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Effects of a Janus kinase inhibitor, pyridone 6, on airway responses in a murine model of asthma

Yuko Matsunaga [a], Hiromasa Inoue [a,b,*], Satoru Fukuyama [a], Hideyuki Yoshida [c], Atsushi Moriwaki [a], Takafumi Matsumoto [a], Koichiro Matsumoto [a], Yukari Asai [a], Masato Kubo [d], Akihiko Yoshimura [c], Yoichi Nakanishi [a]

[a] Research Institute for Diseases of the Chest, Graduate School of Medical Sciences, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan
[b] Department of Pulmonary Medicine, Graduate School of Medical and Dental Sciences, Kagoshima University, 8-35-1 Sakuragaoka, Kagoshima 890-8520, Japan
[c] Department of Microbiology and Immunology, Keio University School of Medicine, 35 Shinanomachi, Shinjuku-ku, Tokyo 160-8582, Japan
[d] Laboratory for Signal Network, Research Center for Allergy and Immunology, RIKEN Yokohama Institute, Suehiro-cho 1-7-22, Tsurumi, Yokohama, Kanagawa 230-0045, Japan

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ABSTRACT

Th2 cytokines and their downstream Janus kinase (JAK)-signal transducer and activation of transcription (STAT) pathways play a critical role in allergic asthma. We studied the effects of a pan-JAK inhibitor, pyridone 6 (P6), on asthmatic responses in a mouse model and investigated the mechanism for its biological effects. Mice were sensitized and challenged by ovalbumin (OVA). P6 treatment during the challenge phase suppressed eosinophilia in bronchoalveolar lavage (BAL) fluids but did not affect airway hyperresponsiveness (AHR). To improve the efficacy of the JAK inhibitor, P6 was encapsulated in polylactic-*co*glycolic acid nanoparticles (P6-PLGA). P6-PLGA treatment just before OVA challenge suppressed both airway eosinophilia and AHR. Although the IL-13 levels in BAL fluids and the OVA-specific IgE levels in serum after the challenge phase treatment with P6-PLGA were similar to those after a sham treatment, the eotaxin levels in BAL fluids and lung mCLCA3/Gob-5 expression were decreased in P6-PLGA-treated mice. Interestingly, the local IL-13 levels and serum OVA-specific IgE were decreased, while IL-17-producing T cells were increased by P6-PLGA treatment during the sensitization plus challenge phases. *In vitro*, P6 strongly suppressed the differentiation of Th2 from naive CD4 T cells, but it partly enhanced Th17 differentiation. P6 potently suppressed IL-13-mediated STAT6 activation and mCLCA3/Gob-5 expression in mouse tracheal epithelial cells. These findings suggest that the JAK inhibitor P6 suppresses asthmatic responses by inhibiting Th2 inflammation and that application of PLGA nanoparticles improves the therapeutic potency of P6.

1. Introduction

Th2 cytokines, including IL-4, IL-5, IL-9, and IL-13, are essential for generating the pathophysiological features of asthma [1]. These cytokines bind to receptors at the cell surface and activate signal transduction pathways, including the Janus-kinase (JAK)-signal transducer and activation of transcription (STAT) pathway. Among Th2 cytokines, IL-13 is now considered particularly critical. IL-13 activates both the IL-4Rα and IL-13Rα1 chains to stimulate JAK1, JAK2, and Tyk2, leading to phosphorylation of STAT6. JAK–STAT pathways play a crucial role in Th2 responses and may be considered as novel targets for asthma [2].

In addition to Th2 cytokines, other cytokines, including IL-2, IL-6, IL-12, and IFN-γ, also activate the JAK–STAT pathways. These respective cytokines signal via JAK1/JAK3-STAT5, JAK1/JAK2/Tyk2-STAT3, JAK2/Tyk2-STAT4, and JAK1/JAK2-STAT1 [3]. A distinct set of cytokines promotes the differentiation processes for Th cell lineage: IL-12/IFN-γ for Th1; IL-4 for Th2; TGF-β/IL-6 (IL-21) for Th17; and TGF-β/IL-2 for regulatory T cells (Tregs) [4,5]. IL-17, which is required during initial allergic sensitization, reduced Th2-dependent inflammation in established diseases in an animal model [6], whereas transfer of Th17 cells together with Th2 cells in naïve mice augmented airway inflammation [7]. Tregs suppress established airway inflammation and airway hyperresponsiveness in mouse models of allergic asthma [8]. Therefore, it is possible that the inhibition of JAK–STAT pathway might suppress the potentially beneficial immune response, and the effects of JAK–STAT inhibition on allergic asthma are not completely clear.

