Suppression of IgE Production by IPD-1151T (Suplatast Tosilate), a New Dimethylsulfonium Agent: (1) Regulation of Murine IgE Response

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ABSTRACT—The effect of IPD-1151T, a new dimethylsulfonium compound, on the IgE response was investigated in the mouse system. The oral administration of IPD-1151T to immunized BALB/c mice suppressed the primary IgE antibody response and depressed the elevation of serum IgE levels, whereas the same treatment did not affect the IgG antibody response. The enhanced expression of low-affinity IgE receptor (FcεRI/CD23) on the spleen cells of immunized mice was also inhibited by IPD-1151T administration. It was further demonstrated from the adoptive transfer experiment that IPD-1151T, administered to hapten-primed B cell donors, but not to carrier-primed T cell donors, exerted its suppressive influence on the hapten-specific secondary IgE antibody response in irradiated syngeneic recipients. Interestingly, IPD-1151T concentration-dependently inhibited the production of interleukin 4 (IL-4) by D10G4.1, known to be a typical Th2 clone. However, IPD-1151T did not suppress the production of IgE and IgG1 by normal splenic B cells stimulated with lipopolysaccharide and IL-4. Moreover, IL-4-induced expression of FcεRI on normal spleen cells was not inhibited by the agent. These results strongly suggest that the IgE-suppressive activity of IPD-1151T is most likely due to the inhibition of IL-4 production at the T cell level.

Keywords: IgE production, IgE receptor (low affinity) (FcεRI/CD23), Interleukin 4, IPD-1151T (suplatast tosilate)

Allergic disorders such as bronchial asthma are mediated by allergen-specific IgE antibodies, and pathological conditions characterized by hyperproduction of IgE antibody exist in allergic patients. In early studies by several investigators (1–5), the IgE antibody response was demonstrated to be regulated not only by antigen-specific helper and suppressor T cells, but also by isotype-specific soluble factors having affinity for IgE. However, more recent studies have clearly indicated the presence of another pathway of IgE regulation. Indeed, T cell-derived interleukin 4 (IL-4) and interferon-γ (IFN-γ) were found to have reciprocal activity on the regulation of the IgE response in mice as well as in humans (6–8). IL-4 plays an essential role in the induction of IgE synthesis, whereas IFN-γ suppresses IL-4-induced IgE synthesis. In addition, IL-4 enhances the expression of low-affinity receptors for IgE (FcεRI/CD23) on B cells, but its enhanced expression is blocked by IFN-γ (9, 10). With regard to the T cell subsets responsible for the production of IL-4 or IFN-γ, Coffman and his group (11) have shown that in the mouse system, CD4+ T cell clones can be distinctly divided into two cell types (Th1 and Th2) on the basis of their pattern of cytokine production; IL-4 is produced by Th2 cells, and IFN-γ is produced by Th1 cells. However, it must be noted that the ability of IFN-γ to suppress the antibody responses is not restricted to the IgE isotype (6–8).

In spite of great progress made in elucidating the regulatory mechanisms of the IgE antibody response, none of the immunological manipulations have been successful for regulating the in vivo human IgE response. Nevertheless, several studies in animal models have shown that some synthetic compounds exert selective influences only on the IgE antibody response. For example, (+)-[2-[(3-ethoxy-2-hydroxypropoxy) phenylcarbamoyl]ethyl] dimethylsulfonium p-toluenesulfonate (IPD-1151T, suplatast tosilate, see structural formula in Fig. 1) was shown to selectively suppress the IgE antibody formation in mice (12). Interestingly, this drug was finally selected from the results of screening experiments with a series of dimethylsulfonium compounds in the search for IgE-suppressive
agents.

The purpose of the present study on the mouse system is to evaluate the IgE-suppressive activity of IPD-1151T and also to explore the regulatory mechanisms involved in the suppression of IgE antibody response by the agent. The present results indicate that IPD-1151T is a very unique drug effective in selectively suppressing the IgE antibody response, possibly by interfering with IL-4 production.

