JTP-27536 [(+) -1,3-Dihydroxy-2-hydroxymethylpropyl-2-ammonium 2-[[ (R)-3-Cyclo-hexyl-1-phenylpropyl]-1,3-dioxo-2,3-dihydro-1H-isoinde-5-carboxylate Monohydrate], a Novel Inhibitor of Immunoglobulins and Interleukin-5 with Anti-Inflammatory Properties in Mouse Allergic Dermatitis Model

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ABSTRACT

We report a novel synthetic compound JTP-27536 [(+) -1,3-dihydroxy-2-hydroxymethylpropyl-2-ammonium 2-[(R)-3-cyclohexyl-1-phenylpropyl]-1,3-dioxo-2,3-dihydro-1H-isoinde-5-carboxylate monohydrate] as an inhibitor of immunoglobulins (Igs) and interleukin (IL)-5 production in vitro and in vivo. JTP-27536 inhibited IgE production in mouse and human B cells with IC50 values of 2.5 and 2.1 μM, respectively, and the inhibition was stronger than that on IgG1 and IgM production (IC50 > 10 μM). JTP-27536 also inhibited IL-5 production in mouse splenocytes and human peripheral blood mononuclear cells with IC50 values of 3.3 and 1.3 μM, respectively, without affecting mouse interferon (IFN)-γ, IL-2, IL-4, IL-10, or human IL-4 production. In contrast, prednisolone not only inhibited mouse IgE production but also mouse IFN-γ, IL-2, IL-4, and IL-10 and human IL-4 and IL-5 production in vitro. The effect of suplatast tosilate, a Th2 cytokine inhibitor, on antibody and cytokine production was less potent than that of JTP-27536. In vivo animal experiments using dinitrophenylated ascariasisensitized mice and 2,4,6-trinitro-1-chrolobenzene-induced chronic dermatitis mice showed that JTP-27536 was more potent than suplatast tosilate and comparable with prednisolone in inhibiting ear swelling, antigen-specific IgE and IL-5 production, and cell infiltrations into the inflamed tissue. These results indicate that JTP-27536 is an inhibitor of Igs, in particular IgE, and of IL-5, which has antiallergic properties in mouse dermatitis model, and suggest that an inhibitor of Igs and IL-5 like JTP-27536 may be useful as a drug for the treatment of allergic diseases.

It has been widely recognized that IgE is a major antibody involved in mediation of allergic diseases. Cross-linking of IgE bound to its high-affinity receptor, FceRI, with antigen initiates the activation of mast cells by promoting the aggregation of FceRI (Turner and Kinet, 1999). This IgE-induced activation results in degranulation, de novo synthesis, and release of proinflammatory lipid mediators, cytokines, and chemokines; thus, the mast cells are major effector cells in IgE-mediated immediate hypersensitivity and allergic diseases (Williams and Galli, 2000). IgE also promotes production of cytokines not only from mast cells (Hart, 2001) but also from basophils (Falcone et al., 2000) and eosinophils (Dombrowicz et al., 2000), and thereby contributes to the persistence and aggravation of allergic diseases. In addition, IgE prolonged survival of mouse mast cells under limited growth factor conditions (Asai et al., 2001; Kalesnikoff et al., 2001). On the other hand, it has been reported that IgG1 binds to FcγR expressed on mast cells and causes degranulation (Woolhiser et al., 2001). Therefore, the control of Igs production, in particular of IgE, is considered to be an important approach to the treatment of allergic diseases.

IL-5 is mainly produced by activated T cells and mast cells and plays an important role in activation, proliferation, and differentiation of B cells and eosinophils (Fattah et al., 1990; Baumann and Paul, 1997). IL-5 facilitates differentiation of bone marrow cells to eosinophils and induces activation and migration of eosinophils into inflammatory tissues (Sandonson, 1988). As a consequence of these processes, allergic inflammation is prolonged and becomes intractable, observed...
as marked accumulation of eosinophils and high levels of histotoxic substances released from eosinophils such as eosinophil cationic protein and major basic protein, in inflamed tissues of patients with asthma, allergic rhinitis, and atopic dermatitis (Peters et al., 1986; Czech et al., 1992; Lönnkvist et al., 2002). Eosinophilic inflammation, the inflammatory changes resulting from the activation of eosinophils, is regarded as an important background factor in delayed-type allergic reactions. The eosinophilic inflammation is refractory to antihistamine or existing antiallergic agents; therefore, IL-5 has become another important target for the treatment of allergic diseases.