Here, we investigated the effects of pan-JAK inhibitor pyridone 6 (P6) on ovalbumin (OVA)-induced mouse models of allergic asthma. P6 competitively binds to the ATP-binding site of JAK1, JAK2, JAK3, and Tyk2 with strong inhibitory characteristics, as IC_{50} for JAK1, JAK2, JAK3, and Tyk2 is 15 nM, 1 nM, 5 nM, and 1 nM, respectively, *in vitro* [9]. It has been reported that P6 is rapidly taken up by cells [10]. To improve the sustainability of the JAK inhibitor, P6 was encapsulated in biodegradable polylactic-*co*glycolic acid (PLGA)

* Corresponding author. Fax: +81 99 275 6482.
E-mail address: inoue-pulm@umin.net (H. Inoue).

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nanoparticles and administered to mice. The PLGA nanoparticle is a drug delivery system for sustained delivery of agents via protection of the drug encapsulated against enzymatic degradation [11].

2. Materials and methods

2.1. Preparation of P6 and P6-encapsulated PLGA nanoparticles

P6 was either obtained from Sigma–Aldrich or synthesized according to the procedure described previously [9] by Fuji Molecular Planning Co. (Yokohama, Japan) with similar results. P6 was prepared as a stock solution of 100 mM DMSO. P6-encapsulated PLGA nanoparticles (P6-PLGA) were generated with an emulsion–solvent evaporation technique, as previously described [12], at Hosokawa Powder Technology Research Institute, Osaka, Japan. PLGA was composed of a polylactic acid to glycolic acid ratio of 75:25. Total PLGA nanoparticles contained 4.6% of P6.

2.2. Antigen sensitization and challenge and measurement of airway responsiveness and BAL

BALB/*c* mice were sensitized with an intraperitoneal injection of 20 μg OVA (Grade V, Sigma–Aldrich, St. Louis, MO) with 2.25 mg aluminum hydroxide on days 1 and 14. On days 26–28, mice were challenged with aerosolized 1% OVA for 30 min. On day 30, 36 h after the last challenge, mice were cannulated via trachea, and airway hyperresponsiveness (AHR) to acetylcholine aerosol was measured as previously described [13]. The increased proportion to baseline intratracheal pressure was plotted at each acetylcholine concentration.

After measurement of AHR, mice were given a lethal dose of pentobarbital, and lungs were gently lavaged one time with 1.0 ml saline via the tracheal cannula. Total cell counts and differential cell counts were performed [13]. The levels of eotaxin, IL-13 (R&D Systems, Inc., Minneapolis, MN), and IFN-γ (Invitrogen, Carlsbad, CA) in the supernatant of BAL fluids were quantified using ELISA kits according to the manufacturer's protocols. All experiments were approved by the institutional animal committee of Kyushu University.

2.3. P6 and P6-encapsulated PLGA nanoparticle treatment

P6 (0.3 mg/kg) or DMSO was diluted in 0.1 ml saline and administered intraperitoneally on days 26 and 28, 1 h prior to each OVA challenge. P6-PLGA (60 mg/kg as P6-PLGA, 3 mg/kg as P6) or empty PLGA nanoparticles were dispersed in 0.1 ml saline and administered intraperitoneally according to the protocol either just before challenge or in both the sensitization plus challenge phases. In the sensitization phase, P6-PLGA was administered intraperitoneally on days 0, 1, 13, and 14. P6-PLGA was administered 2 h prior to OVA sensitization. In the challenge phase, P6-PLGA was administered on days 26 and 28 2 h prior to OVA challenge.

2.4. Serum OVA-specific immunoglobulin-E levels

After the measurement of AHR, a blood sample was collected from the inferior vein. Serum OVA-specific IgE was measured with an ELISA kit (TOYOBO, Osaka, Japan).

2.5. Flow cytometric analysis of lung cells

To determine the percentage of IL-17- and Foxp3-positive cells in mice lungs treated by P6-PLGA at both the sensitization plus challenge phases, intracellular staining was performed as previously described [14]. Single-cell suspensions from the lungs were obtained and used for intracellular IL-17 and Foxp3 staining.

2.6. In vitro T cell differentiation assay

$CD4^+$ $CD25^-$ T cells were isolated from naive C57B6/J murine spleens by negative selection using magnetic beads (Miltenyi Biotech, Gladbach, Germany) (typically 95% purity). $CD4^+$ $CD25^-$ T cells (3×10^5/well) were stimulated with 3 μg/ml plate-bound anti-CD3 mAb (clone 145-2C11) and 1 μg/ml soluble anti-CD28 mAb (eBioscience, San Diego, CA) with cytokines and antibodies required for each differentiation: 20 ng/ml mIL-12p70 (R&D Systems) and 5 μg/ml anti-IL-4 Ab (R&D Systems) for Th1 differentiation, 20 ng/ml mIL-4 (PeproTech, Rocky Hill, NJ) and 5 μg/ml anti-IFN-γ Ab (R&D Systems) for Th2 differentiation, and 2 ng/ml hTGF-β1 (R&D Systems) and 20 ng/ml hIL-6 (R&D Systems) for Th17 differentiation. The hIL-6 was omitted for Treg differentiation. Cells were collected after incubation for 5 days, and used for intracellular staining and culture-supernatant cytokine analysis.