MATERIALS AND METHODS

Animals

Seven-week-old male BALB/c mice and male Wistar rats weighing about 250 g were purchased from Shizuoka Laboratory Animal Center. Male AKR/j mice aged 7–12 weeks were obtained from Seiwa Experimental Animal Center.

Drugs

IPD-1151T (Taiho Pharmaceutical Co.) is a white crystalline powder, highly soluble in water. This agent was dissolved in distilled water and culture medium for in vivo and in vitro use, respectively. Cyclophosphamide (CP) was purchased from Sigma.

Reagents

Mouse recombinant (r) IL-2, IL-4 and IFN-γ were purchased from Genzyme. Biotin-N-hydroxysuccinimide ester was obtained from E.Y. Laboratories, and phycoerythrin-conjugated streptavidin (PE-SA) was from Becton Dickinson. Hanks’ balanced salt solution (HBSS), RPMI 1640 and fetal calf serum (FCS) were obtained from Gibco. Lipopolysaccharide (LPS) from Salmonella typhimurium and cycloheximide (CH) were purchased from Sigma.

Antigens

Ovalbumin (OA), bovine serum albumin (BSA) and conalbumin were purchased from Sigma. OA and BSA were coupled with the 2,4-dinitrophenyl (DNP) group according to the method of Lee and Sehon (13), to produce GNP6-OA and DNP20-BSA. Crude ascaris extract coupled with the DNP group (DNP-As) was prepared by the method of Tada and Okumura (14).

Antibodies

Mouse monoclonal IgE antibody specific for the DNP hapten, biotinylated mouse monoclonal IgE (bi-IgE), two rat monoclonal anti-mouse IgE antibodies (HMK-12 and 6HD5) and anti-Thyl.2 antibody were the same preparations as previously described (15, 16). Two rat monoclonal anti-mouse IL-4 antibodies (BVD4-1D11, IgG2a and BVDG-24G2, IgG1) were obtained from Endogen. Affinity-purified goat anti-mouse IgG antibody was purchased from Cappel Laboratories. Affinity-purified goat anti-mouse IgG1 antibody was from Bethesda Research Laboratories. Horseradish peroxidase (HRP)-conjugated goat anti-rat IgG1 antibody was obtained from Bethyl Laboratories.

Primary IgE antibody response

BALB/c mice were immunized by i.p.-injection of 1 µg of OA absorbed on 2 mg aluminium hydroxide gel (alum), according to the method of Levine and Vaz (17).

Adoptive secondary IgE antibody response

BALB/c mice were immunized with 1 ug of either DNP-As or OA absorbed on alum. After 4 weeks, spleen cells were isolated by centrifugation on a Ficoll-metrizoate gradient. To obtain B cell-enriched fractions, spleen cells from DNP-primed mice were incubated with anti-Thy1.2 antibody for 60 min at 4°C, washed with HBSS, and then incubated for 45 min at 37°C in the presence of guinea pig complement, which had been thoroughly absorbed with normal mouse spleen cells. T cell-enriched fractions were obtained by removing the cells adherent to nylon wool. DNP-primed B cells (5 × 10⁶) were mixed with OA-primed T cells (5 × 10⁶), and the mixtures were transferred i.v. into each syngeneic recipient mouse previously exposed to 6 Gy irradiation. To completely eliminate the influence of the primary IgE antibody response, recipients were immunized by i.p.-injection of 10 µg DNP-OA alone.

Induction of in vitro IgE synthesis

This experiment was performed as described elsewhere (18). Briefly, splenic B cells (1 × 10⁶ cells/ml) from normal BALB/c mice were suspended in RPMI 1640 containing 10% FCS, 100 µg streptomycin, 100 U/ml penicillin, 5

Fig. 1. Chemical structure of (±)-2-[4-(3-ethoxy-2-hydroxypropoxy)phenylcarbamoyl]ethyl] dimethylsulfonium p-toluene-sulfonate (IPD-1151T, suplatast tosilate).
ity-purified goat anti-mouse IgG and IgG1 antibodies. The standard curve of each RIA was constructed by calculating the least-square lines; the sensitivity of the assay was 1 ng/ml for IgE, 10 ng/ml for anti-OA IgG antibody, and 2 ng/ml for IgG1.

