研究成果の刊行に関する一覧表　平成 22 年度(2010)

<雑誌>

研究代表者：中江進

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Yagami A, Kajiwara N, Oboki K, Ohno T, Morita H, Sunnarborg SW, Okumura K, Ogawa H, Saito H, Nakae S.	Amphiregulin is not essential for induction of contact hypersensitivity.	Allergol Int.	59	277-284	2010
Ishii A, Oboki K, Nambu A, Morita H, Ohno T, Kajiwara N, Arae K, Sudo H, Okumura K, Saito H, Nakae S.	Development of IL-17-mediated delayed-type hypersensitivity is not affected by down-regulation of IL-25 expression.	Allergol Int	59	399-408	2010
Nambu A, Nakae S.	IL-1 and Allergy.	Allergol Int.	59	125-135	2010
Oboki K, Ohno T, Kajiwara N, Saito H, Nakae S.	IL-33 and IL-33 receptors in host defense and diseases.	Allergol Int.	59	143-160.	2010
Oboki K, Ohno T, Kajiwara N, Arae K, Morita H, Ishii A, Nambu A, Abe T, Kiyonari H, Matsumoto K, Sudo K, Okumura K, Saito H, Nakae S.	IL-33 is a crucial amplifier of innate rather than acquired immunity.	Proc Natl Acad Sci U S A.	107	18581-18586	2010
Kajiwara N, Oboki K, Ohno T, Ishii A, Sunnarborg SW, Okumura K, Saito H, Nakae S.	Amphiregulin is not essential for ovalbumin-induced acute airway inflammation in mice.	Allergol Int.	59	207-211.	2010

分担研究者：斎藤博久

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Kajiwara N, Sasaki T, Bradding P, Cruse G, Sagara H, Ohmori K, Saito H, Ra C, Okayama Y.	Activation of human mast cells through the platelet-activating factor receptor.	J Allergy Clin Immunol.	125	1137-1145	2010
Yagami A, Orihara K, Morita H, Futamura K, Hashimoto N, Matsumoto K, Saito H, Matsuda A.	IL-33 mediates inflammatory responses in human lung tissue cells.	J Immunol.	185	5743-5750.	2010
Oboki K, Nakae S, Matsumoto K, Saito H.	IL-33 and Airway Inflammation.	Allergy Asthma Immunol Res. 2011;3(2):81-88.	3	81-88	2011
Ebihara T, Azuma M, Oshiumi H, Kasamatsu J, Iwabuchi K, Matsumoto K, Saito H, Taniguchi T, Matsumoto M, Seya T.	Identification of a polyI:C-inducible membrane protein that participates in dendritic cell-mediated natural killer cell activation.	J Exp Med.	207	2675-2687.	2010
Harada M, Hirota T, Jodo AI, Hitomi Y, Sakashita M, Tsunoda T, Miyagawa T, Doi S, Kameda	TSLP Promoter Polymorphisms are Associated with Susceptibility to Bronchial Asthma.	Am J Respir Cell Mol Biol.	—	Jul 23. [Epub ahead of print]	2010

M, Fujita K, Miyatake A, Enomoto T, Noguchi E, Masuko H, Sakamoto T, Hizawa N, Suzuki Y, Yoshihara S, Adachi M, Ebisawa M, Saito H, Matsumoto K, Nakajima T, Mathias RA, Rafaels N, Barnes KC, Himes BE, Duan QL, Tantisira KG, Weiss ST, Nakamura Y, Ziegler SF, Tamari M.					

分担研究者：奥村康

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Kanada S, Nishiyama C, Nakano N, Suzuki R, Maeda K, Hara M, Kitamura N, Ogawa H, Okumura K.	Critical role of transcription factor PU.1 in the expression of CD80 and CD86 on dendritic cells.	Blood.	117	2211-2222.	2011

Maeda K, Nishiyama C, Ogawa H, Okumura K.	GATA2 and Sp1 positively regulate the c-kit promoter in mast cells.	J Immunol.	185	4252-4260.	2010
Niyonsaba F, Ushio H, Hara M, Yokoi H, Tominaga M, Takamori K, Kajiwara N, Saito H, Nagaoka I, Ogawa H, Okumura K.	Antimicrobial peptides human beta-defensins and cathelicidin LL-37 induce the secretion of a pruritogenic cytokine IL-31 by human mast cells.	J Immunol.	184	3526-3534.	2010
Vu AT, Baba T, Chen X, Le TA, Kinoshita H, Xie Y, Kamijo S, Hiramatsu K, Ikeda S, Ogawa H, Okumura K, Takai T.	Staphylococcus aureus membrane and diacylated lipopeptide induce thymic stromal lymphopoietin in keratinocytes through the Toll-like receptor 2-Toll-like receptor 6 pathway.	J Allergy Clin Immunol.	126	985-993	2010
Hirasawa Y, Takai T, Nakamura T, Mitsuishi K, Gunawan H, Suto H, Ogawa T, Wang XL, Ikeda S, Okumura K, Ogawa H.	Staphylococcus aureus extracellular protease causes epidermal barrier dysfunction.	J Invest Dermatol.	130	614-617.	2010

分担研究者：山口正雄

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Toda T, Yamaguchi M, Nakase Y, Sugimoto N, Suzukawa M, Nagase H, Ohta K.	A case of anaphylactic reaction following matsutake mushroom ingestion: demonstration of histamine release reaction of basophils.	Allergol Int.	59	417-419.	2010
Sano Y, Yamada H, Ogawa C, Yamaguchi M.	Some asthmatics show elevation of the peripheral venous oxygen pressure (PvO(2)).	Allergol Int.	60	109-110.	2011

Ⅴ．　研究成果の刊行物・別刷

（主なもの）

Allergology International. 2010;59:207-211
DOI: 10.2332/allergolint.09-OA-0144

ORIGINAL ARTICLE

Amphiregulin Is Not Essential for Ovalbumin-Induced Acute Airway Inflammation in Mice

Naoki Kajiwara[1,2], Keisuke Oboki[1], Tatsukuni Ohno[1], Akina Ishii[1], Susan W Sunnarborg[3], Ko Okumura[2], Hirohisa Saito[1,2] and Susumu Nakae[1,2,4]

ABSTRACT

Background: The number of amphiregulin (AR)-positive mast cells in the bronchial mucosa and the levels of AR in sputum from asthmatic patients have been reported to be increased. In addition, AR can promote mucin gene expression in human epithelial cells, suggesting that AR contributes to the pathogenesis of allergic asthma.

Methods: To elucidate the role of AR in the pathogenesis of asthma, we immunized AR-deficient mice with ovalbumin (OVA) and then induced airway inflammation in them after OVA inhalation. The OVA-induced airway inflammation was assessed on the basis of the lung histology, number of leukocytes in the bronchoalveolar lavage (BAL) fluid, Th2 cytokine levels in the BAL fluid and OVA-specific IgG1 and IgE levels in the serum and compared between AR-sufficient and -deficient mice.

Results: The OVA-induced airway inflammation was comparable in the AR-sufficient and -deficient mice.

Conclusions: Amphiregulin is not essential for induction of acute airway inflammation by OVA in mice.

KEY WORDS

amphiregulin, asthma, EGF, IgE, mast cells

INTRODUCTION

Allergic asthma is considered to be a typical Th2-type cytokine-mediated chronic pulmonary disease.[1,2] Th2-cell-derived IL-4 and IL-13 are essential for IgE class-switching and production by B cells. Subsequently, IgE provokes antigen-specific mast cell activation to induce degranulation and cytokine secretion, including Th2-type cytokines such as IL-4 and IL-13.[1,2] IL-5 and IL-13, and IL-9, are key cytokines for inducing accumulation and activation of eosinophils and mast cells, respectively, in sites of inflammation.[1,2] Therefore, these Th2-type cytokines orchestrate the activation of various types of immune cells, leading to exacerbation of airway inflammation and airway hyperresponsiveness. In particular, Th2 cells and mast cells, as sources of Th2-type cytokines, are deeply involved in the pathogenesis of asthma.

We previously demonstrated that human mast cells can produce amphiregulin (AR), which is a member of the EGF family of growth factors/cytokines, after IgE-FcεRI crosslinking.[3] AR is known to be physio/pathologically involved in mammary gland development, blastocyst implantation, bone formation and nerve regeneration, and to contribute to the progression of breast cancer.[4,5] In addition, AR can activate keratinocytes and epidermal hyperplasia, suggesting that it is involved in the pathogenesis of psoriasis.[5,6] However, the precise contributions of AR to the immune responses are poorly understood. Regarding this, we reported for the first time that the number of AR-expressing mast cells was increased in bronchial mucosa from patients with asthma.[3] Moreover, AR promoted expression of the mucin gene such as MUC2 and MUC5AC in human epithelial cells[3] and proliferation of human lung fibroblasts.[7] In addition to mast cells, it was shown that Th2 cells, but not Th1 cells, expressed AR, and that Th2-cell-derived AR en-

[1]Department of Allergy and Immunology, National Research Institute for Child Health and Development, [2]Atopy Research Center, Juntendo University, [4]Frontier Research Initiative, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan and [3]Department of Biochemistry and Biophysics, School of Medicine, University of North Carolina, NC, USA.
Correspondence: Susumu Nakae, PhD, Frontier Research Initiative, The Institute of Medical Science, The University of Tokyo, 4-6-1 Shirokanedai, Minato, Tokyo 108-8639, Japan.
Email: snakae@ims.u-tokyo.ac.jp
Received 4 September 2009. Accepted for publication 6 January 2010.

hanced resistance to nematode infection.[8] These observations suggest that AR produced by mast cells and/or Th2 cells potentially contributes to the development of immune responses to infections and allergies. In support of this notion, Kim *et al.*[9] very recently reported that the levels of AR in the sputum from patients with asthma were increased and correlated with an increased number of eosinophils in the sputum. However, the precise role of AR in the pathogenesis of asthma remains to be elucidated. Therefore, in the present study, we used AR-deficient mice to investigate the contribution of AR in the development of allergen-induced airway inflammation.