Steroids have been widely used in the treatment of allergic diseases and are reported to reduce eosinophilic inflammation by inhibiting IL-5, IL-8, RANTES, eosinotaxin, MCP-1, MCP-3, and MIP-1α (Barnes et al., 1998). Immunosuppressive drugs, such as FK506 (tacrolimus) and cyclosporine A, have recently been applied to allergic patients because of their expected inflammatory cytokine inhibition. However, the usefulness of steroids and immunosuppressive drugs has been limited due to associated adverse effects resulting from excessive immunosuppression by IL-2 or interferon (IFN)-γ inhibition and intrinsic toxicities to kidneys (Granelli-Piperno, 1990; Mihatsch et al., 1998; Nash et al., 2000). It is, therefore, considered that inhibition of IgE and IL-5, key factors in allergic processes, would provide great advantage in the treatment of allergic diseases.

We focused on screening inhibitors of IgS and IL-5 production to find an antiallergic drug and discovered JTP-27536 (Fig. 1). We report in this article that JTP-27536 inhibits IgE and IL-5 production and shows antiallergic properties in vivo. The eosinophilic inflammation is refractory to antihistamine or existing antiallergic agents; therefore, IL-5 has become another important target for the treatment of allergic diseases.

**Materials and Methods**

**Animals.** Female BALB/c mice (7 weeks) were obtained from Charles River Japan, Inc. (Yokohama, Japan) and maintained under specific pathogen-free conditions. Female BALB/c mice (7 weeks) were obtained from Charles River Japan, Inc. (Yokohama, Japan) and maintained under specific pathogen-free conditions at a room temperature of 23 ± 3°C and air humidity of 55 ± 15% in a 12-/12-h light/dark cycle environment.

**Reagents.** JTP-27536 and sulfaestolate tosylate [\(\{\pm\}-2-[4-(3-ethoxy-2-hydroxypropoxy)-phenylcarbamoyl] ethyl \] dimethylsulfoxonium p-toluenesulfonate) were synthesized by Japan Tobacco Inc. (Osaka, Japan). Prednisolone was purchased from Sigma-Aldrich (St. Louis, MO). These test compounds were dissolved in dimethylsulfoxide, which was kept at a concentration of 0.1% in vitro. Lipopolysaccharide (LPS) from *Salmonella typhosa*, Histopaque 1119, and phorbol 12-myristate 13-acetate (PMA) were purchased from Sigma-Aldrich. Ficoll-Paque was purchased from Pfzer, Inc. (Täby, Sweden). CD3 monoclonal antibody (OKT-3) was purchased from cBiocience (San Diego, CA). Microbeads for anti-mouse CD90 and CD11b and human B cell isolation kit were purchased from Daiichi Pure Chemicals (Tokyo, Japan). Recombinant mouse (rm) IL-4 was purchased from PeproTech (Rocky Hill, NJ). Anti-DNP-specific mouse IgE antibody and mouse IgE enzyme-linked immunosorbent assay (ELISA) kit were purchased from Yamasa Sboyu (Chiba, Japan). ELISA kits for mouse IgG1 and IgM were purchased from Rougier Bio-Tech (Montreal, Canada). Cocanovalin A (Con.A) was purchased from Vector Laboratories (Peterborough, UK). ELISA kits for mouse IFN-γ, IL-2, IL-4, IL-5, and IL-10 and human IL-5 were from Applied Biosystems (Foster City, CA). Anti-mouse IgE antibody and anti-human CD40 monoclonal antibody were from BD Biosciences Pharmingen (San Diego, CA). ELISA kits for human IL-4, recombinant human (rh) IL-10, and rhIL-4 were from Genzyme (Cambridge, MA). Human IgE and IgM ELISA kits were from MBL (Nagoya, Japan) and IBL (Tokyo, Japan), respectively. Methylcellulose was from Nacalai Tesque (Kyoto, Japan) and used as a solvent (0.5% solution in distilled water) for oral treatment of test compounds in vivo. DNP-ascaris extract and alum were from DSL (Tokyo, Japan). Reagent 2,4,6-trinitro-1-chlorobenzene (TNCB) was from Tokyo Kasei Co. (Tokyo, Japan) and was used as a solution in acetone. Block-ace was from Dainippon Pharmaceutical (Osaka, Japan). Horseradish peroxidase (HRP)-labeled anti-mouse IgG1 and HRP-anti-mouse IgG2a were from Zymed Laboratories (South San Francisco, CA). HRP-anti-mouse IgM was from Cappel Laboratories (Durham, NC). Peroxidase-conjugated streptavidin was from DAKO JAPAN (Kyoto, Japan). TMB substrate was from InVitrogen (Carlsbad, CA).