2.7. Intracellular cytokine staining

Intracellular staining was performed as described previously [14]. For cytokine staining, cells were restimulated for 8 h with 50 nM PMA (Sigma–Aldrich), 1 μg/ml ionomycin (Sigma–Aldrich), and 1 μM brefeldin A (eBioscience). After surface staining by CD4-perCP (BD Pharmingen, San Diego, CA) or CD4-PE (eBioscience) with/without CD25-FITC (BD Pharmingen) according to the protocol, cells were incubated with unlabeled anti-CD16/32 mAb (BD Pharmingen). The cells were fixed, permeabilized, and incubated with anti-IFN-γ-FITC (BD Pharmingen), anti-IL-4-PE (eBioscience), anti-IL-17-PE (eBioscience), and anti-Foxp3-APC (eBioscience). A FACS calibur with CELLQuest software (BD Biosciences) was used for analysis.

2.8. Cytokine production in in vitro cultured supernatants

To determine cytokine production in *in vitro* differentiated cultured supernatants, viable cells (1×10^6 cells/ml) at each condition were restimulated with 10 μg/ml plate-bound anti-CD3 mAb and 1 μg/ml anti-CD28 mAb in the absence of any additional cytokines. Supernatants were collected after re-stimulation for 24 h, and the production of cytokines was measured using an ELISA kit (R&D Systems).

2.9. Histological assessment

Formalin-fixed lung sections were stained with Alcian blue/periodic acid-Schiff (AB/PAS) to determine the presence of mucin glucoconjugates. Numeric scores for the PAS-positive cells in each airway were determined as previously described [15].

2.10. Epithelial cell culture

TGMBE-02-3 cells, a mouse tracheal epithelial cell line [15], were cultured in a D-MEM/F-12 (GIBCO BRL, Rockville, MD) supplemented with 10% FCS, 1% ITES (2 mg/ml insulin, 2 mg/ml transferrin, 122 ng/ml ethanolamine, and 9.14 ng/ml sodium selenite; Wako, Osaka, Japan), and 10 ng/ml murine EGF (Wako) as previously described [15].

2.11. Western blotting for STAT6 and phosphorylated STAT6

TGMBE-02-3 cells were pretreated with 1 μM P6 in a serum-free D-MEM/F-12 medium for 1 h; then, 10 ng/ml mIL-13 (Peprotech) at 10 ng/ml was added, and samples were collected

at indicated periods. Immunoblotting was performed using anti-phospho-STAT6 (New England Biolabs, Beverly, MA) or anti-STAT6 (Santa Cruz Biotechnology, Santa Cruz, CA) antibodies as previously described [15].

2.12. Determination of eotaxin production and mRNA expression of CLCA and MUC5AC

TGMBE-02-3 cells were pretreated with the indicated concentration of P6 or DMSO for 1 h, and murine IL-13 was then added at 10 ng/ml and incubated for 24 h. Eotaxin levels in the supernatants were measured using an ELISA kit (R&D Systems). The cells were collected and used for RNA extraction and PCR analysis. The following primer pairs were used as previously described [15]: mCLCA3, 5′-GCCAAGGAGCCTCGCCTATTCTCAGG-3′ and 5′-GAAGCT CTCCCGTGGTCGTAG-3′; MUC5AC, 5′-CAGCCGAGAGGAGGGTTTGA TCT-3′ and 5′-AGTCTCTCTCCGCTCCTCTCAAT-3′; β-actin as an internal control, 5′-TCCTGTGGCATCCATGAAACT-3′ and 5′-GAAGCACTT GCGGTGCACGAT-3′.

2.13. Statistical analysis

All data were analyzed by the paired Student's *t* test, except for semi-quantitative counting of goblet cell hyperplasia, which was analyzed with the Kruskal–Wallis test and Mann–Whitney *U* test. A *p*-value of <0.05 was considered to be significant. The values of all measurements were expressed as the means ± SE.

3. Results

3.1. Effect of treatment with unencapsulated P6 on allergic asthmatic responses

Th2 cytokines activate signal transduction pathways, including JAK–STAT signaling, and we first studied the effects of unencapsulated P6 on airway responses during the challenge phase of allergic asthma. Treatment with P6 during the OVA challenge phase significantly reduced eosinophilia in BAL fluids compared to the treatment with vehicle but failed to affect AHR (Fig. 1A and B). The concentration of eotaxin in BAL fluids was reduced, but neither IL-13 and IFN-γ nor the serum OVA-specific IgE levels were affected by P6 (Fig. 1C). The levels of PAS-positive cells in the airways were not affected by P6 treatment (Fig. 1D).

