

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

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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 anti-

gens, 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- γ R1 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- γ ^{-/-}) mice showed attenuated DTH responses to KLH,^{5,6} but aggravated

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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 differentiation¹¹ 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°C for 72 hours. The mBSA-specific LN cell proliferative responses were determined by pulsing with 0.25 µCi [³H]-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°C 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°C 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	GAAGTGGCAAAAGGATGGTGA	TGTGGGTTGTTGACCTCAAAC
IL-1 β	CAACCAACAAGTGATATTCTCCATG	GATCCACACTCTCCAGCTGCA
IL-4	TCCAAGGTGCTTCGCATATTTT	CAGCTTATCGATGAATCCAGGC
IL-5	CCCTCATCCTCTTCGTTGCAT	ATGTGATCCTCCTGCGTCCAT
IL-6	GAGGATACCACTCCCAACAGACC	AAGTGCATCATCGTTGTTTCATACA
IL-13	GGCAGCAGCTTGAGCACATT	GGCATAGGCAGCAAACCATG
IL-17	CCGCAATGAAGACCCTGATAGAT	AGAATTCATGTGGTGGTCCAGC
IL-21	TTGCACAGCAGTCTTGAACCTC	ACACCAGTGGCAACCTTCATG
IL-25	GGCATTCTACTCAGGAACGGA	GGTGGAGAAAGTGCCTGTGC
IL-33	CAGGCCTTCTTCGTCCTTCAC	TCTCCTCCACTAGAGCCAGCTG
TNF	GCCTCCCTCTCATCAGTTCT	CACTTGGTGGTTTGCTACGA
TSLP	CAATCCTATCCCTGGCTGCC	TGTGCCATTTCTGAGTACCGT
KC	CACGTGTTGACGCTTCCCTT	TGAACGTCTCTGTCCCAGGC
MIP-2	AACTGACCTGGAAGGAGGAGC	ACTCTCAGACAGCGAGGCACAT

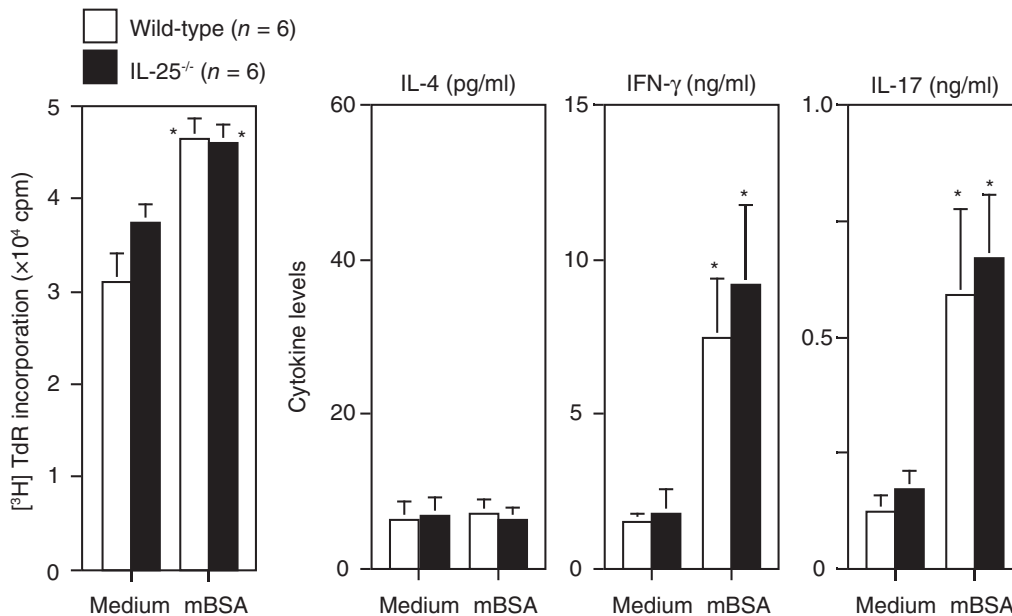


Fig. 1 Normal antigen-specific LN cell responses in IL-25^{-/-} mice. BALB/c-wild-type (open columns, $n = 6$) and -IL-25^{-/-} (closed columns, $n = 6$) mice were immunized subcutaneously with mBSA emulsified in CFA. Six days later, inguinal LNs were collected, and LN cells were cultured in the presence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