**Antibody Production in Murine Splenic B Cells.** Mice were sacrificed under light anesthesia, and spleens were collected under aseptic conditions. After straining the spleens through wire mesh in phosphate-buffered saline (PBS) containing 2 mM EDTA, the splenocytes were enriched for splenic B cells using a magnetic-activated cell separator with anti-mouse CD90 and CD11b microbeads. The enriched B cells were transferred to a 96-well round-bottom plate at 1 × 10⁶ cells/well in culture medium (RPMI-1640 supplemented with 10% fetal calf serum, 100 IU/ml penicillin/streptomycin, 2 mM L-glutamine, and 50 μM 2-mercaptoethanol) and were stimulated with LPS (final concentration 20 μg/ml) for 24 h at 37°C in humidified incubator with 5% CO₂. Various concentrations of test compounds or vehicle were added 10 min before the stimulation with IL-4 (final concentration 20 ng/ml), and the cells were incubated for 7 days at 37°C in humidified incubator with 5% CO₂ under the presence of the compounds. The amounts of IgE, IgG1, and IgM in the supernatants of the culture were measured using the respective ELISA kit.

**Cytokine Production in Mouse Splenocytes.** Mouse splenocytes were obtained as described above and adjusted to 1 × 10⁶ cells/ml in culture medium. For the evaluation of test compounds on IL-2 and IFN-γ production, the splenocytes were cultured at 1 × 10⁶ cells/well in a 96-well round-bottom plate and stimulated with Con.A (final concentration 5 μg/ml) for 24 h at 37°C in a humidified incubator with 5% CO₂. Various concentrations of test compounds or vehicle were added to the culture 10 min before the Con.A stimulation. The supernatants were collected for the measurement of IL-2 and IFN-γ using the corresponding ELISA kit. For the evaluations of test compounds on IL-4, IL-5, and IL-10 production, the splenocytes cultured at 1 × 10⁷ cells/ml in a 75-cm² flask were stimulated with Con.A (final concentration 5 μg/ml) for 4 days at 37°C in humidified incubator with 5% CO₂. The cells were collected, and dead cells were removed by density gradient centrifugation using a Histopaque 1119 (800g for 15 min at room temperature), cultured at 1 × 10⁶ cells/well in a 96-well round-bottom plate, and further stimulated with Con.A (final concentration 5 μg/ml) and PMA (final concentration 1 ng/ml) for 24 h at 37°C in humidified incubator with 5% CO₂. Various concentrations of test compounds or vehicle were added to the culture 10 min before the Con.A + PMA stimulation. The amounts of cytokines in the supernatants were measured using the respective ELISA kit.

**Antibody Production in Human Peripheral B Cells.** Human peripheral blood mononuclear cells (hPBMCs) were isolated from heparinized blood of normal volunteers by Ficoll-Paque density gradient centrifugation (800g for 20 min at room temperature). The
hPBMC layer was harvested, washed twice with PBS, and resuspended in culture medium. B cells were enriched using a magnetic-activated cell separator human B cell isolation kit and cultured at 2 x 10^5 cells/well in a 96-well round-bottom plate. Various concentrations of test compounds or vehicle were added 10 min before the stimulation with anti-human CD40 monoclonal antibody, rhIL-10, and rhIL-4 at final concentrations of 3 μg/ml, 10 ng/ml, and 10 ng/ml, respectively. The cells were incubated in the presence of the compounds for 14 days at 37°C in a humidified incubator with 5% CO2, and the supernatants were collected for the measurement of IgE and IgM with the corresponding ELISA kit.