METHODS

MICE

Amphiregulin-deficient ($AR^{-/-}$) mice on a 129 × B6 mixed background were originally generated as described elsewhere.[10] Littermates ($AR^{+/-}$ and $AR^{-/-}$ mice) were used in all experiments. By ELISA, the levels of AR in lung homogenates were comparable between $AR^{+/+}$ and $AR^{+/-}$ mice (data not shown). The mice were housed under specific pathogen-free conditions at the National Research Institute for Child Health and Development, and the animal protocols were approved by the Institutional Review Board of the National Research Institute for Child Health and Development.

OVA-INDUCED AIRWAY INFLAMMATION

OVA-induced airway inflammation was established in the mice as described elsewhere.[11] Briefly, mice were immunized intraperitoneally with 10 μg OVA (Grade V, SIGMA-Aldrich, St Louis, MO, USA) in 0.2 ml PBS on days 1, 3, 5, 7, 9, 11 and 13. On days 41, 44 and 47, the animals were treated intranasally with 200 μg OVA in 20 μl PBS or with 20 μl PBS alone.

HISTOLOGY

At 24 hours after the last OVA or PBS inhalation, lungs were harvested and fixed in Carnoy's fluid. The fixed lungs were embedded in paraffin, and 5-μm-thick serial sections were stained with hematoxylin and eosin (H&E).

BAL FLUID

At 24 hours after the last OVA or PBS inhalation, BAL fluid and cells were collected as described previously.[12] The total cell number in the BAL fluids was counted, and cytospin specimens were prepared to quantify the proportions of the different cell types after staining with Hansel's stain.

CYTOKINE ELISA

IL-4, IL-5, eotaxin, TSLP and AR levels in the BAL fluids were measured using mouse IL-4 and IL-5 ELISA kits (eBioscience, San Diego, CA, USA) and mouse eotaxin, TSLP and amphiregulin and human/mouse TGF-β1 ELISA kits (R&D Systems, Minneapolis, MN, USA).

OVA-SPECIFIC Ig DETECTION

At 24 hours after the last OVA or PBS inhalation, blood was collected, and the serum was separated and stored at -80℃ until assay. OVA-specific IgG1 and IgE levels in sera were determined as described elsewhere.[13]

STATISTICAL ANALYSES

Data show the mean + SEM and were evaluated for statistical significance using the two-tailed Student's *t* test.

RESULTS

AMPHIREGULIN IS NOT ESSENTIAL FOR THE DEVELOPMENT OF IgE- AND MAST CELL-MEDIATED OVA-INDUCED AIRWAY INFLAMMATION

It is known that the requirement for mast cells and IgE in the development of mouse airway inflammation induced by OVA differs as a result of whether the sensitization with OVA is performed in the presence or absence of aluminum hydroxide (alum). That is, mast cells and IgE are required for sensitization in the absence of alum, but not in the presence of alum.[14] Since we reported that AR is produced by mast cells after IgE-FcεRI crosslinking, we used AR-deficient mice to investigate the role of AR in IgE- and mast cell-mediated OVA-induced airway inflammation. After OVA sensitization without alum, mice were treated intranasally with OVA or PBS. At 24 hours after the last OVA challenge, the numbers of total cells, eosinophils, macrophages, lymphocytes and neutrophils in the BAL fluid were comparable between $AR^{+/-}$ and $AR^{-/-}$ mice (Fig. 1). Consistent with those BAL cell profiles, airway inflammation such as leukocyte recruitment, goblet and epithelial cell hyperplasia and mucus secretion assessed on the basis of the lung histology was also similar in the $AR^{+/-}$ and $AR^{-/-}$ mice after the OVA challenge (Fig. 2). These observations indicate that AR is not essential for IgE- and mast cell-mediated OVA-induced airway inflammation in mice.

AMPHIREGULIN-DEFICIENT MICE SHOWED IDENTICAL LEVELS OF Th2 CYTOKINES IN BAL FLUIDS DURING MURINE ASTHMA

We next examined the contribution of AR to the production of Th2 cytokines such as IL-4, IL-5 and IL-13 and AR-receptor expressing epithelial cell-derived cytokines such as eotaxin, TGF-β1 and TSLP during IgE- and mast cell-mediated OVA-induced airway inflammation. At 24 hours after the last OVA inhalation, the levels of IL-4 and eotaxin were significantly increased in BAL fluid from OVA-challenged mice in comparison with PBS-treated mice (Fig. 3). However,

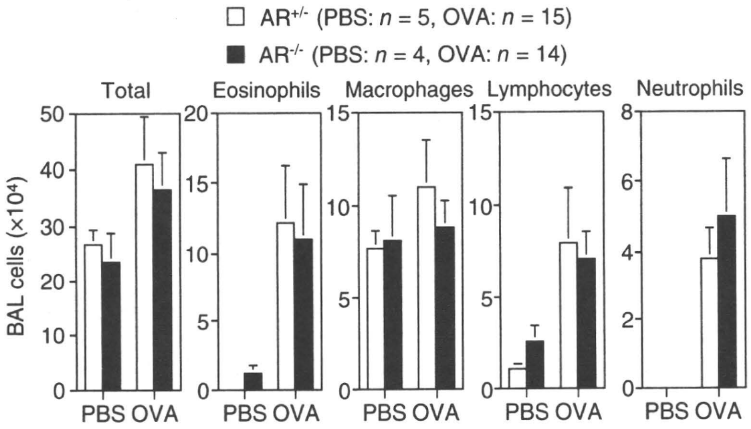

Fig. 1 Number of BAL cells in amphiregulin-deficient mice during OVA-induced airway inflammation. OVA-sensitized mice were treated intranasally with OVA or PBS alone. At 24 hours after the last OVA or PBS inhalation, BAL fluids were collected. Then the number and types of cells in the BAL fluids were determined. Open columns = $AR^{+/-}$ mice (PBS: n = 5; OVA: n = 15), and closed columns = $AR^{-/-}$ mice (PBS: n = 5; OVA: n = 14). Data show the mean + SEM.

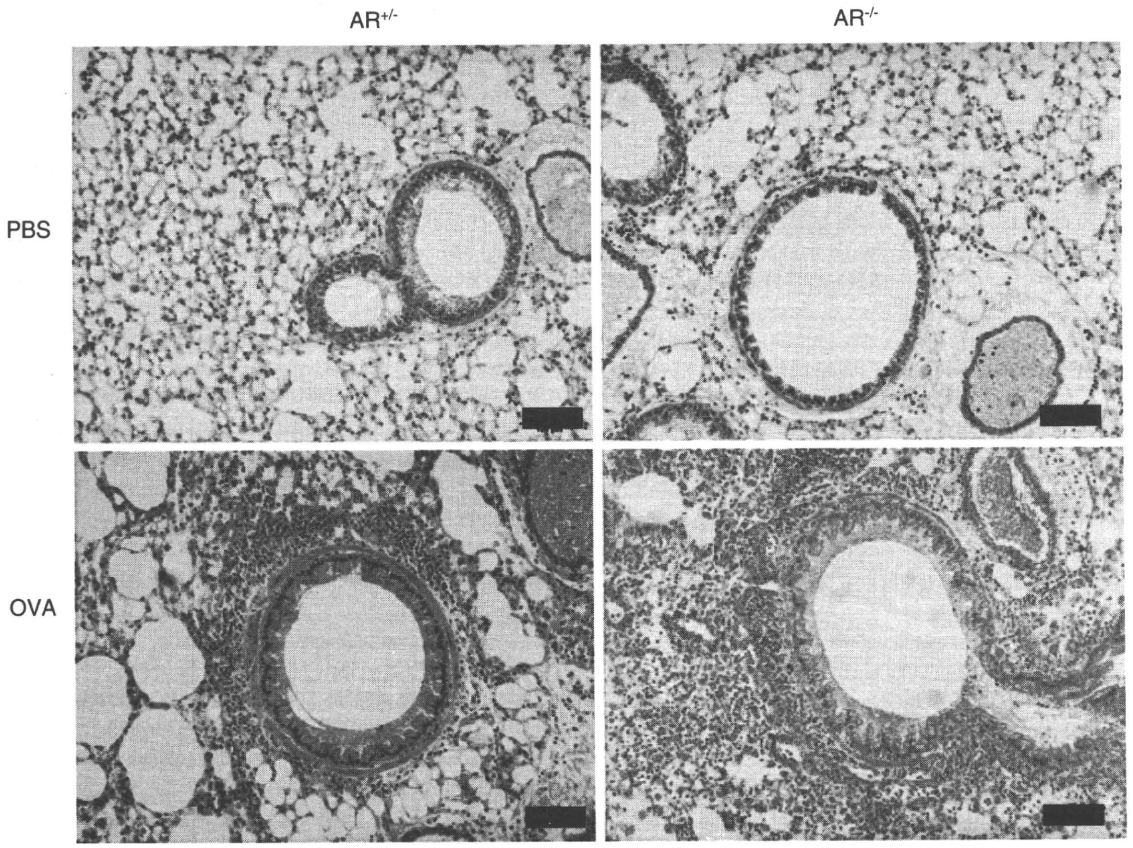

Fig. 2 Airway inflammation was similarly observed in amphiregulin-deficient mice during OVA-induced airway inflammation. At 24 hours after the last OVA or PBS inhalation, lungs were harvested from mice as shown in Figure 1. The sections of lungs were stained with H & E. Scale bars = 100 μm. Data show a representative result.