3.2. Effect of P6-PLGA treatment on allergic asthmatic responses

To improve sustainability of the JAK inhibitor, P6 was encapsulated in PLGA nanoparticles. P6-PLGA treatment just before OVA

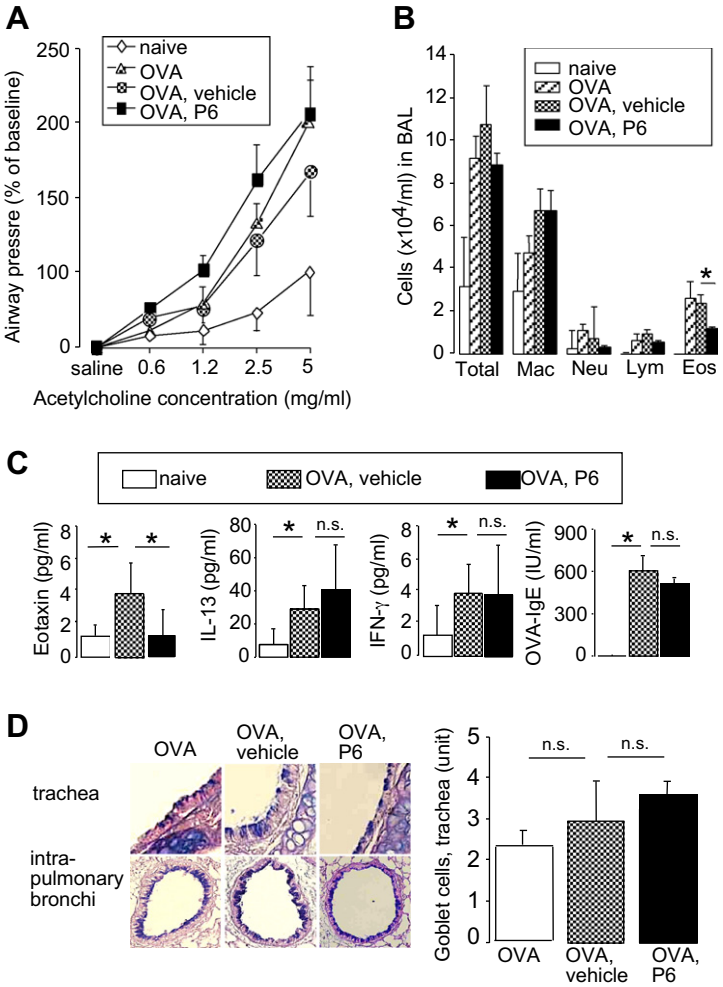

Fig. 1. Effects of treatment with unencapsulated pyridone 6 (P6) on allergic asthmatic responses. P6 or vehicle was administered intraperitoneally during the OVA challenge in sensitized mice. (A) Airway hyperresponsiveness (AHR) in response to inhaled acetylcholine. (B) Effect of P6 on cell counts in bronchoalveolar lavage (BAL) fluids. (C) Cytokine levels in BAL fluids and serum OVA-IgE levels. All data mean ± SEM of 5–8 mice per group. $p < 0.05$. (D) Alcian blue-periodic acid-Schiff (AB/PAS) staining of lungs (×100, *left panel*). Semiquantitative analysis of the PAS-positive cells (*right panel*). The numeric scores for the PAS-positive cells in each airway were determined as follows: 0, 5% PAS-positive cells; 1, 5–25%; 2, 25–50%; 3, 50–75%; 4, 75%.

