses in BALF (see Fig E13, D) and LN (see Fig E13, E) cultures, and serum OVA-specific IgE levels (see Fig E13, F). These results indicate that the immunomodulatory effect of the OVA/FlaB mixture on asthma progression was mediated by a CD25+ Treg cell–dependent mechanism.

HDM/FlaB mixture ameliorates HDM-induced asthma

HDM is the most common allergen in human asthma, and the development of a therapeutic modality for HDM-induced asthma would have high clinical effect. We assessed whether an HDM/FlaB mixture would inhibit HDM-induced asthma in a mouse model (see Fig E14, A, in this article’s Online Repository at www.jacionline.org). Treatment with a 10-μg FlaB mixture with HDM reduced HDM-induced AHR (see Fig E14, B), eosinophilic and neutrophilic airway inflammation (see Fig E14, C) and IL-4, IL-13, and IFN-γ levels in BALF (see Fig E14, D) and LN (see Fig E14, E) cultures and enhanced the production of IL-10 and TGF-β in BALF (see Fig E14, D) and LN (see Fig E14, E) cultures. Together, the HDM/FlaB mixture inhibited HDM-induced asthma and Th1/Th2 responses while enhancing the Treg cell response, similar to what was observed in the OVA sensitization model.
Effect of FlaB on rDCs and Treg cells is reproduced in peripheral blood from HDM-sensitive asthmatic patients

After concluding the animal model study, we questioned whether the effect of FlaB on DCs and T cells in the mouse experiments could be reproduced in human PBMCs. We tested the effect of FlaB-treated autologous DCs on HDM-stimulated CD4+ T-cell functions by using PBMCs obtained from patients with HDM-sensitive asthma (see Table E1). In response to FlaB treatment, monocyte-derived CD11c+ DCs generated from PBMCs manifested rDC phenotypes. DCs treated in vitro for 48 hours with 1 μg/mL FlaB increased IL-10 production (Fig 5, A). Treatment with FlaB, but not 0.1 μg/mL LPS, increased the expression of HLA-G on DCs (Fig 5, B), which is a representative marker of human rDCs.28 FlaB treatment of DCs from asthmatic patients resulted in significantly increased TGF-β production but decreased production of IL-6, IL-12, and IL-1β (see Fig E15, A, in this article’s Online Repository at www.jacionline.org). Interestingly, IL-10 production by untreated DCs from asthmatic patients was less than half that by DCs from healthy subjects. The reduced IL-10 levels in asthmatic patients returned to normal after the FlaB treatment. In healthy control subjects FlaB treatment did not affect IL-10 and TGF-β production (Fig 5, A, and see Fig E15, A). Basal TLR5 expression on DCs was much lower in patients than in control subjects. After FlaB treatment, the TLR5 expression in DCs from asthmatic patients recovered to that in DCs from control subjects (see Fig E15, B).

Subsequently, we tested whether FlaB-treated autologous DCs have the potential to induce Treg cells. Either FlaB-treated or mock–treated control DCs were cocultured with CD4+ T cells in the presence of HDM extracts. FlaB-treated DCs significantly induced CD4+ Foxp3+ cells from HDM-stimulated CD4+ T cells (Fig 5, C, and see Fig E16 in this article’s Online Repository at www.jacionline.org). It was also noted that mRNA expression of CD25, Foxp3, and lymphocyte activation gene 3 was induced after cocultures (see Fig E15, C). The CD4+ Foxp3+ cell induction from peripheral CD4+ cells was prevented by an anti–IL-10 receptor mAb (Fig 5, D), suggesting that FlaB-treated rDCs induced Treg cells through an IL-10–dependent mechanism. The FlaB treatment of DCs suppressed the production of IL-13 and IFN-γ but increased the production of IL-10 and TGF-β in the HDM-stimulated CD4+ T-cell cocultures (Fig 5, E). Intracellular cytokine staining also showed that coculturing of FlaB-treated DCs inhibited the production of IL-4, IL-5, IL-13, and IFN-γ from HDM-stimulated CD4+ T cells but increased the production of IL-10 (Fig 5, F, and see Fig E16). These data collectively corroborate that FlaB treatment generated rDCs in PBMCs from patients with allergic asthma, which in turn suppressed Th1/Th12 responses and enhanced Treg cells in an IL-10–dependent manner.

DISCUSSION

A substantial proportion of asthmatic patients’ symptoms are poorly controlled by currently available treatments. This subgroup is defined as having severe refractory asthma. Blood CD14+ monocyte–derived DCs from patients with allergic asthma could be driven toward rDC functionality by FlaB treatment. We speculate that these rDCs can be used for cell-based asthma therapy. We propose that flagellin-treated autologous rDC cell therapy can be applied to the treatment of severe refractory asthma. Regulatory immune cell therapy has already been attempted in the treatment of immunologic pathologies, such as rheumatoid arthritis and other autoimmune diseases.30 Additionally, SIT is the only currently used treatment modality that has been shown to modify the natural progression of asthma in patients with mild-to-moderate disease. However, not all asthmatic patients benefit from SIT, and for those who do benefit, the magnitude of effect might not be very substantial.31 SIT requires a long course of therapy (>3 years). Therefore the development of more effective SIT is paramount. Because SIT is known to modulate immune reactions through increased production of Treg cells,32 an agent that effectively generates Treg cells could be a potent adjuvant in the development of a new SIT. Flagellin could be a powerful candidate based on our findings. Recently, sublingual SIT had become available. Flagellin, having very effective mucosal adjuvant activity,31 might prove effective for sublingual SIT in human subjects.