**Cytokine Production in hPBMCs.** hPBMCs at 4 x 10^5 cells/well in a 96-well round-bottom plate were stimulated with anti-human OKT-3 (final concentration 1 μg/ml) 10 min after addition of test compounds or vehicle and incubated for 24 h at 37°C in a humidified incubator with 5% CO2. The supernatants were collected for the measurement of IL-4 and IL-5 with the respective ELISA kit.
TABLE 1

Effects of JTP-27536, suplatast tosilate, and prednisolone on antibody production in mouse and human B cells. Each value represents the average IC_{50} from three to four independent experiments.

<table>
<thead>
<tr>
<th>Inhibitors</th>
<th>Mouse IC_{50} (μM)</th>
<th>Human IC_{50} (μM)</th>
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<tbody>
<tr>
<td>JTP-27536</td>
<td>2.5</td>
<td>10</td>
</tr>
<tr>
<td>Suplatast</td>
<td>&gt;100</td>
<td>&gt;100</td>
</tr>
<tr>
<td>Prednisolone</td>
<td>13.5</td>
<td>&gt;100</td>
</tr>
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orbital sinus 6 h after the antigen application on day 35, and serum samples were prepared for the measurements of antibodies and IL-5 to be performed by ELISA. The pinna of each mouse was fixed in 10% neutral buffered formalin, embedded in paraffin, cut into 6-μm sections, and stained with hematoxin-eosin and toluidine blue for counting eosinophils and mast cells microscopically. Eosinophils were identified as polymorphonucleated cells with eosin-stained granule. Mast cells were identified in hematoxin-eosin stained preparation by their widely varying cytoplasmic contours, being round, irregularly oval, spindle-, or star-shaped cells, and confirmed by toluidine blue-stained granules. The cells were counted under 400-fold magnification in a blind manner.

Measurement of Antigen-Specific Antibodies in Mouse Serum. The levels of antigen-specific IgE, IgG1, IgG2a, and IgM in DNP-ascaris-sensitized mice and TNCB-sensitized mice were determined by sandwich ELISA. Because TNCB-specific antibodies recognize DNP-ascaris, TNCB-specific antibodies were measured as DNP-specific antibodies and described as antigen-specific antibodies. For the measurement of antigen-specific IgG1, IgM, and IgG2a, a 96-well flat-bottom plate was coated with 30 μg/ml DNP-ascaris in 0.05 M carbonate buffer, pH 9.6, and incubated at 37°C for 1 h. After washing the plate (×3 with 0.05% Tween 20 in PBS), wells were blocked with Block-ace at room temperature for 1 h. The plates were then washed again, and serum samples were added and incubated at room temperature for 1 h. Plates were washed again and incubated with HRP-anti-mouse IgG1 (225 ng/ml), HRP-anti-mouse IgM (500 ng/ml), or HRP-anti-mouse IgG2a (255 ng/ml) at room temperature for 1 h. After another washing, TMB solution was added at 100 μl/well, and incubated for 30 min at room temperature. The reaction was stopped by adding 2 mol/l sulfuric acid at 50 μl/well; 15 min later, the absorbance was measured at 450 nm with a plate reader (MT-MAX, Wako Bioproducts, Richmond, VA). For the measurement of antigen-specific IgE, a 96-well flat-bottom plate was coated with anti-mouse IgE antibody at 4 μg/ml in 0.05 M carbonate buffer, pH 9.6, and incubated at 37°C for 1 h. The plate was washed and incubated at room temperature for 1 h in the presence of Block-ace. After again washing the plate, serum samples were added and incubated at room temperature for 1 h. Biotin-labeled DNP-BSA (20 ng/ml) was added to each well after washing, and the plate was incubated for 1 h at room temperature. Peroxidase-conjugated streptavidin (80 ng/ml) was added after another washing, and the plate was incubated at room temperature for 1 h. TMB solution was added at 100 μl/well and incubated for 30 min at room temperature. The reaction was stopped by adding 2 mol/l sulfuric acid at 50 μl/well; 15 min later, the absorbance was measured at 450 nm with the plate reader. The concentration of antigen-specific IgE was calculated from the known concentration of anti-DNP-specific mouse IgE as a standard, and expressed as nanograms per milliliter. Because there was no commercially available DNP-specific IgG1, IgG2a, and IgM, at the time of the study, concentrations of these antibodies were calculated as relative amounts of vehicle control.

Statistical Analysis. Values are expressed as mean ± S.E.M. The differences between control and compound values were analyzed by analysis of variance with Dunnett’s test or Student’s t test. P < 0.05 was considered statistically significant.