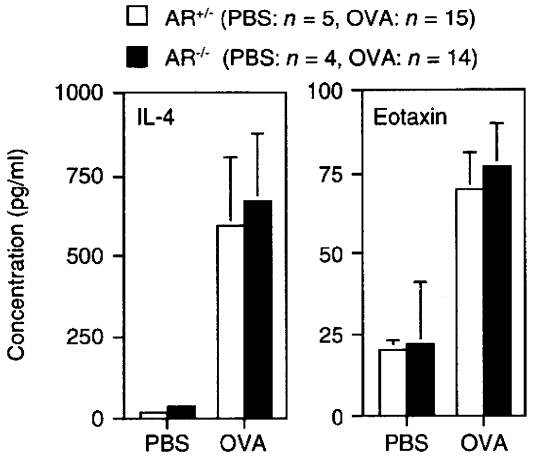

Fig. 3 IL-4 and eotaxin levels in BAL fluids from amphiregulin-deficient mice during OVA-induced airway inflammation. At 24 hours after the last OVA or PBS inhalation, BAL fluids were collected from mice as shown in Figure 1. IL-4 and eotaxin levels in the BAL fluids were determined by ELISA. Open columns = $AR^{+/-}$ mice (PBS: n = 5; OVA: n = 15), and closed columns = $AR^{-/-}$ mice (PBS: n = 5; OVA: n = 14). Data show the mean + SEM.

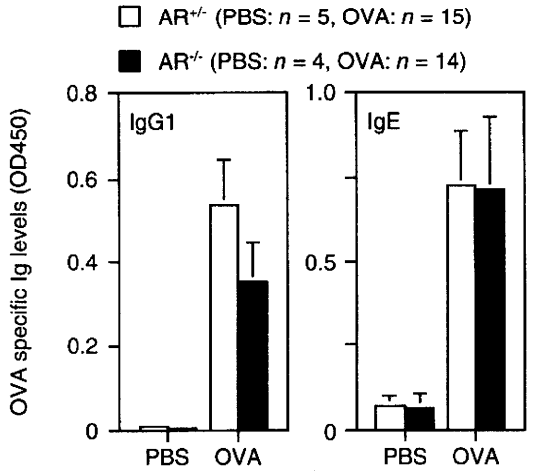

Fig. 4 Serum OVA-specific IgG1 and IgE levels in amphiregulin-deficient mice during OVA-induced airway inflammation. At 24 hours after the last OVA or PBS inhalation, sera were collected from mice as shown in Figure 1. OVA-specific IgG1 and IgE levels in the sera were determined by ELISA. Open columns = $AR^{+/-}$ mice (PBS: n = 5; OVA: n = 15), and closed columns = $AR^{-/-}$ mice (PBS: n = 5; OVA: n = 14). Data show the mean + SEM.

the amounts of AR, IL-5, IL-13, TGF-β1 and TSLP in the BAL fluids were below the limit of detection by ELISA in the tested settings (data not shown). We found that the levels of IL-4 and eotaxin in BAL fluids were indistinguishable between the OVA-challenged $AR^{-/-}$ mice and the OVA-challenged $AR^{+/-}$ mice (Fig. 3), indicating that AR is not necessary for IL-4 and eotaxin production during IgE- and mast cell-mediated OVA-induced airway inflammation in mice.

OVA-SPECIFIC Ig PRODUCTION IS EQUIVALENT IN AMPHIREGULIN-DEFICIENT MICE DURING MURINE ASTHMA

OVA-specific IgE is important for the pathogenesis of the murine asthma model. At 24 hours after the last OVA inhalation, the levels of OVA-specific IgG1 and IgE were markedly increased in sera from OVA-challenged mice compared with sera from PBS-treated mice (Fig. 4). However, the Ig levels were not statistically different between $AR^{+/-}$ and $AR^{-/-}$ mice (Fig. 4). These observations indicate that AR is not responsible for Th2-cytokine-dependent IgG1 and IgE production during IgE- and mast cell-mediated OVA-induced airway inflammation in mice.

DISCUSSION

Amphiregulin, a member of the EGF family of growth factors/cytokines, was first identified in supernatants from breast cancer cell lines stimulated with PMA.[15] AR has dual functions: it both enhances and inhibits the growth of certain cell lines.[4,5] In addition to its role as a growth factor, AR also acts as a cytokine in immune systems.[8] Indeed, Th2-cell-derived AR is crucial for host defense against nematode infection.[8] Moreover, IgE-FcεRI-stimulated mast cells produce AR, and AR enhances mucin gene expression in human epithelial cells and proliferation of human lung fibroblasts.[3,7] These observations suggest that AR may contribute to the pathogenesis of Th2 cell- and mast cell-mediated immune responses, such as nematode infection, and allergic disorders. In support of this, we previously showed that the number of AR-expressing mast cells is increased in bronchial mucosa from patients with asthma.[3] In addition, AR levels are increased in the sputum from asthmatic patients.[9] However, in the present study, we showed that even AR-deficient mice similarly developed Th2 cytokine- and mast cell-mediated airway inflammation in response to OVA. That is, OVA-induced airway inflammation accompanied by eosinophilia, OVA-specific IgG1 and IgE production, and IL-4 and eotaxin production was equivalently observed even in AR-deficient mice. These observations indicate that AR is not essential for OVA induction of Th2- and mast cell-mediated airway inflammation. However, the precise role of AR in the pathogenesis of allergic asthma may not be fully explained by the animal model used in our study, in which treatment with steroids can result in attenuation of acute airway inflammation. Accordingly, we originally identified AR as an upregulated gene in IgE-stimulated human mast cells even in the presence of glucocorticoids.[3]

These observations suggest that AR may be involved in the pathogenesis of steroid-resistant chronic asthma associated with airway remodeling rather than the pathogenesis of steroid-responsive acute asthma. Therefore, the role of AR in the pathogenesis of chronic airway inflammation should be investigated by using AR-deficient mice in a future study.

In summary, we demonstrated that AR is not essential for induction of acute airway inflammation by OVA in mice.

ACKNOWLEDGEMENTS

We thank Shuhei Fukuda, Hiromi Wakita, Michiko Yamada, and Noriko Hashimoto for their excellent technical assistance, Akane Nakamura for animal care, and Dr. David Lee for providing amphiregulin-deficient mice. This work was supported by a grant to H.S. from the National Institute of Biomedical Innovation and by Grants-in-Aid for Young Scientists (B) to N.K., K.O. and S.N., and by the Program for Improvement of Research Environment for Young Researchers, The Special Coordination Funds for Promoting Science and Technology to S.N. from the MEXT, Japan and by a US National Institute of Health grant CA43793 to S.W.S.

REFERENCES

1. Wills-Karp M. Immunologic basis of antigen-induced airway hyperresponsiveness. *Annu Rev Immunol* 1999;**17**: 255-81.
2. Cohn L, Elias JA, Chupp GL. Asthma: mechanisms of disease persistence and progression. *Annu Rev Immunol* 2004;**22**:789-815.
3. Okumura S, Sagara H, Fukuda T, Saito H, Okayama Y. FceRI-mediated amphiregulin production by human mast cells increases mucin gene expression in epithelial cells. *J Allergy Clin Immunol* 2005;**115**:272-9.
4. McBryan J, Howlin J, Napoletano S, Martin F. Amphiregulin: role in mammary gland development and breast cancer. *J Mammary Gland Biol Neoplasia* 2008;**13**: 159-69.
5. Willmarth NE, Ethier SP. Amphiregulin as a novel target for breast cancer therapy. *J Mammary Gland Biol Neoplasia* 2008;**13**:171-9.
6. Piepkorn M, Pittelkow MR, Cook PW. Autocrine regulation of keratinocytes: the emerging role of heparin-binding, epidermal growth factor-related growth factors. *J Invest Dermatol* 1998;**111**:715-21.
7. Wang SW, Oh CK, Cho SH *et al.* Amphiregulin expression in human mast cells and its effect on the primary human lung fibroblasts. *J Allergy Clin Immunol* 2005;**115**: 287-94.
8. Zaiss DM, Yang L, Shah PR, Kobie JJ, Urban JF, Mosmann TR. Amphiregulin, a TH2 cytokine enhancing resistance to nematodes. *Science* 2006;**314**:1746.
9. Kim KW, Jee HM, Park YH, Choi BS, Sohn MH, Kim KE. Relationship between amphiregulin and airway inflammation in children with asthma and eosinophilic bronchitis. *Chest* 2009;**136**:805-10.
10. Luetteke NC, Qiu TH, Fenton SE *et al.* Targeted inactivation of the EGF and amphiregulin genes reveals distinct roles for EGF receptor ligands in mouse mammary gland development. *Development* 1999;**126**:2739-50.
11. Williams CM, Galli SJ. Mast cells can amplify airway reactivity and features of chronic inflammation in an asthma model in mice. *J Exp Med* 2000;**192**:455-62.
12. Nakae S, Lunderius C, Ho LH, Schafer B, Tsai M, Galli SJ. TNF can contribute to multiple features of ovalbumin-induced allergic inflammation of the airways in mice. *J Allergy Clin Immunol* 2007;**119**:680-6.
13. Nakae S, Komiyama Y, Yokoyama H *et al.* IL-1 is required for allergen-specific Th2 cell activation and the development of airway hypersensitivity response. *Int Immunol* 2003;**15**:483-90.
14. Oboki K, Ohno T, Saito H, Nakae S. Th17 and allergy. *Allergol Int* 2008;**57**:121-34.
15. Shoyab M, McDonald VL, Bradley JG, Todaro GJ. Amphiregulin: a bifunctional growth-modulating glycoprotein produced by the phorbol 12-myristate 13-acetate-treated human breast adenocarcinoma cell line MCF-7. *Proc Natl Acad Sci U S A* 1988;**85**:6528-32.