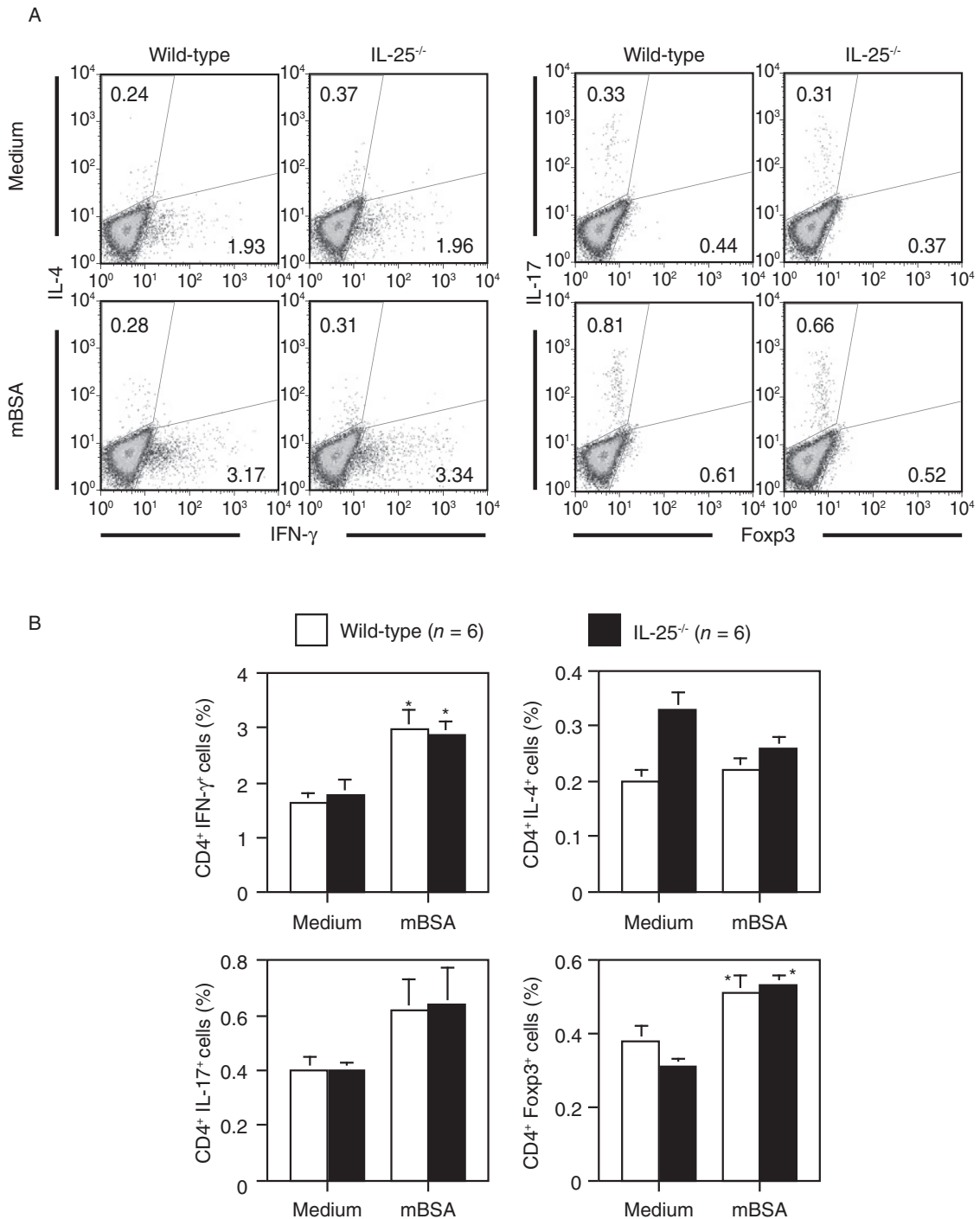


Fig. 2 Normal antigen-specific Th subset differentiation in IL-25^{-/-} mice. LN cells cultured as described for Figure 1 were further stimulated with PMA plus ionomycin in the presence of monensin for 4 hours. Then the profiles of intracellular cytokine (IFN-γ, IL-4 and IL-17) and transcription factor (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 tempera-

ture 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

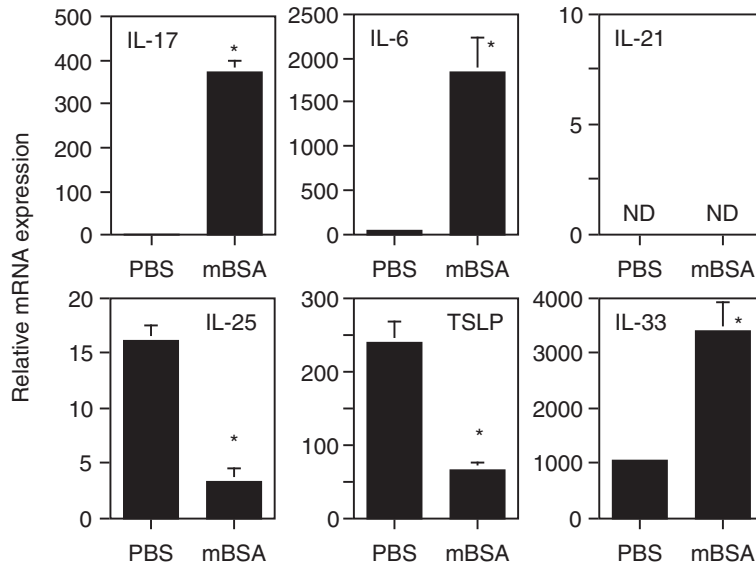


Fig. 3 Decreased local IL-25 expression during mBSA-induced DTH. BALB/c-wild-type mice were immunized subcutaneously with mBSA emulsified in CFA. Six days later, the mice were