Results

Effects of Test Compounds on Antibody Production in Mouse Splenic and Human Peripheral Blood B Cells. Figures 2 and 3 and Table 1 show the effects of JTP-27536, suplatast tosilate, and prednisolone on antibody production in isolated mouse splenic and human peripheral blood B cells. JTP-27536 inhibited IgE production both in cultures of activated mouse and human B cells, and the effect was stronger than that on IgG1 and IgM. Prednisolone inhibited mouse IgE production without affecting IgG1 and IgM; conversely, however, production of human IgE and IgM was enhanced. Suplatast tosilate did not show any effect on all the antibodies measured in these experiment systems.

Effects of Test Compounds on Cytokine Production in Mouse Spleenocytes and hPBMCs. Figures 4 and 5 and Table 2 show the effects of JTP-27536, suplatast tosilate, and prednisolone on mouse and human cytokine production. JTP-27536 inhibited IL-5 production but showed little effect on human IL-4 production stimulated by combination of Con.A and PMA in mouse splenocytes. Supernatant from cultured cells was analyzed for cytokine production by enzyme immunoassay. The cytokine production in vehicle control for IFN-γ, IL-2, IL-4, IL-5, and IL-10 was 99.15 ± 23.82, 14,018 ± 2549, 1275 ± 389, 834 ± 188, and 3294 ± 649 pg/ml, respectively. Each value represents the mean ± S.E.M. of three independent experiments.

![Fig. 4. Effects of JTP-27536, suplatast tosilate, and prednisolone on cytokine production stimulated by combination of Con.A and PMA in mouse splenocytes. Supernatant from cultured cells was analyzed for cytokine production by enzyme immunoassay. The cytokine production in vehicle control for IFN-γ, IL-2, IL-4, IL-5, and IL-10 was 99.15 ± 23.82, 14,018 ± 2549, 1275 ± 389, 834 ± 188, and 3294 ± 649 pg/ml, respectively. Each value represents the mean ± S.E.M. of three independent experiments.](image-url)
duction even at 10 μM. Prednisolone strongly inhibited all the cytokines measured in mice and humans. Suplatast tosilate did not show any inhibition up to 100 μM for all cytokines.

**Effects of Test Compounds on Antibody Production in DNP-Ascaris-Sensitized Mice.** JTP-27536 showed dose-dependent inhibition of antigen-specific IgE in DNP-ascaris immunized mice, and the effect was significant from 10 to 100 mg/kg (Fig. 6A). IgG1 production was also significantly inhibited by JTP-27536 at 30 and 100 mg/kg (Fig. 6B), but antigen-specific IgG2a and IgM production was not affected (Fig. 6, C and D). Suplatast tosilate significantly inhibited IgE and IgG1 but not IgG2a and IgM at 100 mg/kg (Fig. 6, A–D).

**Effects of Test Compounds in TNCB-Induced Chronic Dermatitis Model.** Repeated application of TNCB on mouse ear induced ear swelling on day 7, which increased thereafter (Fig. 7). JTP-27536 (10–100 mg/kg) significantly inhibited the TNCB-induced ear swelling from day 17 and afterward over the experimental period in a dose-dependent manner. JTP-27536 showed significant inhibition of antigen-specific IgE production in a dose-dependent manner, whereas it did not show significant inhibition on other antibody subclasses (Fig. 8, A–D). Serum IL-5 production at 6 h after antigen application on day 35 was also significantly inhibited by JTP-27536 at 30 and 100 mg/kg (Fig. 8E). Prednisolone significantly inhibited ear swelling from days 7 to 35, and productions of antigen-specific IgE, IgG1, and IgG2a (Fig. 8, A–D) but did not inhibit IL-5. Histological study revealed that repeated TNCB treatment caused remarkable increase of eosinophil and mast cell numbers in inflamed ear, which were significantly and dose-dependently inhibited by JTP-27536 (10, 30, and 100 mg/kg) and prednisolone (5 mg/kg) (Fig. 9, A and B). Only marginal increases of neutrophils and lymphocytes were observed in the tissue; thus, effects of test compounds on these cells were not evaluated. There were no significant body weight differences between vehicle- and JTP-27536-treated mice (10–100 mg/kg), whereas prednisolone-treated mice (5 mg/kg) showed significant weight decrease from day 24 (data not shown).