Allergology International. 2010;59:399-408
DOI: 10.2332/allergolint.10-OA-0218

ORIGINAL ARTICLE

Development of IL-17-mediated Delayed-Type Hypersensitivity Is Not Affected by Down-Regulation of IL-25 Expression

Akina Ishii[1], Keisuke Oboki[1], Aya Nambu[2], Hideaki Morita[1,3], Tatsukuni Ohno[1], Naoki Kajiwara[1,2], Ken Arae[1,4], Hajime Sudo[2], Ko Okumura[2], Hirohisa Saito[1,2] and Susumu Nakae[1,2,5]

ABSTRACT

Background: IL-25, which is a member of the IL-17 family, induces Th2 cell differentiation and Th2 cytokine production, contributing to induction of Th2-type immune responses and diseases, as a result of which it suppresses Th1- and Th17-type immune responses.

Methods: To elucidate the role of IL-25 in the pathogenesis of IL-17-mediated delayed-type hypersensitivity (DTH), IL-25-deficient mice were sensitized with methylated BSA (mBSA), and then a DTH reaction was induced by mBSA challenge. mBSA-specific T-cell induction was assessed on the basis of cell proliferation and cytokine production. The DTH reaction was evaluated on the basis of tissue swelling, histology and inflammatory mediator expression.

Results: IL-25 expression was markedly reduced in local DTH lesions. However, mBSA-specific Th1, Th2 and Th17 cell induction, and the mBSA-induced DTH reaction were comparable in IL-25-deficient and wild-type mice.

Conclusions: IL-25 is not essential for differentiation of Th1, Th2 and Th17 cells in the sensitization phase or induction of local inflammation in the elicitation phase of the mBSA-induced DTH reaction.

KEY WORDS

allergy, delayed-type hypersensitivity, helper T cell, interleukin-17 family

INTRODUCTION

Delayed-type hypersensitivity (DTH) reactions, which are classified as a type IV allergy, can be experimentally induced in rodents by immunization with exogenous antigens such as pathogens (*Mycobacterium*, *Leishmania* and viruses), cells (allogenic splenocytes and red blood cells from other species) and protein antigens (methylated bovine serum albumin [mBSA], key hole limpet hemocyanin [KLH] and ovalbumin [OVA]).[1] During sensitization of rodents with such antigens, antigen-specific memory T cells develop in draining lymph nodes (LNs). After challenge of the sensitized rodents with the same antigens, neutrophil-dominant inflammation is induced at local sites dependent on activation of antigen-specific memory T cells. Among the T cell subsets, $CD4^+$ helper T cells (Th cells) are considered to be effectors while $CD8^+$ cytotoxic T cells (Tc cells) are negative regulators in the induction of DTH.[1] Although it is thought that IFN-γ-producing Th1 cells are important for induction of DTH, the contribution of IFN-γ differs depending on the antigen. Mice deficient in IFN-γ or IFN-γR1 showed decreased immune responses during DTH induced by herpes simplex virus type-1[2,3] but not *Mycobacterium tuberculosis*.[4] In addition, IFN-γ-deficient (IFN-$\gamma^{-/-}$) mice showed attenuated DTH responses to KLH,[5,6] but aggravated

[1]Department of Allergy and Immunology, National Research Institute for Child Health and Development, [2]Atopy Research Center, Juntendo University, [3]Department of Pediatrics, Keio University, School of Medicine, [4]Department of Immunology, Faculty of Health Science, Kyorin University and [5]Frontier Research Initiative, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan.

Correspondence: Susumu Nakae, PhD, Frontier Research Initiative, The Institute of Medical Science, The University of Tokyo, 4–6–1 Shirokanedai, Minato, Tokyo 108–8639, Japan.
Email: snakae@ims.u-tokyo.ac.jp
Received 20 April 2010. Accepted for publication 17 May 2010.

responses to OVA and mBSA.[7,8] On the other hand, we previously demonstrated that IL-17$^{-/-}$ mice showed the reduced DTH reaction induced by mBSA and *Mycobacterium tuberculosis*.[9,10] Therefore, in certain cases, Th17 cells rather than Th1 cells seem to be crucial for the development of DTH.

IL-25 (also called IL-17E), which is a member of the IL-17 family of cytokines, can enhance Th2 cell differentiation[11] and Th2 cytokine production,[12] contributing to the induction of Th2 cytokine/cell-mediated immune responses such as to nematode infection[13,14] and diseases such as allergic asthma.[15,16] Conversely, IL-25-mediated Th2 cytokines suppress Th1-type immune responses. IL-25 is also known to suppress Th17 cell differentiation and Th17 cell-mediated autoimmune disorders.[17] These observations suggest that IL-25 may influence the pathogenesis of Th1 and/or Th17 cytokine/cell-mediated type IV allergies such as DTH. However, the role of IL-25 in these responses is poorly understood. In the present study, we investigated the role of IL-25 in Th17 cytokine/cell-mediated DTH induced by mBSA in IL-25$^{-/-}$ mice.

METHODS

MICE

Chimeric mice were generated from *il25*-targeted 129 ES cells, which were deleted exon 1 containing an initial codon and exon 2 of *il25* genes, by Lexicon Pharmaceuticals (The Woodlands, TX, USA). Male age-matched BALB/cA-IL-25$^{-/-}$ mice (N8) and -wild-type mice (Sankyo Lab, Tsukuba, Japan) were used. The mice were housed under specific pathogen-free conditions at the National Research Institute for Child Health and Development, and the animal protocols were approved by the Institutional Review Board of the National Research Institute for Child Health and Development.

mBSA-INDUCED DTH

mBSA-induced DTH was established as described elsewhere.[9,18,19] In brief, after shaving the dorsal hair at the base of the tail with clippers, mice were sensitized with 200 μl of 1.25 mg/ml mBSA (Sigma-Aldrich, MO, USA) emulsified in complete Freund's adjuvant (CFA; Difco Laboratories, MI, USA) by subcutaneous injection to the shaved site. Six days later, the mice were challenged by intradermal injection of 20 μl of 10 mg/ml mBSA in PBS into one footpad and 20 μl of PBS alone into another footpad as a control. For a secondary DTH reaction, mice were re-challenged as described above 6 days after the first challenge. The footpad thickness was measured before (0 hours or 0 days) and after mBSA or PBS challenge using an engineer's calipers (Ozaki, Tokyo, Japan) by an investigator who was blinded to the mouse genotypes. The data were calculated as follows: increase in footpad thickness (mm) = (footpad thickness after challenge) - (footpad thickness before challenge).

LN CELL CULTURE

Mice were subcutaneously sensitized with 200 μl of 1.25 mg/ml mBSA emulsified in CFA as described above. Six days later, inguinal LNs were collected, and the LN cells (4×10^5 cells/well of 96-well flat-bottom plates) were cultured in the presence and absence of 40 μg/ml mBSA at 37℃ for 72 hours. The mBSA-specific LN cell proliferative responses were determined by pulsing with 0.25 μCi [^{3}H]-labeled thymidine for 6 hours. For flow cytometry, LN cells (5×10^6 cells/well of 24-well plates) were cultured in the presence and absence of 40 μg/ml mBSA at 37℃ for 72 hours and then stimulated with 1 μg/ml ionomycin (Sigma-Aldrich) and 0.1 μg/ml PMA in the presence of 1 μM monensin (Sigma-Aldrich) for 4 hours. After washing, the cells were incubated on ice with anti-CD16/CD32 mAb (2.4G2; BD Biosciences, CA, USA) in FACS buffer (Hanks' buffer containing 2% FCS) for FcR blocking for 15 min and then with APC-conjugated anti-mouse CD4 mAb (GK1.5; eBiosciences, CA, USA) for 30 min. After washing, the cells were treated with Fix Buffer I (BD Biosciences) at room temperature for 15 min. Then the cells were washed with 0.1% saponin (Sigma-Aldrich) in FACS buffer and incubated with FITC anti-mouse Foxp3 mAb (FJK-16s; eBiosciences) and PE anti-mouse IL-17 mAb (TC11-18H10; BD Biosciences) or FITC anti-mouse IFN-γ mAb (XMG1.2; eBiosciences) and PE anti-mouse IL-4 mAb (11B11; BD Biosciences) at 4℃ for 30 min. IL-17 and Foxp3 expressions as well as IFN-γ and IL-4 expressions in CD4$^+$ T cells were analyzed on a FACSCalibur (BD Biosciences) using CellQuest (BD Biosciences) or FlowJo (Tree Star, OR, USA) software.

CYTOKINE ELISA

Cytokine levels in the culture supernatants of mBSA-specific LN cells were determined with mouse IFN-γ, IL-4, IL-17 and IL-17E/IL-25 ELISA kits obtained from BD Biosciences, eBiosciences or R&D systems.