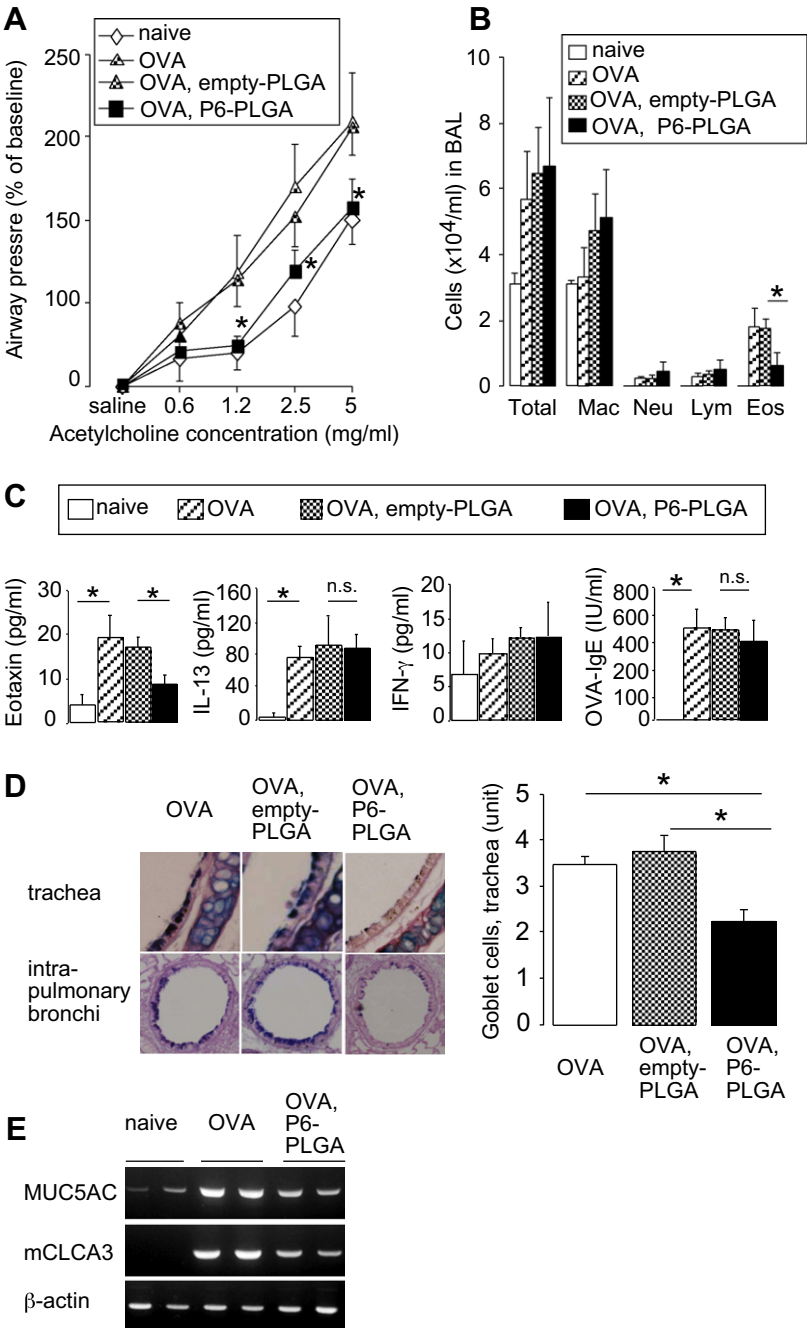

Fig. 2. Effects of polylactic-*co*glycolic acid (PLGA)-encapsulated P6 (P6-PLGA) on allergic airway responses. P6-PLGA or empty-PLGA was administered during OVA challenge. (A) AHR as determined by acetylcholine-dependent change in airway pressure. (B) Effect of P6-PLGA on cell counts in BAL fluids. (C) Cytokine levels in BAL fluids and serum OVA-IgE levels. All data are the mean ± SEM of 6–7 mice per group. $^{*}p < 0.05$. (D) AB/PAS staining of lung tissue (×100, *left panel*). Semiquantitative analysis of PAS-positive cells (*right panel*). $^{*}p < 0.05$. (E) MUC5AC and mCLCA3/Gob-5 mRNA expression in lung determined by RT-PCR. β-actin is shown as an internal control.

challenge markedly suppressed AHR compared to empty-PLGA treatment in addition to suppressing the eosinophil counts in BAL fluids (Fig. 2A and B). The eotaxin levels in BAL fluids were reduced, but neither the IL-13 and IFN-γ levels in BAL fluids nor the serum OVA-specific IgE levels were affected by P6 treatment (Fig. 2C). There was a reduction in PAS-positive cells (Fig. 2D) as well as in the expression of the goblet cell secretory granule marker mCLCA3/Gob-5 and the mucin gene MUC5AC in the airways after P6 treatment (Fig. 2E). The empty-PLGA treatment had no effect on the AHR, the profiles of BAL fluids, and PAS-positive cells.

Next, we administered P6-PLGA during the sensitization plus challenge phases to evaluate the effects of a JAK inhibitor in the development of allergic asthma. Interestingly, P6-PLGA treatment during the sensitization plus challenge phases did not suppress AHR or eosinophilia but significantly reduced the IL-13 and eotaxin levels in BAL fluids and the serum OVA-specific IgE level compared to empty-PLGA treatment (Fig. 3A–C). The IFN-γ levels in BAL fluids were not affected (Fig. 3C).

3.3. Effect of P6 on Th17 and Foxp3^{+} T cells in lungs

Despite the significant decrease in IL-13 and eotaxin levels by P6-PLGA treatment during the sensitization plus challenge phases, AHR and airway eosinophilia were not inhibited. Therefore, we