challenged by injection of mBSA into one footpad and PBS alone into another footpad. Twenty-four hours after challenge, mRNA 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 differentiation¹¹ 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-

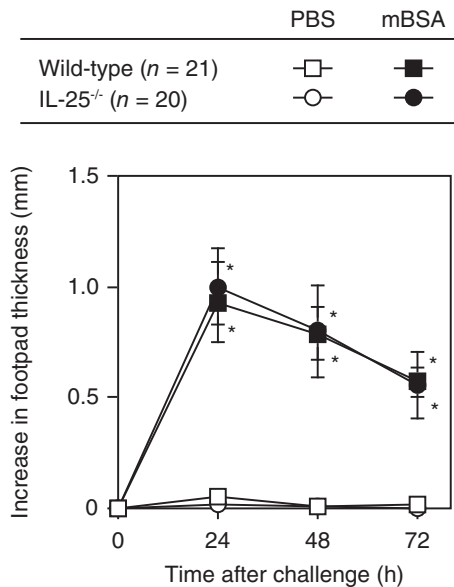


Fig. 4 Normal DTH reaction in IL-25^{-/-} mice. BALB/c-wild-type (squares, *n* = 21) and -IL-25^{-/-} (circles, *n* = 20) mice were immunized subcutaneously with mBSA emulsified in CFA. Six days later, the mice were challenged by injection of mBSA into one footpad (closed symbols) and PBS alone into 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 $\pm$ SD. **p* < 0.05 vs. PBS-injected footpads.