QUANTITATIVE PCR

Twenty-four hours after mBSA or PBS challenge, the footpads were harvested and homogenized. Total RNA in the homogenates was isolated using ISOGEN (NIPPON GENE, Tokyo, Japan) and RNeasy Mini Kit (QIAGEN, Hilden, Germany). Using the isolated RNA, cDNA was obtained by RT-PCR with an iScript cDNA Synthesis Kit (Bio-Rad, CA, USA). Quantitative real-time PCR was performed with THUNDERBIRD SYBR qPCR Mix (TOYOBO, Osaka, Japan) and an Applied Biosystems 7300 real-time PCR system (Applied Biosystems, CA, USA). The relative gene expression was determined against GAPDH gene expression. PCR primers were designed as shown in

Table 1 Primer design

Gene	Forward (5'-3')	Reverse (5'-3')
GAPDH	CCCACTCTTCCACCTTCGATG	AGGTCCACCACCCTGTTGCT
IFN-γ	GAACTGGCAAAAGGATGGTGA	TGTGGGTTGTTGACCTCAAAC
IL-1β	CAACCAACAAGTGATATTCTCCATG	GATCCACACTCTCCAGCTGCA
IL-4	TCCAAGGTGCTTCGCATATTTT	CAGCTTATCGATGAATCCAGGC
IL-5	CCCTCATCCTCTTCGTTGCAT	ATGTGATCCTCCTGCGTCCAT
IL-6	GAGGATACCACTCCCAACAGACC	AAGTGCATCATCGTTGTTCATACA
IL-13	GGCAGCAGCTTGAGCACATT	GGCATAGGCAGCAAACCATG
IL-17	CCGCAATGAAGACCCTGATAGAT	AGAATTCATGTGGTGGTCCAGC
IL-21	TTGCACAGCAGTCTTGAACCTC	ACACCAGTGGCAACCTTCATG
IL-25	GGCATTTCTACTCAGGAACGGA	GGTGGAGAAAGTGCCTGTGC
IL-33	CAGGCCTTCTTCGTCCTTCAC	TCTCCTCCACTAGAGCCAGCTG
TNF	GCCTCCCTCTCATCAGTTCT	CACTTGGTGGTTTGCTACGA
TSLP	CAATCCTATCCCTGGCTGCC	TGTGCCATTTCCTGAGTACCGT
KC	CACGTGTTGACGCTTCCCTT	TGAACGTCTCTGTCCCGAGC
MIP-2	AACTGACCTGGAAAGGAGGAGC	ACTCTCAGACAGCGAGGCACAT

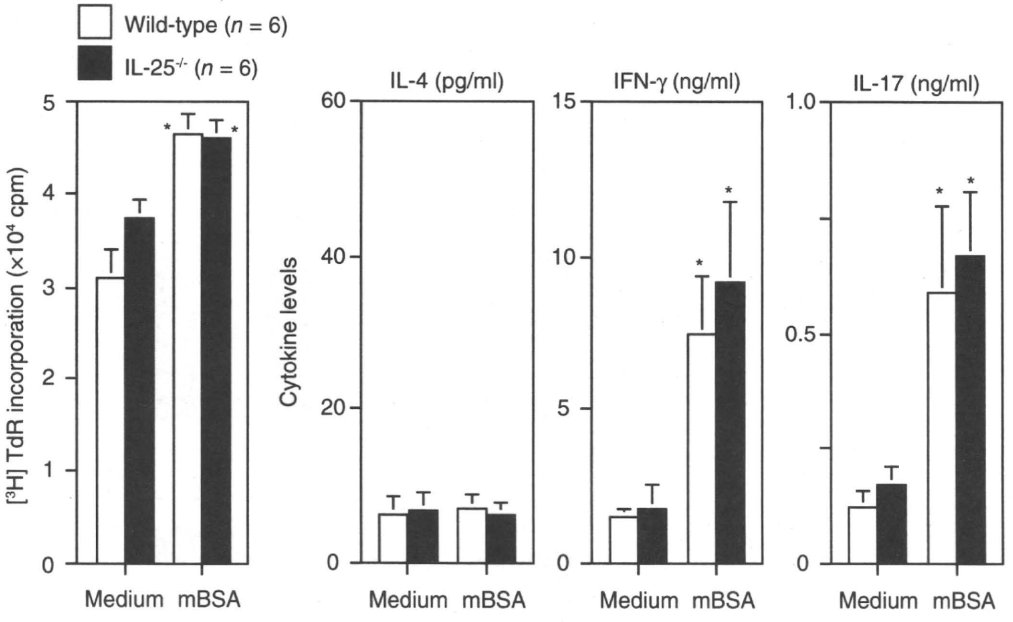

Fig. 1 Normal antigen-specific LN cell responses in IL-25$^{-/-}$ mice. BALB/c-wild-type (open columns, $n = 6$) and -IL-25$^{-/-}$ (closed columns. $n = 6$) mice were immunized subcutaneously with mBSA emulsified in CFA. Six days later, inguinal LNs were collected, and LN cells were cultured in the presence and absence of 40 μg/ml mBSA for 72 hours. Proliferative responses were determined by [3H]-TdR incorporation. The levels of IL-4, IFN-γ and IL-17 in the culture supernatants were measured by ELISA. Data show the mean + SE. *$p < 0.05$ vs. medium alone.

Table 1.

HISTOLOGY

Twenty-four hours after mBSA or PBS challenge, the footpads were harvested and fixed in 4% paraformaldehyde, decalcified in Morse's solution (10% sodium citrate and 22.5% formic acid) and embedded in paraffin. Sections were prepared and stained with hematoxylin-eosin.

DETECTION OF mBSA-SPECIFIC IgGs

Sera were collected 6 days after challenge for measurement of mBSA-specific IgG levels. ELISA plates (Nunc, 442404) were coated overnight with 10 μg/ml mBSA in PBS at 4℃. After blocking with PBS containing 10% FCS, optimally diluted serum samples were applied, and the plates were incubated at room temperature for 1 hour. After washing, biotinylated

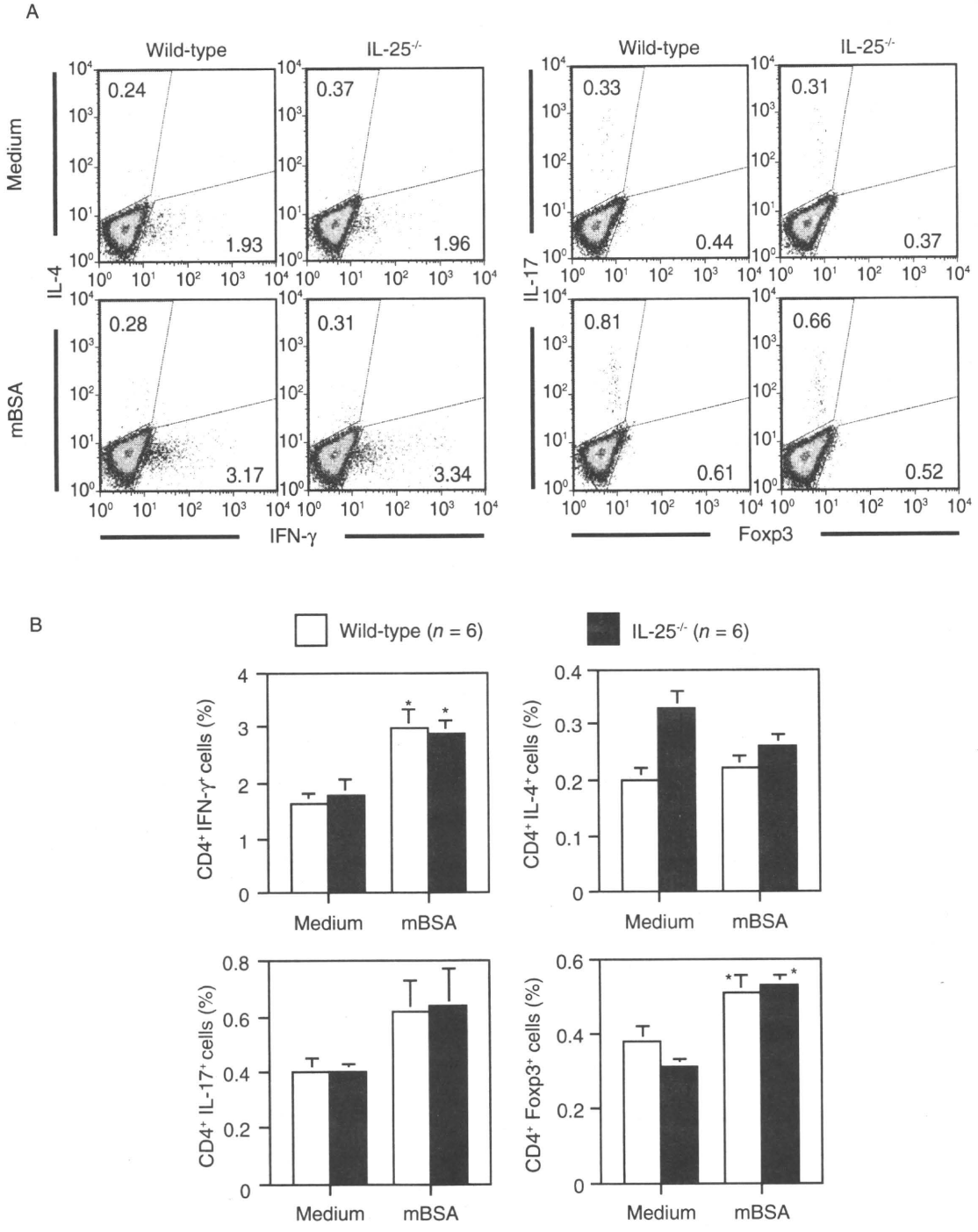

Fig. 2 Normal antigen-specific Th subset differentiation in IL-25$^{-/-}$ mice. LN cells cultured as described for Figure 1 were further stimulated with PMA plus ionomycin in the presence of monensin for 4 hours. Then the profiles of intracellular cytokine (IFN-γ, IL-4 and IL-17) and transcription factor (Foxp3) expression were determined by flow cytometry. (**A**) Representative flow cytometry data. (**B**) The mean + SE (n = 6) in (**A**). *p < 0.05 vs. medium alone.