Measurement of IL-4

IL-4 was quantitatively measured by an enzyme-linked immunosorbent assay (ELISA) which was newly established by us. Briefly, 96-well flat-bottom plates were coated with 100 µl of 1 µg/ml BVD4-1D11 at pH 9.6. After washing, the wells were incubated with 150 µl of diluted Block-Ace (Dainippon Pharmaceutical Co.) for 1 hr at 37°C. The plates were washed thoroughly, and a 100-µl sample was added to each well. The wells were incubated for 2 hr at 37°C, washed again, and then filled with BVDG-24G2. After incubation for 1 hr at 37°C, the wells were washed, and then incubated with HRP-conjugated goat anti-rat IgG1 antibody for 1 hr at 37°C. The washed wells were developed by the enzyme substrate (HRP-color development). Mouse rIL-4 was used as a standard, and the sensitivity of the assay was usually 0.1 ng/ml. This assay was not affected by the addition of mouse rIL-2, rIL-3, rIL-5, rIL-6 and rGM-CSF (data not shown).

RESULTS

Effect on in vivo IgE antibody response

Since IPD-1151T has been reported to be effective in selectively suppressing the IgE antibody response in mice immunized with DNP-As (12), a preliminary experiment was first performed to re-examine the IgE-suppressive activity of the drug using BALB/c mice immunized with OA. IPD-1151T (10 or 100 mg/kg/day) was administered p.o. for 14 days to immunized mice, and the serum levels

| Table 1 | Effect of IPD-1151T and CP on IgE and IgG antibody formation in BALB/c mice immunized with alum-absorbed OA |
|---------|-------------------------------------------------------------------------------------------------|
| Group   | Dose (mg/kg/day) | Anti-OA antibody              | Total IgE (µg/ml) |
|         |                   | IgE (PCA titer) | IgG (µg/ml)          |                                    |
| Normal  | <10                | <0.01          | 0.1                   |
| Control | 1280               | 4.6            | 2.9                   |
| IPD-1151T | 10   | 1280          | 5.5                  | 2.7                   |
|         | 100                | 320            | 5.3                  | 1.8                   |
| CP      | 20                 | <10            | 0.2                  | 0.2                   |

IPD-1151T and CP were given p.o and i.p. for 14 and 5 days after the immunization, respectively. Measurements were performed with the sera pooled from 3 mice sacrificed at day 14. Each value indicates the mean of duplicate assays. The mean coefficient of value was less than 10%.
of anti-OA IgE and IgG antibodies were determined at day 14 after the immunization. As shown in Table 1, IPD-1151T given at 100 mg/kg/day but not 10 mg/kg/day was effective in suppressing the IgE antibody response without affecting the IgG antibody response. Moreover, the elevation of serum total IgE levels was also depressed by the drug (100 mg/kg/day). In contrast to IPD-1151T, CP administered i.p. at 20 mg/kg/day for 5 days strongly suppressed the production of both IgE and IgG antibodies. We next investigated the effect of IPD-1151T on the generation of memory T and B cells by using an adoptive transfer system. In this experiment, OA-primed T cells were transferred i.v. together with DNP-primed splenic B cells into irradiated syngeneic recipient mice. Recipients were further boosted by i.p.-injection of DNP-OA alone in order to induce only the secondary DNP-specific IgE antibody response mediated by memory T and B cells. IPD-1151T was administered p.o. at 10 to 100 mg/kg/day either to DNP-primed B cell donor mice or to OA-primed T cell donors for 3 weeks after the immunization. As shown in Table 2, IPD-1151T, given at 50 or 100 mg/kg/day to donor mice of DNP-primed B cells, but not to those of OA-primed T cells, clearly suppressed the adoptive secondary anti-DNP IgE antibody response in recipients.