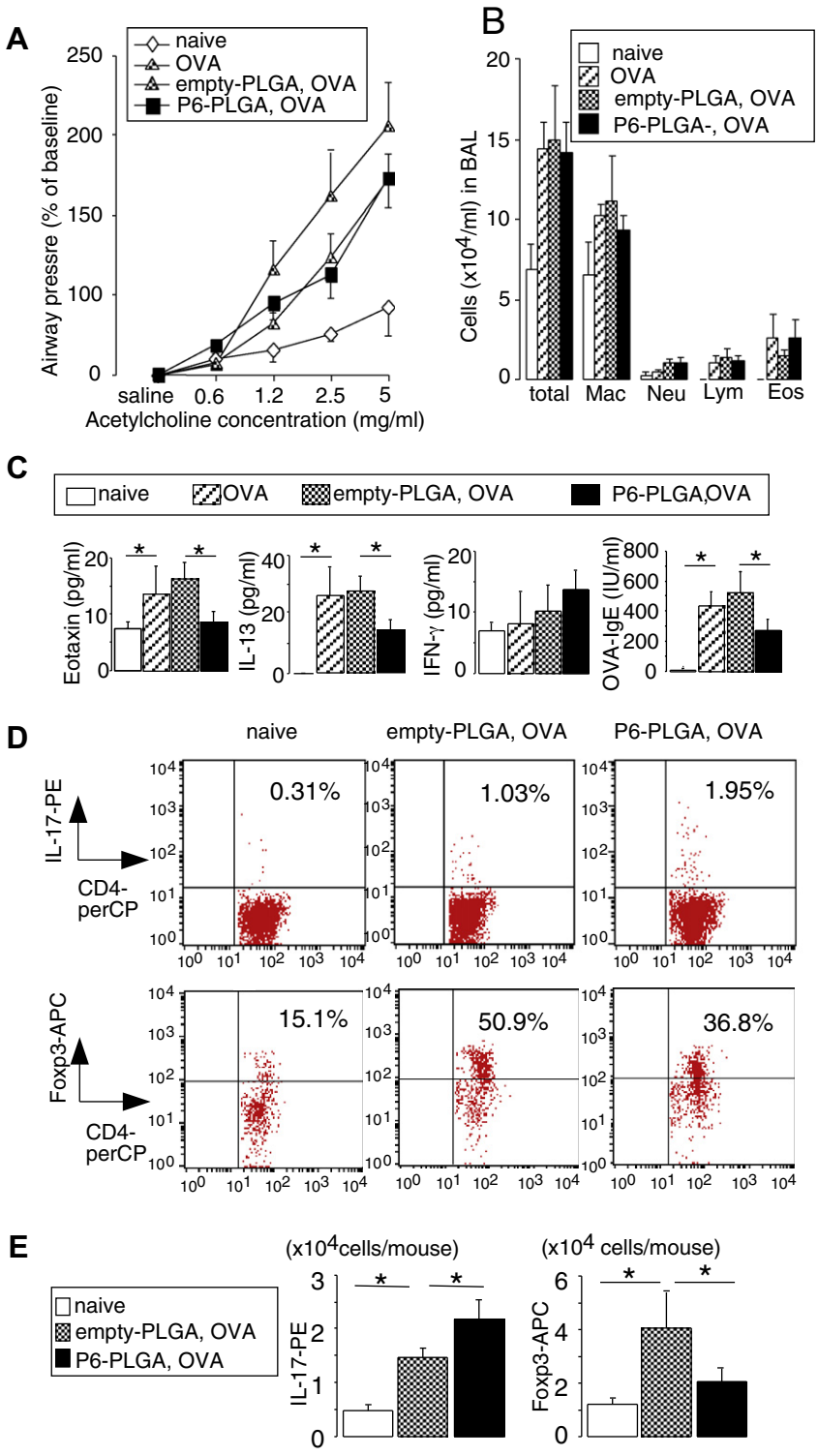

Fig. 3. Effect of P6-PLGA administered during both the OVA sensitization plus challenge phases on allergic airway responses. (A) AHR in response to inhaled acetylcholine. (B) Effect of P6-PLGA on cell counts in BAL fluids. (C) Cytokine levels in BAL fluids and serum OVA-IgE levels. All data are the mean ± SEM of 6–8 mice per group. * $p < 0.05$. (D and E) Effect of P6 on Th17 and Foxp3-expressing regulatory T cells (Foxp3$^+$ Tregs) in lungs. (D) Typical plots of IL-17-producing or Foxp3$^+$ CD4$^+$ T cells in the lungs. The quadrant percentiles of positive cells for the IL-17 or Foxp3 are shown. (E) Average numbers of IL-17- or Foxp3$^+$ CD4$^+$ T cells in lung cells. All data are the mean ± SEM from 4 to 6 mice. * $p < 0.05$.

speculated that another mechanism was at work for enhancing asthmatic responses by P6-PLGA, such as IL-17 overproduction and decreases in Foxp3$^+$ Tregs.

We examined the percentage of Th17 cells and Foxp3$^+$ Tregs in the lungs of P6-PLGA treated mice during the sensitization plus challenge phases by flow cytometry. P6-PLGA treatment significantly increased Th17 cells and reciprocally decreased Foxp3$^+$ Tregs more than empty-PLGA treatment (Fig. 3C and D). These results indicate that the inhibitory effect of P6 on airway Th2-driven inflammation may be reversed by an increase in Th17 cells and a decrease in Tregs.