anti-mouse IgG1 (A85-1; BD Biosciences), IgG2a (R19-15; BD Biosciences), IgG2b (R12-3; BD Biosciences) or IgG3 (R40-82; BD Biosciences) mAb was added, followed by incubation at room temperature for 1 hour. Then, after washing, HRP-conjugated streptavidin (BD Biosciences) was added, followed by incubation at room temperature for 30 minutes. TMB substrate (KPL, MD, USA) was used as the

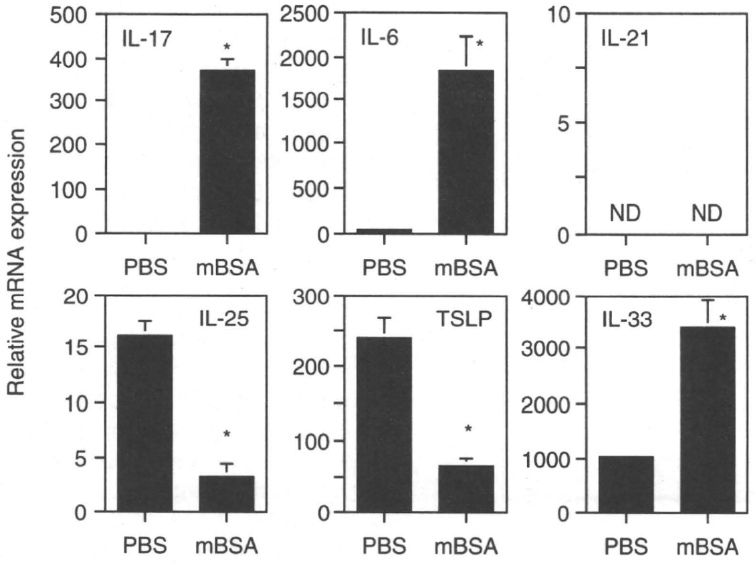

Fig. 3 Decreased local IL-25 expression during mBSA-induced DTH. BALB/c-wild-type mice were immunized subcutaneously with mBSA emulsified in CFA. Six days later, the mice were challenged by injection of mBSA into one footpad and PBS alone into another footpad. Twenty-four hours after challenge, mRNA was extracted from the mBSA- and PBS-injected footpads. The expressions of IL-6, IL-17, IL-21, IL-25, IL-33 and TSLP were determined by quantitative PCR. Data show the mean + SE (n = 5). *p < 0.05 vs. PBS-injected footpads.

substrate for enzymatic reaction. The reaction was stopped by addition of 1N HCl, and then the absorbance was measured using a plate reader at 450 nm. Data show the absorbance value at 450 nm.

STATISTICAL ANALYSES

Data show the mean with SD (for measurement of footpad swelling) or SE (for other experiments) and were evaluated for statistical significance using the two-tailed Student's t test.

RESULTS

IL-25 IS NOT REQUIRED FOR Th CELL DIFFERENTIATION AFTER mBSA SENSITIZATION

It has been shown that IL-25 can enhance Th2 cell differentiation[11] but conversely suppresses Th1 cell and Th17 cell differentiation/activation.[13,17] These observations suggest that IL-25 may influence the differentiation and/or activation of Th cell subsets during IL-17-mediated, mBSA-induced DTH. To elucidate this, LN cells from mBSA-sensitized IL-25$^{-/-}$ and wild-type mice were cultured in the presence and absence of mBSA *in vitro*. The mBSA-specific proliferative responses of LN cells from IL-25$^{-/-}$ mice were comparable to those from wild-type mice (Fig. 1). Moreover, the levels of IFN-γ and IL-17, but not IL-4, in the culture supernatants were similarly increased in both mouse strains after stimulation with mBSA (Fig. 1). The levels of IL-25 in the setting were below the limit of detection of ELISA (data not shown). Consistent with this, the proportions of IFN-γ$^+$ and IL-17$^+$ CD4$^+$ T cells were also equivalent in mBSA-stimulated LN cells from IL-25$^{-/-}$ and wild-type mice (Fig. 2A, B). The proportion of IL-4-producing cells was not increased in LN cell cultures from both strains after mBSA stimulation, while that of Foxp3-expressing cells was significantly increased (Fig. 2A, B). However, the intensity of Foxp3 expression in that setting was relatively low compared with in CD4$^+$CD25$^+$ cells that were freshly harvested from mouse spleen (data not shown). Thus, these observations indicate that IL-25 is not required for differentiation and activation of Th1 and Th17 cells after sensitization with mBSA.

IL-25 IS NOT ESSENTIAL FOR INDUCTION OF LOCAL INFLAMMATION DURING mBSA-INDUCED DTH

Although IL-25 is dispensable for induction of mBSA-specific Th cells in the sensitization phase of DTH (Fig. 1, 2), IL-25 may be involved in the elicitation of local inflammation in DTH by affecting Th cell activation. Therefore, we examined the expressions of Th17 cell-related and Th2 cytokine-inducible cytokines in local inflammatory lesions of wild-type mice during mBSA-induced DTH. The mRNA expressions of IL-17 and IL-6 (a crucial cytokine for Th17 cell dif-

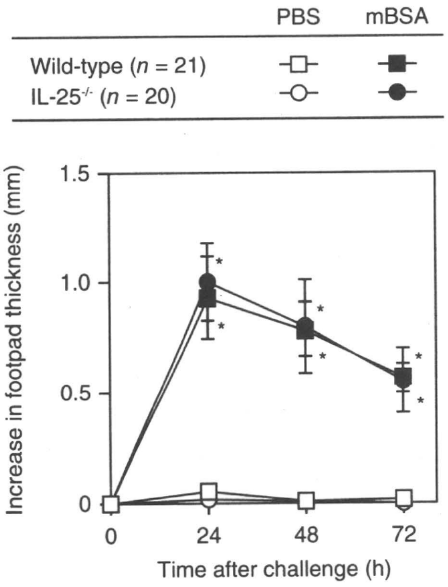

Fig. 4 Normal DTH reaction in IL-25$^{-/-}$ mice. BALB/c-wild-type (squares, n = 21) and -IL-25$^{-/-}$ (circles, n = 20) mice were immunized subcutaneously with mBSA emulsified in CFA. Six days later, the mice were challenged by injection of mBSA into one footpad (closed symbols) and PBS alone into another footpad (open symbols). Before (indicated as "0 h") and after challenge, the thickness of each footpad was measured with an engineer's calipers. Data show the mean ± SD. *p < 0.05 vs. PBS-injected footpads.

ferentiation[20]) were markedly increased in the mBSA-challenged footpads compared with the PBS-injected footpads (Fig. 3). Like IL-6, IL-21 is known to be involved in Th17 cell differentiation,[21-23] and it is also important for follicular Th cell development[24,25] and Th2 cell-mediated immune responses.[26] In the above setting, the expression of IL-21 mRNA was below the limit of detection of our quantitative PCR system (Fig. 3). Similar to IL-25, TSLP and IL-33 are known to induce Th2 cytokine production by Th2 cells.[27] The expression of IL-33 mRNA was increased in the mBSA-challenged footpads compared with the PBS-injected footpads (Fig. 3). On the other hand, interestingly, mRNA expression for IL-25 and TSLP was significantly decreased after the challenge with mBSA (Fig. 3). These observations suggest that IL-25 may play a role in the induction of DTH. However, as shown in Figure 4, the development of DTH in IL-25$^{-/-}$ mice was comparable to that in wild-type mice after mBSA challenge (Fig. 4). Consistent with this, local inflammatory cell infiltration determined by histological analysis was also similarly observed in wild-type mice and IL-25$^{-/-}$ mice (Fig. 5). In wild-type mice during the DTH reaction, mRNA expression of both Th1 (IFN-γ) and Th2 (IL-4, IL-5 and IL-13) cytokines was significantly increased in the mBSA-injected footpads compared with the PBS-injected footpads (Fig. 6). Although IL-25 is well-known to induce Th2 cytokine production,[12] IL-25-deficiency did not have any effect on mRNA expression levels and patterns for the Th1