ferentiation²⁰) 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

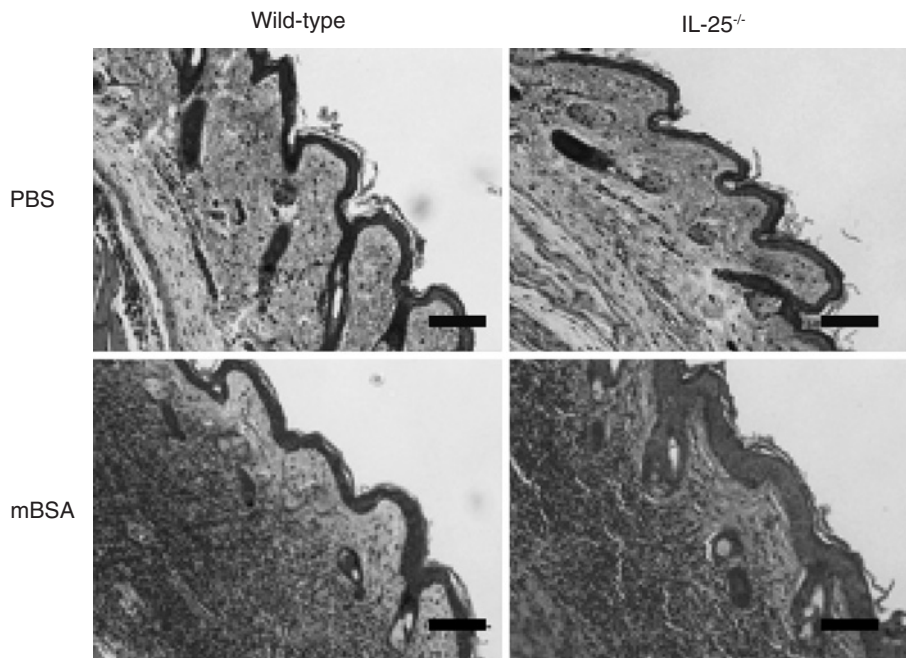


Fig. 5 Histological analysis of local lesions of IL-25^{-/-} mice during DTH. Twenty-four hours after challenge as in Figure 4, mBSA- and PBS-injected footpads from BALB/c-wild-type and -IL-25^{-/-} mice were harvested, and sections were stained with hematoxylin and eosin. The data show representative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