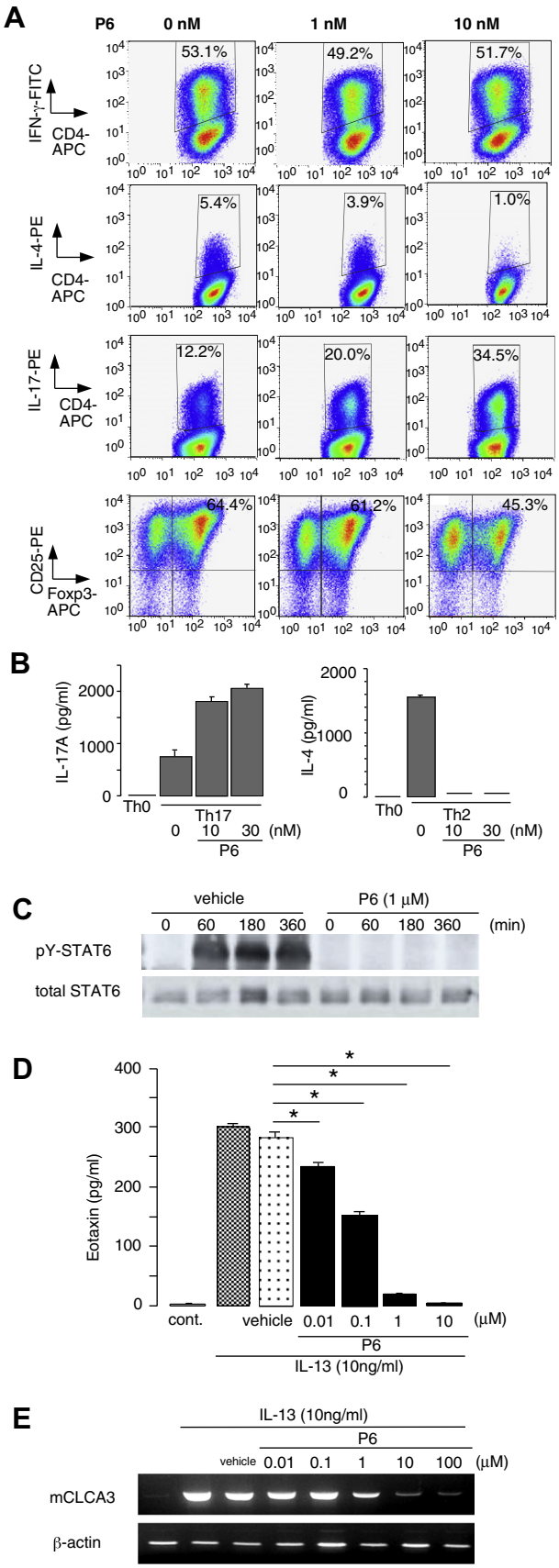

Fig. 4. Changes in naive CD4⁺ T cell differentiation and cytokine production by P6 *in vitro*. (A) Typical plots of cytokines and Foxp3 determined by intracellular staining. Cells were activated *in vitro* under Th1, Th2, Th17, and Treg conditions as described in Section 2. (B) Cytokine production from *in vitro* differentiated CD4⁺ T cells. After 5 days of culture under Th2 and Th17 conditions, cells were restimulated by non-specific TCR Abs. Data indicate the mean SD of triplicate cultures in one representative experiment out of three independent experiments. $^{*}p < 0.05$. (C–E) Effect of P6 on IL-13-induced mCLCA3/Gob-5 expression *in vitro*. Mouse airway epithelial cells (TGMBE-02-3) were pretreated with P6 or DMSO for 1 h. The cells were stimulated with IL-13. (C) STAT6 was activated by IL-13, and P6 suppressed STAT6 activation. (D) Eotaxin levels in the supernatant were increased after IL-13 stimulation. P6 suppressed eotaxin expression dose-dependently. (E) After IL-13 stimulation for 24 h, mCLCA3 mRNA expression was determined by RT-PCR. β-actin is shown as an internal control.

3.4. Effect of P6 on Th cell differentiation in vitro

To confirm Th2/Treg inhibition and Th17 enhancement by JAK inhibitors, naive CD4⁺ T cells were differentiated *in vitro*. By flow cytometrical analysis, P6-treated CD4⁺ T cells displayed a marked reduction in IL-4-producing cells under the Th2 condition. At the same concentration, P6 enhanced Th17 development under the Th17 condition. Consistent with Th17 enhancement, P6 partly reduced TGF-β-mediated iTreg induction. P6 treatment did not change IFN-γ production (Fig. 4A). P6 significantly enhanced IL-17 and reduced IL-4 production in culture supernatant collected after restimulation with anti-CD3 mAb and anti-CD28 mAb (Fig. 4B).

3.5. Effect of P6 on IL-13-induced mCLCA3/Gob-5 expression in vitro

Compared with the inhibitory effects on airway eosinophilia, unencapsulated P6 treatment did not inhibit AHR and goblet cell hyperplasia, suggesting that AHR and goblet cell hyperplasia in asthmatic phenotypes may be induced by a different mechanism from that for eosinophilic inflammation. Therefore, we investigated whether the inhibitory potency of P6 on IL-13-driven mCLCA3/Gob-5 expression is different from that on IL-13-induced eotaxin production in the mouse tracheal epithelial cell line TGMBE-02-3 *in vitro*. Phosphorylated STAT6 was induced following IL-13 stimulation, which was suppressed by P6 (Fig. 4C). IL-13 increased eotaxin production and mCLCA3/Gob-5 mRNA expression, which were inhibited by pretreatment with P6 in a dose-dependent manner (Fig. 4D and E). Importantly, 1 μM of P6 was sufficient to almost complete inhibition of eotaxin production, whereas 10–100 μM of P6 was required to do so for mCLCA/Gob-5 expression.