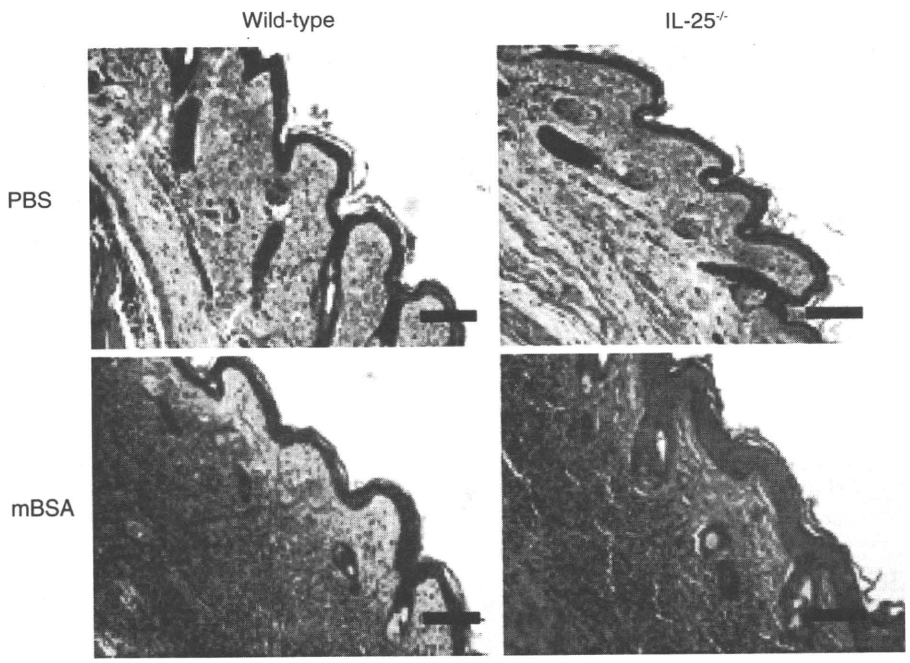

Fig. 5 Histological analysis of local lesions of IL-25$^{-/-}$ mice during DTH. Twenty-four hours after challenge as in Figure 4, mBSA- and PBS-injected footpads from BALB/c-wild-type and -IL-25$^{-/-}$ mice were harvested, and sections were stained with hematoxylin and eosin. The data show representative results from each mouse strain. Scale bar = 100 μm.

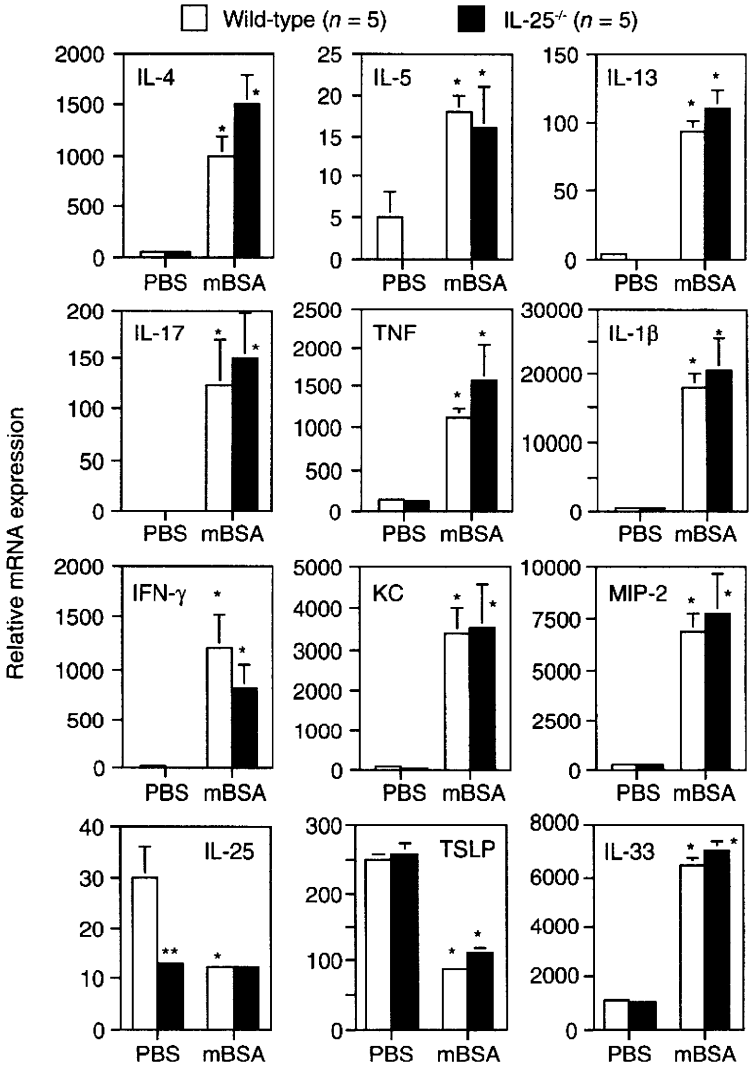

Fig. 6 Normal local expression of proinflammatory mediators in IL-25$^{-/-}$ mice during DTH. Twenty-four hours after challenge as in Figure 4, mRNA was isolated from mBSA- and PBS-injected footpads of BALB/c-wild-type and -IL-25$^{-/-}$ mice. The expressions of cytokines and chemokines were determined by quantitative PCR. Data show the mean + SE (n = 5). *p < 0.05 vs. PBS-injected footpads, and **p < 0.05 vs. wild-type mice.

or Th2 cytokines and TSLP and IL-33 in the local sites during mBSA-induced DTH (Fig. 6). Like TNF and IL-1, IL-17, a Th17 cytokine, is known to be crucial for infiltration of neutrophils, which are a major infiltrating leukocyte population in local inflammatory lesions of DTH. Expression of these proinflammatory cytokines and neutrophil chemoattractant factors such as KC and MIP-2 was also comparably increased in both wild-type and IL-25$^{-/-}$ mice after mBSA challenge (Fig. 6). These observations clearly indicate that IL-25 is dispensable for the elicitation of local inflammation during DTH induced by mBSA.

IL-25 IS NOT NECESSARY FOR THE DEVELOPMENT OF SECONDARY DTH REACTIONS

One week after mBSA challenge, we collected sera from wild-type and IL-25$^{-/-}$ mice and determined the levels of mBSA-specific IgGs by ELISA. The levels of mBSA-specific IgG1 and IgG3 as well as that of mBSA-specific IgG2a were partially, but not significantly, decreased or increased in sera from the IL-25$^{-/-}$ mice in comparison with the wild-type mice (Fig. 7). These observations suggest that IL-25 may contribute to the induction of secondary DTH, and mice were re-challenged with mBSA 6 days after the first mBSA injection to investigate this. However, the degree of footpad swelling was identical between wild-

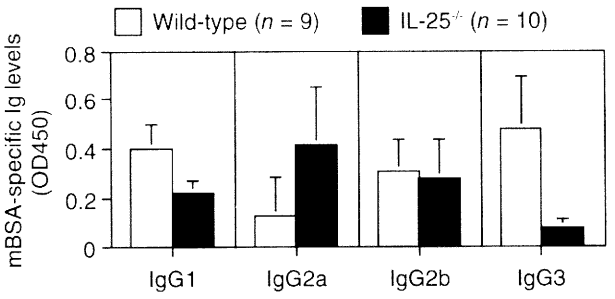

Fig. 7 No significant effect of IL-25-deficiency on antigen-specific Ig production during DTH. Six days after challenge as in Figure 4, sera were collected from BALB/c-wild-type (open columns, n = 9) and -IL-25$^{-/-}$ (closed columns, n = 10) mice. The levels of mBSA-specific IgG1, IgG2a, IgG2b and IgG3 were determined by ELISA. The data show the mean + SE.

type and IL-25$^{-/-}$ mice in the experimental setting (Fig. 8). Taken all together, our results indicate that IL-25 is not a major mediator in the induction of Th17 cell-mediated DTH reactions.

DISCUSSION

Expression of IL-25 mRNA has been constitutively detected in various mouse tissues such as the colon and lung.[12] In immune cells, IL-25 mRNA and/or proteins were detected in CD4$^+$ and CD8$^+$ T cells in the cecal patch (but not in peripheral LNs),[13] *in vitro*-skewed Th2 cells,[12] bone marrow-derived mast cells,[28] alveolar macrophages,[29] eosinophils and basophils.[30] Both administration of recombinant IL-25 and infection by adenovirus carrying IL-25 cDNA in mice result in elevation of Th2 cytokine (IL-4, IL-5 and IL-13) expression and serum IgA, IgE and IgG1 levels, and increase eosinophils in the blood and tissues.[12] IL-25 induces Th2 cytokine production by myeloid cells[12,31] and differentiation of Th2 cells from naïve T cells dependent on IL-4 and STAT-6.[11] Therefore, IL-25 is surmised to suppress Th1 cell differentiation and Th1 cell-mediated immune responses by enhancing Th2 cell differentiation and activation. In addition, IL-4 can inhibit Th17 cell differentiation,[32] suggesting that IL-25-induced Th2 cytokines are involved in the regulation of Th17 cytokine-mediated immune responses. In support of this, IL-25$^{-/-}$ mice showed reduced Th2-type immune responses but increased Th1 cell and Th17 cell activation.[13,17] In particular, IL-13 induced by IL-25 inhibited dendritic cell expression of mRNA for IL-1, IL-6 and IL-23, which are required for Th17 cell differentiation.[17] These observations suggest that IL-25 is a key mediator for Th2-type immune responses and an important regulatory cytokine for induction of Th1- and/or Th17-type immune responses.