**Discussion**

Recently, IgE, and IL-5 have drawn attention as targets in allergy treatment. Since most of the mediator release from mast cells in asthma is considered to be IgE-dependent, an attractive approach is to block the activation of IgE using blocking antibodies that would not result in mast cell activation (Owen, 2002). In fact, anti-IgE therapy with a recombinant humanized monoclonal antibody reduces the incidence of asthma exacerbations even after inhaled corticosteroids are markedly reduced or completely withdrawn (de Amato, 2003; Ruffin and Busch, 2004). On the other hand, IL-5 is crucial in orchestrating eosinophilic inflammation in asthma (Egan et al., 1996). It was shown that blocking antibodies to IL-5 inhibits eosinophilic inflammation and airway hyperresponsiveness in animal models of asthma (Hamelmann et al., 1997). Furthermore, it has been demonstrated that a
single intravenous infusion of humanized monoclonal antibodies to IL-5 markedly reduced blood eosinophils for several weeks and prevented eosinophil recruitment into the airways after allergen challenge in patients with mild asthma (Leckie et al., 2000). However, this treatment had no significant effect on early or late response to the allergen challenge or on baseline airway hypersensitivity, suggesting that eosinophils may not be of critical importance for these responses in humans. However, recent investigations suggest that eosinophils are associated with more chronic aspects of asthma, such as airway remodeling (Cho et al., 2004; Kumar et al., 2004) in mice, by showing that a blocking anti-IL-5 antibody prevented the increased collagen deposition in airways associated with repeated allergen exposure.

We discovered JTE-27536 by screening our library of compounds to find inhibitors of Igs and IL-5 in vitro. JTP-27536 showed stronger inhibition on IgE production than on IgG1 and IgM production in mouse splenic B cells and human peripheral B cells. In addition, JTP-27536 inhibited IL-5 production from mouse splenocytes without affecting IL-2, IFN-γ, IL-4, or IL-10 and from hPBMCs without affecting IL-4. We first evaluated JTP-27536 in a DNP-ascaris-induced antibody production model because this animal model was hyper antigen-specific in IgE production and had been

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**Fig. 6.** Effects of JTP-27536 and suplatast tosilate on antigen-specific IgE (A), IgG1 (B), IgG2a (C), and IgM (D) levels in serum from BALB/c mice immunized with DNP-ascaris. Each value represents the mean ± S.E.M. of eight animals. *, p < 0.05; ***, p < 0.01 compared with vehicle-treated sensitized control (PC; positive control) by Student’s t test.

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**Fig. 7.** Effects of JTP-27536 and prednisolone on mouse ear swelling induced by repeated-TNCB application onto ear. Each value represents the mean ± S.E.M. of eight animals. ***, p < 0.01; ###, p < 0.001 compared with vehicle-treated sensitized control (PC) by Student’s t test.
used for evaluation of suplatast tosilate (Matsuura, 1994). Although the inhibitory effect of JTP-27536 on IgE production was observed, IgG1 was also inhibited, which may reflect weak inhibition of IgG1 observed in vitro. It is also possible that the inhibition of IgG1 may be due to the indirect action of JTP-27536 on B cells though IL-5 inhibition. It is reported that IgG1 production was observed when B cells were stimulated in combination with rhIL-5 and anti-CD38 antibody (Mizoguchi et al., 1999). On the other hand, IL-4 induces antibody class switching to IgG1 and IgE in B cells, and suplatast tosilate was able to reduce IL-4 production at higher concentrations. Therefore, we speculate that inhibition of IgG1 production by JTP-27536 and suplatast tosilate was the result of inhibiting IL-5 and/or IL-4 production.