4. Discussion

In this study, we demonstrated that, during the challenge phase of allergic asthma, unencapsulated P6 treatment suppressed airway eosinophilia, and P6-PLGA treatment suppressed eosinophilia, AHR, and goblet cell hyperplasia. These findings suggest that P6-PLGA treatment during allergen challenge suppresses asthmatic phenotypes.

Application of PLGA nanoparticles improved the therapeutic potency of P6 in this asthma model. Unencapsulated P6 treatment inhibited airway eosinophilia but not AHR or goblet cell hyperplasia. This limited potency was successfully overcome by encapsulation of P6 with PLGA nanoparticles. To further elucidate the underlying mechanisms, we focused on the difference in effective doses of P6 on each asthma phenotype. Local production of eotaxin contributes to airway eosinophilia in asthma [16]. The mCLCA3/Gob-5 plays an important role in allergic airway inflammation and AHR in addition to induction of goblet cell hyperplasia [15,17], and Th2 cytokines induce mCLCA3/Gob-5 expression in the airways [15,18]. In the

present study, effective inhibition of mCLCA3/Gob-5 expression required more than 10 times the dosage of P6 compared to that of eotaxin *in vitro*. However, hydrophobicity of P6 is a major obstacle to its dose escalation for *in vivo* administration. By means of nanoparticle formulation, P6 was easily dispersed in saline, and a 10-time higher dose than the maximum dose of unencapsulated P6 *in vivo* could be given. Moreover, the nanoparticle formulation could demonstrate its efficacy with only two injections, just before the first and the last day of OVA challenge, whereas unencapsulated P6 had to be injected five times before, during, and after the OVA challenge. Encapsulation of P6 with PLGA nanoparticles may attain a high concentration in the airway tissues, leading to marked inhibition of AHR and goblet cell hyperplasia.

Treatment with P6-PLGA during the sensitization plus challenge phases decreased the serum IgE and IL-13 levels in BAL fluids but failed to suppress airway eosinophilia and AHR, which were accompanied with increased Th17 cells and decreased $Foxp3^+$ Tregs in lungs. The functional impairment of Tregs in the sensitization or the challenge phase is known to enhance asthmatic responses [19]. Even if P6-PLGA treatment attenuates Th2 inflammation at both the sensitization and challenge phases, this effect might be reversed by concurrent inhibition of Tregs.

On the other hand, the functional consequence of increased Th17 may be complex. IL-17 expression by both T cells and eosinophils was increased in the airway of asthmatic subjects, which was associated with neutrophilic airway inflammation [20]. In mouse models of allergic asthma, IL-17 showed dual actions [6]. Thus, IL-17 was demonstrated to be essential in the sensitization phase, but, once allergic asthma was established, it attenuated the allergic response [6]. It has been also reported that airway inflammation was deteriorated when Th17 cells were transferred with Th2 cells to naïve mice [7]. The blockade of IL-17 by mAb at the challenge phase demonstrated that IL-17 was responsible for neutrophilic airway inflammation in the chronic asthma model [21]. However, in the present study, P6-PLGA did not affect the neutrophil count in BAL fluids. P6-PLGA administered in the challenge phase only attenuated Th17 differentiation (data not shown). These results suggest that the pan JAK inhibitor attenuates both Th2 and Th17 airway responses in established asthma but might contribute to Th17-mediated responses during the development of asthma, an issue which needs to be further investigated.

The differentiation of Tregs/Th17 requires TGF-β, and Tregs/Th17 are regulated in a reciprocal way via other cytokines or directly through RORγt and Foxp3 interaction [14,22]. IL-6 promotes the generation of Th17 cells but inhibits that of Tregs. In contrast, IL-2 promotes the generation of Tregs, whereas it inhibits that of Th17 cells via STAT5 [23]. In the present study, P6 enhanced Th17 differentiation but suppressed Treg, suggesting differential regulation of Th commitment by P6. We observed strong suppression of STAT1 and STAT5 by P6, while STAT3 activation was not significantly reduced (data not shown). Thus, enhanced Th17 development could be due to suppression of Th17-inhibitory signals (STAT1 and STAT5) by P6 but relatively weak suppression of Th17-promoting signals (STAT3). JAK1 is thought to be the most relevant for IL-6 signaling [24], and JAK1 and JAK3, for IL-2 signaling [25]. Although P6 is originally reported be a pan JAK inhibition, its relative potency against each JAK might affect Th differentiation.

In conclusion, we showed that treatment with the pan JAK inhibitor P6-encapsulated in PLGA nanoparticles during local allergen challenge suppresses asthmatic responses. Nanoparticle loading is a promising drug delivery system for intracellular kinase targeting.

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