It was shown that development of DTH induced by mBSA was attenuated in IL-17$^{-/-}$ mice[9] but exacerbated in IFN-$\gamma^{-/-}$ mice,[8] suggesting that IL-17 is a

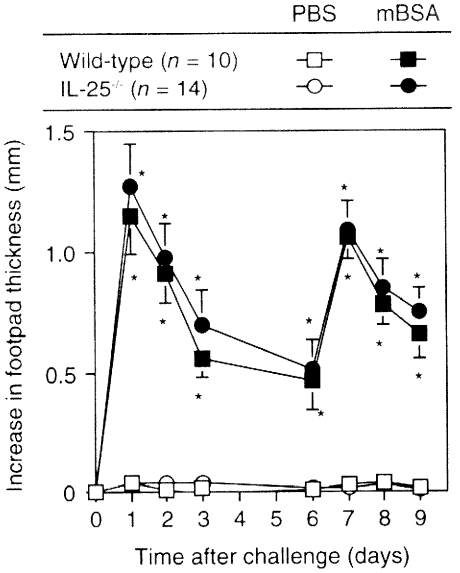

Fig. 8 Normal secondary DTH reaction in IL-25$^{-/-}$ mice. BALB/c-wild-type (squares, n = 10) and -IL-25$^{-/-}$ (circles, n = 14) mice were immunized subcutaneously with mBSA emulsified in CFA. Six days later, the mice were challenged by injection of mBSA into one footpad (closed symbols) and PBS alone into another footpad (open symbols). Six days after the first challenge, mice were re-challenged by injection of mBSA or PBS into the respective footpads. Before (indicated as "0 days") and after challenge, the thickness of each footpad was measured with an engineer's calipers. Data show the mean + or - SD. *p < 0.05 vs. PBS-injected footpads.

proinflammatory cytokine for the event and that IFN-γ is an anti-inflammatory cytokine. As noted above, since IL-25 can regulate both Th1- and Th17-type immune responses, it could be readily expected that IL-25 would influence the pathogenesis of mBSA-induced DTH. However, we found that mBSA-specific Th1, Th2 and Th17 cell differentiation and activation were similarly observed between IL-25$^{-/-}$ and wild-type mice after sensitization with mBSA, since IL-25 was barely detectable in the setting(Fig. 1, 2). These observations indicate that IL-25 is not essential for induction of antigen-specific memory T cells in the sensitization phase of DTH induced by mBSA.

As expected, we found that expression of both Th17-related cytokines (IL-17, IL-6 and IL-1β) and Th 1 cytokine (IFN-γ) was profoundly increased in the inflamed lesion during mBSA-induced DTH(Fig. 3, 6). Interestingly, the expression of such Th2 cytokines as IL-4, IL-5 and IL-13 was also up-regulated in the setting, although expression of both IL-25 and TSLP was decreased (Fig. 3). Therefore, it seemed that down-regulation of IL-25 expression in the local lesion may contribute to induction of local inflammation during mBSA-induced DTH. However, we

showed here that comparable tissue swelling was observed in both IL-25$^{-/-}$ and wild-type mice during mBSA-induced DTH (Fig. 4). Consistent with this, inflammation as assessed by histology and the expression of proinflammatory cytokines and chemokines in the local lesion were also identical in these mouse strains after mBSA challenge(Fig. 5, 6). The production of serum mBSA-specific IgG1, IgG2a and IgG3 was partly, but not significantly, influenced by IL-25-deficiency (Fig. 7), but those changes did not affect induction of DTH by mBSA (Fig. 8). Therefore, our observations indicate that IL-25 is not responsible for the induction of local inflammation in the elicitation phase of DTH induced by mBSA, although IL-25 expression was down-regulated in the setting.

In summary, IL-25 is not essential for differentiation of Th1, Th2 and Th17 cells in the sensitization phase or induction of local inflammation in the elicitation phase of mBSA-induced DTH.

ACKNOWLEDGEMENTS

We thank Noriko Hashimoto, Shuhei Fukuda, Hiromi Wakita, and Michiko Yamada for their excellent technical assistance. This work was supported by Takeda Science Foundation, the Long-Range Research Initiative, the Japan Chemical Industry Association, Grants-in-Aid for Young Scientists (B) and the Program for Improvement of Research Environment for Young Researchers, The Special Coordination Funds for Promoting Science and Technology from the Ministry of Education, Culture, Sports, Science and Technology, Japan (S.N.), and by a grant from the National Institute of Biomedical Innovation and Health and Labour Sciences Research Grant for Research on Allergic Disease and Immunology from the Ministry of Health, Labour and Welfare, Japan (H.S.).

REFERENCES

1. Grabbe S, Schwarz T. Immunoregulatory mechanisms involved in elicitation of allergic contact hypersensitivity. *Immunol Today* 1998;**19**:37-44.
2. Bouley DM, Kanangat S, Wire W, Rouse BT. Characterization of herpes simplex virus type-1 infection and herpetic stromal keratitis development in IFN-γ knockout mice. *J Immunol* 1995;**155**:3964-71.
3. Molesworth-Kenyon SJ, Oakes JE, Lausch RN. A novel role for neutrophils as a source of T cell-recruiting chemokines IP-10 and Mig during the DTH response to HSV-1 antigen. *J Leukoc Biol* 2005;**77**:552-9.
4. Cooper AM, Dalton DK, Stewart TA, Griffin JP, Russell DG, Orme IM. Disseminated tuberculosis in interferon-γ gene-disrupted mice. *J Exp Med* 1993;**178**:2243-7.
5. Akahira-Azuma M, Szczepanik M, Tsuji RF *et al.* Early delayed-type hypersensitivity eosinophil infiltrates depend on T helper 2 cytokines and interferon-γ via CXCR3 chemokines. *Immunology* 2004;**111**:306-17.
6. Gao D, Kasten-Jolly J, Lawrence DA. The paradoxical effects of lead in interferon-γ knockout BALB/c mice. *Toxicol Sci* 2006;**89**:444-53.
7. Feuerer M, Eulenburg K, Loddenkemper C, Hamann A, Huehn J. Self-limitation of Th1-mediated inflammation by IFN-γ. *J Immunol* 2006;**176**:2857-63.
8. Irmler IM, Gajda M, Brauer R. Exacerbation of antigen-induced arthritis in IFN-γ-deficient mice as a result of unrestricted IL-17 response. *J Immunol* 2007;**179**:6228-36.
9. Nakae S, Komiyama Y, Nambu A *et al.* Antigen-specific T cell sensitization is impaired in IL-17-deficient mice, causing suppression of allergic cellular and humoral responses. *Immunity* 2002;**17**:375-87.
10. Umemura M, Yahagi A, Hamada S *et al.* IL-17-mediated regulation of innate and acquired immune response against pulmonary Mycobacterium bovis bacille Calmette-Guerin infection. *J Immunol* 2007;**178**:3786-96.
11. Angkasekwinai P, Park H, Wang YH *et al.* Interleukin 25 promotes the initiation of proallergic type 2 responses. *J Exp Med* 2007;**204**:1509-17.
12. Fort MM, Cheung J, Yen D *et al.* IL-25 induces IL-4, IL-5, and IL-13 and Th2-associated pathologies in vivo. *Immunity* 2001;**15**:985-95.
13. Owyang AM, Zaph C, Wilson EH *et al.* Interleukin 25 regulates type 2 cytokine-dependent immunity and limits chronic inflammation in the gastrointestinal tract. *J Exp Med* 2006;**203**:843-9.
14. Fallon PG, Ballantyne SJ, Mangan NE *et al.* Identification of an interleukin (IL)-25-dependent cell population that provides IL-4, IL-5, and IL-13 at the onset of helminth expulsion. *J Exp Med* 2006;**203**:1105-16.
15. Tamachi T, Maezawa Y, Ikeda K *et al.* IL-25 enhances allergic airway inflammation by amplifying a TH2 cell-dependent pathway in mice. *J Allergy Clin Immunol* 2006;**118**:606-14.
16. Ballantyne SJ, Barlow JL, Jolin HE *et al.* Blocking IL-25 prevents airway hyperresponsiveness in allergic asthma. *J Allergy Clin Immunol* 2007;**120**:1324-31.
17. Kleinschek MA, Owyang AM, Joyce-Shaikh B *et al.* IL-25 regulates Th17 function in autoimmune inflammation. *J Exp Med* 2007;**204**:161-70.
18. Nambu A, Nakae S, Iwakura Y. IL-1β, but not IL-1α, is required for antigen-specific T cell activation and the induction of local inflammation in the delayed-type hypersensitivity responses. *Int Immunol* 2006;**18**:701-12.
19. Ishigame H, Kakuta S, Nagai T *et al.* Differential roles of interleukin-17A and -17F in host defense against mucoepithelial bacterial infection and allergic responses. *Immunity* 2009;**30**:108-19.
20. Veldhoen M, Hocking RJ, Atkins CJ, Locksley RM, Stockinger B. TGF-β in the context of an inflammatory cytokine milieu supports de novo differentiation of IL-17-producing T cells. *Immunity* 2006;**24**:179-89.
21. Nurieva R, Yang XO, Martinez G *et al.* Essential autocrine regulation by IL-21 in the generation of inflammatory T cells. *Nature* 2007;**448**:480-3.
22. Korn T, Bettelli E, Gao W *et al.* IL-21 initiates an alternative pathway to induce proinflammatory TH17 cells. *Nature* 2007;**448**:484-7.
23. Zhou L, Ivanov II, Spolski R *et al.* IL-6 programs TH-17 cell differentiation by promoting sequential engagement of the IL-21 and IL-23 pathways. *Nat Immunol* 2007;**8**:967-74.
24. Vogelzang A, McGuire HM, Yu D, Sprent J, Mackay CR, King C. A fundamental role for interleukin-21 in the generation of T follicular helper cells. *Immunity* 2008;**29**:127-37.
25. Nurieva RI, Chung Y, Hwang D *et al.* Generation of T follicular helper cells is mediated by interleukin-21 but independent of T helper 1, 2, or 17 cell lineages. *Immunity*