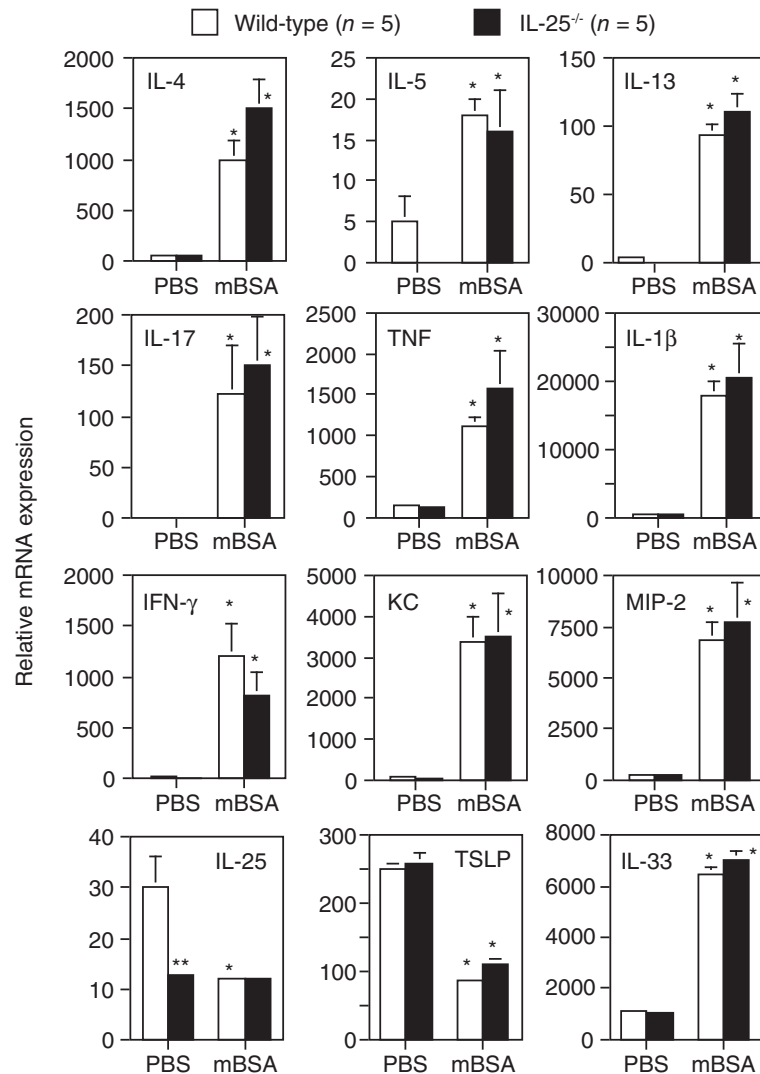


Fig. 6 Normal local expression of proinflammatory mediators in IL-25^{-/-} mice during DTH. Twenty-four hours after challenge as in Figure 4, mRNA was isolated from mBSA- and PBS-injected footpads of BALB/c-wild-type and -IL-25^{-/-} mice. The expressions of cytokines and chemokines were determined by 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-

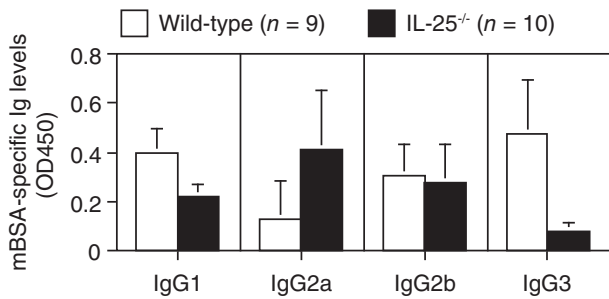


Fig. 7 No significant effect of IL-25-deficiency on antigen-specific Ig production during DTH. Six days after challenge as in Figure 4, sera were collected from BALB/c-wild-type (open columns, $n = 9$) and -IL-25^{-/-} (closed columns, $n = 10$) mice. The levels of mBSA-specific IgG1, IgG2a, IgG2b and 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^{-/-} mice⁹ but exacerbated in IFN- γ ^{-/-} mice,⁸ suggesting that IL-17 is a

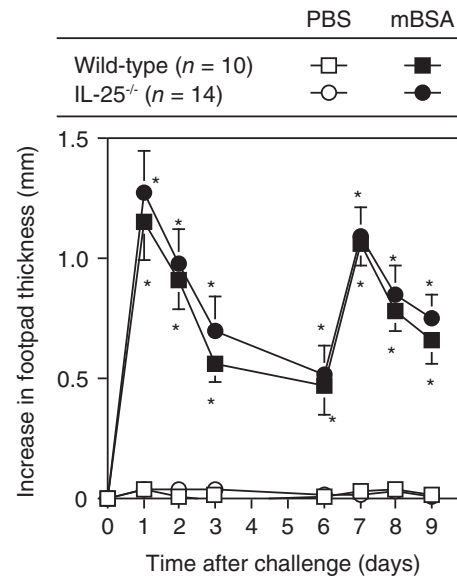


Fig. 8 Normal secondary DTH reaction in IL-25^{-/-} mice. BALB/c-wild-type (squares, $n = 10$) and -IL-25^{-/-} (circles, $n = 14$) mice were immunized subcutaneously with mBSA emulsified in CFA. Six days later, the mice were challenged by injection of mBSA into one footpad (closed symbols) and PBS alone 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-1 β) and Th1 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.

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