Safety would be a concern for any flagellin-based immunomodulatory therapy. Recent studies have shown contradictory results on the effect of flagellin,10,33 suggesting that flagellin can promote asthma or atopic dermatitis. However, both of these studies10,33 are based on experiments in which flagellin doses were 1/50th to 1/1000th of those used in this study. We recapitulated the asthma-promoting activity of lower doses of flagellin in our system. Higher doses of flagellin should be used to avoid possible asthma exacerbation. However, the safety margin of flagellin might become narrower in individual asthmatic patients. Cell-based immunotherapy with rDCs induced in vitro by flagellin treatment will be a safer choice in cases of more severe asthma to avoid the risk of reactogenicity. Additionally, there could be a worry that a probable overproduction of Treg cells might promote the exacerbation of infectious diseases or cancer. However, the present patients’ PBMC studies showed that flagellin treatment restored the defective functions of rDCs34 and Treg cells35 to healthy control levels.

The molecular mechanism of how TLR5 signaling mediates the development of rDCs and Treg cells remains elusive. The immunomodulatory activity of flagellin has been reported to occur primarily through DCs.21 We show that in vivo flagellin-treated mouse bronchial LN DCs and in vitro flagellin-treated human DCs both produce IL-10, which is consistent with the results of similar studies.13,36 IL-10–producing DCs or IL-10–differentiated DCs induce Treg cells.32–40 Myeloid DCs might be the major player rather than plasmacytoid DCs.13,36 Ubiquitin edition enzyme A20, a quiescent factor against activation counteracting nuclear factor κB signaling, might play a role in the development of rDCs.41 Treatment of DCs with high LPS concentrations appeared to upregulate A20 and consequently suppressed allergic asthma.42 TLR activation with high-dose agonists can similarly activate immunomodulatory pathways in antigen-presenting cells. Baseline characteristics of DCs might differ between asthmatic patients and healthy subjects. The present and other studies14 revealed the reduction of TLR5 expression on DCs in asthmatic patients, which can be explained as follows: healthy subjects might have more potentially tolerogenic DCs expressing high-level TLR5 on the surface, whereas asthmatic patients might have less. We have previously noted in mice that flagellin treatment results in a significant induction of TLR5 expression on DCs, presumably through a positive feedback mechanism.11 Hence, after flagellin treatment, TLR5 expression on DCs should have been restored, and more tolerogenic DCs should have been generated. However,
other TLR5-expressing cells might also be activated by flagellin. Very low doses of flagellin have been reported to produce thymic stromal lymphopoietin from skin keratinocytes or lung epithelial cells.\textsuperscript{10,33} Thus we cautiously hypothesize that lower doses of flagellin might promote the T\textsubscript{i}2 response by activating epithelial cells, whereas higher doses should induce the Treg cell response by inducing rDCs.

We previously showed that flagellin is an excellent mucosal adjuvant for augmenting IgA production in both the mucosal and systemic compartments.\textsuperscript{11} IgA production is thought to be related to TGF-\beta regulation, which is crucial for induction of Treg cells.\textsuperscript{3,44} Treg cells have been reported to be the major encourager cells for IgA responses to flagellin in the mouse gut.\textsuperscript{35} Otherwise, flagellin could directly act on T cells and induce Treg cells. The treatment of lymphocytes with flagellin has been reported to induce Treg cells in cats.\textsuperscript{36} Human Treg cells have been reported to express TLR5, with flagellin treatment enhancing the function of Treg cells.\textsuperscript{37} However, in our system the direct action of flagellin on T cells did not play a major role because DC adoptive transfer conferred the inhibitory effect almost completely.

We thank Dr Shizuo Akira of Osaka University for providing C57BL/6 TLR5 knockout mice.

Key messages

- A therapeutic dose of flagellin inhibits allergic asthma by generating rDCs and Foxp\textsuperscript{3} Treg cells in a TLR5-dependent manner in mice with OVA- and HMDS-induced asthma.

The immunomodulatory effect of flagellin could be reproduced in PBMCs from HMDS-sensitive asthmatic patients.

REFERENCES

47. Crellin NK, Garcia RV, Hadsfar O, Allan SE, Steiner TS, Levings MK. Human CD4+ T cells express TLR5 and its ligand flagellin enhances the suppressive capacity and expression of FOXP3 in CD4+CD25+ regulatory cells. J Immunol 2005;175:8051-9,