The TNCB-induced dermatitis model in mice appears to mimic many, if not all, events occurring (hyperkeratosis, inflammatory cell infiltration, and increase in mast cells) within the regional skin in patients with chronic allergic dermatitis (Kitagaki et al., 1995). Because antiallergic effects of prednisolone on this dermatitis model were well examined (Inoue et al., 2002) and the effect of suplatast tosilate in vivo was not strong enough, we used prednisolone as positive control in this model. There was a slight difference in onset of ear swelling inhibition between JTP-27536 and prednisolone. In TNCB-sensitized mice, cell populations were reported to shift from Th1 to Th2 about 15 days after primary immunization (Kitagaki et al., 1995). Prednisolone can inhibit Th1 cells as suggested from inhibition of IL-2 and IFN-γ in vitro; therefore, it is suggested that Th1-mediated response is involved in early swelling, which was inhibited by prednisolone, but not by JTP-27536. We also demonstrated in vivo that JTP-27536 significantly inhibited antigen-specific IgE production in a dose-dependent manner without affecting other antibodies. In contrast, prednisolone appeared to show significant inhibition on antigen-specific IgE, IgG1, and IgG2a, whereas it did not reduce IL-5 quantity in serum. These results are inconsistent with the observations that prednisolone inhibited IL-5 production much more strongly than IgE from mouse splenocytes or splenic B cells, respectively. As shown in Fig. 8, A and E, the evaluation range for
serum IL-5 in sham and vehicle treatment was much narrower than for IgE, which might have caused different sensitivity to their inhibitory effects. In addition, the inhibition of ear swelling by prednisolone at 5 mg/kg on day 35 is almost equivalent to that of JTP-27536 at 10 mg/kg, and neither showed significant inhibition in serum IL-5. Therefore, we speculate that prednisolone at 5 mg/kg was a critical dose for observing clear inhibition of serum IL-5 compared with that of serum IgE in vivo.

Histological evaluation of the TNCB-induced dermatitis model revealed drastic increase in eosinophils and mast cells in inflamed ear and significant effect of JTP-27536 on the infiltration of inflammatory cells. IgE not only activates mast cells but also basophils and eosinophils (Dombrowicz et al., 2000; Falcone et al., 2000; Hart, 2001) and produces cytokines and chemokines that can induce cell migration into inflamed tissues. IL-5 is involved in differentiation of bone marrow cells to eosinophils and induces activation and migration of eosinophils into inflammatory tissues (Sanderson, 1988). It is, therefore, suggested that the reduction of eosinophil and mast cell numbers in the inflamed tissue by JTP-27536 reflects IgE and IL-5 inhibition by JTP-27536.

We used suplatast tosilate and prednisolone as positive control drugs in our experiments in vitro and in vivo because these drugs have been used in the treatment of allergic diseases by inhibiting Th2 cytokines. A Th2 inhibitor, suplatast tosilate, was reported to inhibit IL-4 production from mouse D10G4.1 cells at 10 to 100 μM (Yanagihara et al., 1993); however, we did not observe obvious effect up to 100 μM in IL-4 production from mouse splenic B cells, whereas the inhibition was apparent at 1000 μM in human PBMCs in our experiment. This indicates that the activity of suplatast tosilate is not powerful in primary cells under our experimental conditions. Difference among cells and stimulations tested in the experiments may be the explanation for the discrepancy between the previous report and our experiment.

Steroids are currently the most effective treatment for allergic diseases, including asthma, allergic rhinitis, and atopic dermatitis, and high doses of oral corticosteroids can control the disease in almost every atopic patient. However, the dosages that can be given over long periods are limited due to systemic side effects in part through the mechanism of cytokine and antibody inhibition (Uesugi et al., 1996; Barnes, 1998). The inhibitory effect of prednisolone on cytokine production was strong enough, as we demonstrated in this paper; however, there is no selectivity in the inhibition of cytokines, such as IL-2 and IFN-γ, which may lead to immunosuppression. Prednisolone was also shown to inhibit IgE production without affecting IgG1 or IgM in mouse B cells. In contrast to those results, prednisolone accelerated production of IgE and IgM in hPBMC-derived B cells. It was reported that steroids accelerate antibody production by increasing CD11a expression on human monocytes, which leads to activation of B cells in antibody production through ICAM-1/LFA-1 interaction (Fischer and König, 1991). It was, therefore, speculated that prednisolone induced CD11a expression on the monocytes in our experiment, thereby causing increase in antibody production.

In conclusion, we demonstrated that JTP-27536 inhibited Igs, in particular IgE, and IL-5 in vitro and in vivo and had beneficial effects on allergic dermatitis in mice. Although the cytokine (IL-4) measured for human cells in this experiment was not enough to show the selectivity of JTP-27536 in humans, the results from mouse cells in vitro and the effect in mouse dermatitis model suggest that inhibitors of Igs and IL-5 like JTP-27536 are promising as new drugs for the treatment of allergic diseases, and, unlike prednisolone, do not cause immunosuppression.
Acknowledgments

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References


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JTP-27536, an Inhibitor of Immunoglobulins and IL-5 301