Effect on in vivo and in vitro Fc ε RII expression

We previously reported that immunization of BALB/c mice with alum-absorbed antigen induces a clear increase in the in vivo expression of Fc ε RII on splenic B cells and that this increase correlates well with elevation of serum IgE levels (16). To determine whether IPD-1151T inhibits the in vivo enhanced expression of Fc ε RII, BALB/c mice immunized with OA were orally administered IPD-1151T (10 or 100 mg/kg/day) for 14 days after the immunization, and spleen cells from normal and immunized mice were then analyzed for the Fc ε RII expression by means of single-color FACS analysis. One typical example out of three independent experiments is shown in Fig.

| Administration (mg/kg/day) | Anti-DNP IgE antibody (PCA titer) |
|---------------------------|----------------------------------|
| T cell donor | B cell donor | | |
| Vehicle | Vehicle | 80 |
| IPD-1151T | Vehicle | 80 |
| 10 | Vehicle | 80 |
| 50 | Vehicle | 80 |
| 100 | Vehicle | 80 |
| Vehicle | Vehicle | 80 |
| Vehicle | IPD-1151T | 10 |
| Vehicle | 50 | 20 |
| Vehicle | 100 | 20 |

BALB/c mice were immunized with either OA or DNP-As. After 4 weeks, OA-primed splenic T cells were transferred i.v. together with DNP-As-primed B cells into irradiated syngeneic recipient mice. Recipients were boosted by i.p. injection of DNP-OA alone. IPD-1151T was given p.o. to T or B cell donors for 3 weeks. Measurements were performed with the sera pooled from 5 mice sacrificed at 10 days after the adoptive transfer.

Fig. 2. Effect of IPD-1151T and CP on the induction of Fc ε RII expression in BALB/c mice immunized with alum-absorbed OA. IPD-1151T and CP were given p.o. and i.p. for 14 and 5 days after the immunization, respectively. The Fc ε RII expression on spleen cells pooled from 3 mice was analyzed at day 14 by single-color FACS.
2. Immunization with antigen obviously enhanced the fluorescence intensity of Fc ε RII+ cells, and it tended to augment the number of Fc ε RII+ cells (44.6±0.8% in normal mice versus 51.0±3.1% in immunized mice, mean±S.E. of three experiments). IPD-1151T at the dose (100 mg/kg/day) capable of suppressing the IgE antibody response induced a clear decrease in the enhanced fluorescence intensity of Fc ε RII+ cells in immunized mice, and it also showed a tendency to decrease the number of Fc ε RII+ cells (46.3±4.5%). CP having the suppressive activity on the formation of both IgE and IgG antibodies displayed the marked inhibition of the Fc ε RII induction when administered i.p. at 20 mg/kg/day for 5 days after the immunization. Note that CP also induced a significant decrease in the number of Fc ε RII+ cells (28.5±2.5%, P<0.05) and that this decrease was below the normal level.

We next tested whether IPD-1151T affects rIL-4-induced in vitro expression of Fc ε RII on normal spleen cells. As seen in Fig. 3, IPD-1151T at concentrations of 1 and 10 µg/ml showed no effect on rIL-4-induced Fc ε RII expression, indicating that this agent has no antagonistic action on IL-4. However, the drug at concentrations higher than 50 µg/ml induced non-specific inhibition in some experiments, including the cell proliferation mediated by B and T cell mitogens (data not shown).

**Effect on rIL-4-induced IgE synthesis in vitro**

To determine whether IPD-1151T acts directly on B cells, we further studied its effect on rIL-4-induced in vitro IgE synthesis by splenic B cells from normal BALB/c mice. In this experiment, normal B cells were stimulated with LPS for 24 hr, and the cells were then cultured with rIL-4 for 6 days. As summarized in Table 3, rIL-4-induced IgE production was not suppressed by 0.1 to 10 µg/ml of IPD-1151T even when added with LPS or rIL-4, indicating that this agent inhibits neither LPS-induced B cell activation nor IL-4-mediated IgE class switch in LPS-activated B cells. Moreover, IgG1 production by B cells stimulated with LPS and rIL-4 was also not suppressed by the agent. In a separate experiment, rIFN-γ (100 U/ml) completely abrogated the production of both IgE and IgG1 when added together with rIL-4 (data not shown).

**Effect on rIL-4 production in vitro**

D10G4.1, a Th2 clone specific to conalbumin, was employed to determine whether IPD-1151T inhibits IL-4 production.
production by T cells. Upon stimulation of D10G4.1 with the specific antigen in the presence of irradiated APC from AKR/j mice, the level of IL-4 in the culture supernatant amounted to approximately 0.6 ng/ml after 24 hr. As shown in Table 4, IL-4 production was inhibited in a concentration-dependent manner by 0.5 to 50 µg/ml of IPD-1151T, and significant inhibition was obtained with 5 and 50 µg/ml of the drug. On the other hand, CH (0.3 µg/ml), one of the protein synthesis inhibitors, completely inhibited IL-4 production by D10G4.1 stimulated with the specific antigen (less than 0.1 ng/ml), indicating that antigen-induced IL-4 production is not mediated by the passive release of preformed IL-4.

**DISCUSSION**

The data in the present study confirm the previous report (12) that IPD-1151T acts selectively on the IgE antibody response in mice immunized with DNP-As. Indeed, the oral administration of IPD-1151T to BALB/c mice immunized with alum-absorbed OA was effective in suppressing the primary IgE antibody response as well as in depressing the elevation of serum IgE levels. In contrast, the primary IgG antibody response was not affected by IPD-1151T. It is therefore clear that the suppression of antibody responses by the drug is IgE class-specific and antigen-nonspecific. The adoptive transfer experiment further demonstrates that IPD-1151T, administered orally to hapten-primed B cell donor mice, but not to carrier-primed T cell donors, has a suppressive influence on the secondary anti-hapten IgE antibody response in irradiated syngeneic recipients. These results indicate that IPD-1151T inhibits the generation of IgE-producing plasma cells as well as that of IgE memory B cells in immunized mice.

More interesting is the present observation that IPD-1151T also inhibits the in vivo enhanced expression of Fc ε RII on spleen cells from immunized mice. In two series of our previous experiments (15, 16), we reported that not only immunization with alum-absorbed antigen but also parasite infection induces a clear increase in the Fc ε RII expression on spleen cells of high IgE responder strains such as BALB/c mice. Such enhanced expression of Fc ε RII in immunized or infected mice was detected in B cells with B220, surface IgM and IgD, but not detected in T cells. In addition, the regulation of the Fc ε RII expression on B cells in immunized mice was shown to correlate well with the magnitude of the IgE response. For example, the suppressive factor for allergy (SFA), known to be one of the IgE class-specific suppressor factors derived from T cells (20), was demonstrated to inhibit the enhanced expression of Fc ε RII on spleen cells, without significantly depressing the number of Fc ε RII⁺ cells. Iso-type-nonspecific immunosuppressive drugs such as CP were also shown to induce a marked decrease not only in the Fc ε RII expression but also in the number of Fc ε RII⁺ cells. Since Fc ε RII has been identified as a B cell differentiation marker, CD23 antigen, constitutively expressed on mature B cells with surface IgM and IgD (21), a remarkable decrease in the number of Fc ε RII⁺ B cells may induce the nonspecific suppression of B cell development; this is especially likely in the mouse system where, in contrast to the human system with two isoforms of Fc ε RII (named a and b) (22), only one form of Fc ε RII, i.e., Fc ε RIIa, has been demonstrated by means of the cDNA cloning (23). Indeed, CP markedly depressed the number of Fc ε RII⁺ cells in immunized mice, and this depression was obviously below the normal level. In contrast to CP, IPD-1151T exerted its inhibitory influence on the enhanced but not constitutive expression of Fc ε RII. A similar result was obtained with SFA as previously reported (16). From these findings, it is strongly suggested that the specific suppression of the IgE response by IPD-1151T is fully associated with the decrease in the enhanced expression of Fc ε RII on B cells.

Evidence has been accumulated that T cell-derived IL-4 plays an essential role in the induction of Fc ε RII expression as well as the induction of IgE synthesis (6–10). IPD-1151T did not inhibit rIL-4-induced Fc ε RII expression on normal B cells, indicating that this agent has no directly antagonistic action on IL-4. Nevertheless, IPD-1151T showed a selective influence on the in vivo IgE antibody response in mice. Recent observations have demonstrated that IL-4 is required for the induction and maintenance of the IgE antibody response (24). Moreover, the reciprocal activity of IL-4 and IFN-γ has been well-documented both in mice and humans (6–8). However, it should be emphasized that the IgE-suppressive activity of IFN-γ is not restricted only to the IgE isotype. These findings give rise to the possibility that the selective suppres-

| Group  | Concentration (µg/ml) | IL-4 production (ng/ml) |
|--------|----------------------|-------------------------|
| Control |                      | 0.63±0.04               |
| IPD-1151T | 0.5                  | 0.52±0.04               |
|         | 5                    | 0.41±0.02*              |
|         | 50                   | 0.30±0.04*              |

Mixtures of D10G4.1 and irradiated APC were stimulated in the absence or presence of IPD-1151T with conalbumin for 24 hr. Measurement was performed by an ELISA system. Each value indicates the mean±S.E. of 5 cultures. *: Statistically significant difference from the control at P<0.01 (Student's t-test).
sion of the IgE response by IPD-1151T may be due either to the inhibition of IL-4-induced IgE class switching at the B cell level or to the inhibition of IL-4 production at the T cell level.

We first tested whether rIL-4-induced IgE production by LPS-activated B cells is suppressed by IPD-1151T. In this system, IPD-1151T was added to B cell cultures together with LPS or rIL-4. As a result, IPD-1151T did not affect the production of IgE and IgG1 by B cells stimulated with LPS and rIL-4, even when added with either LPS or with rIL-4. This result indicates that IPD-1151T has no ability to inhibit LPS-induced B cell activation and IL-4-mediated IgE class switching in LPS-activated B cells. Consistent with this result is the fact that IPD-1151T does not inhibit rIL-4-induced expression of FcεRII on B cells. Therefore, the possibility that B cells may be targets for the action of IPD-1151T has been excluded by these experiments.

We next studied whether IPD-1151T inhibits IL-4 production by T cells. Although an attempt was made to induce IL-4 production using antigen-stimulated spleen cells of immunized mice, it was not detectable in the culture supernatant (data not shown). On the other hand, studies with murine CD4⁺ T cell clones have clearly indicated that there exist two cell types (Th1 and Th2) on the basis of their pattern of cytokine production (11). Upon stimulation with specific antigen, Th1 clones produce IFN-γ, lymphotoxin and IL-2, whereas Th2 clones produce IL-4, IL-5 and IL-6. On the basis of these findings, we further investigated the effect of IPD-1151T on IL-4 production by D10G4.1, known to be a typical Th2 clone. As expected, IPD-1151T showed concentration-dependent inhibition of antigen-induced IL-4 production by D10G4.1. This in vitro finding strongly suggests that IPD-1151T acts at the T cell level. Therefore, the suppression of the in vivo IgE antibody response and FcεRII expression by IPD-1151T is most likely due to the inhibition of endogeneous IL-4 production by antigen-primed Th2 cells. However, this suggestion does not completely rule out another possibility that IPD-1151T may also act on APC, since soluble protein antigens are well-known to be recognized by T cells via T cell receptors in the context of major histocompatibility complex class II molecules (25). Further studies are required to elucidate the exact target cells for the action of IPD-1151T and to clarify the molecular mechanism by which IPD-1151T inhibits IL-4 production.

Taken collectively, the present study indicates that IPD-1151T is a very unique dimethylsulfonium agent capable of selectively suppressing the IgE antibody response in the mouse system. The data further demonstrate that whereas IPD-1151T shows no antagonistic action on IL-4, it has an inhibitory effect on IL-4 production by a Th2 clone. Therefore, the inhibition of IL-4 production at the T cell level is suggested to be responsible for the IgE-suppressive activity of IPD-1151T.

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