Sensitivity Difference to the Suppressive Effect of Prostaglandin E\(_2\) Among Mouse Strains: A Possible Mechanism to Polarize Th2 Type Response in BALB/c Mice

Etsushi Kuroda, Tsutomu Sugiura, Kazuya Zeki, Yasuhiro Yoshida and Uki Yamashita

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Mechanism to Polarize Th2 Type Response in BALB/c Mice

Etsushi Kuroda, Tsutomu Sugiura, Kazuya Zeki, Yasuhiro Yoshida, and Uki Yamashita

PGE₂ has been shown to play a prominent role in regulating Th1 and Th2 type responses. We studied the role of PGE₂ in IFN-γ production by Staphylococcus aureus Cowan I-stimulated spleen cells from several mouse strains such as BALB/c, C3H/HeN, and C57BL/6. When spleen cells were pretreated with indomethacin (cyclooxygenase (COX)-1 and COX-2 inhibitor) or NS-398 (COX-2-specific inhibitor), S. aureus Cowan I-induced IFN-γ production was increased more markedly in spleen cells from BALB/c mice than from C3H/HeN and C57BL/6 mouse. However, PGE₂ production was not significantly different among spleen cells from three mouse strains. When various concentrations of PGE₂ were exogenously added to spleen cells, PGE₂ showed a stronger suppressive effect on IFN-γ production in spleen cells from BALB/c mice than from other strains of mice. This suppressive effect of PGE₂ in BALB/c mice mainly depended on IL-12p70 production by APCs. More PGE₂ binding sites were found in BALB/c spleen cells than in C3H/HeN spleen cells, indicating that the sensitivity difference to the suppressive effect of PGE₂ was due to the difference of the number of PGE₂ receptors. The administration of NS-398 into BALB/c mice enhanced Ag-specific IFN-γ production, but not IL-4 production. This effect is the same as the IL-12 administration in vivo. From these results, we propose that the modulation of PGE₂ is important for Th1 activation via IFN-γ and IL-12p70 production in vitro and in vivo and that PGE₂ is one of the pivotal factors in the Th2-dominant immune response in BALB/c mice.

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Department of Immunology, University of Occupational and Environmental Health, School of Medicine, Kitakyushu, Japan

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Address correspondence and reprint requests to Dr. Uki Yamashita, Department of Immunology, University of Occupational and Environmental Health, School of Medicine, 1-1 Iseigaoka, Yabatani-shi, Kitakyushu, 807-8555 Japan. E-mail address: yama-uki@med.uoeh-u.ac.jp

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Immune responses are composed of two phases. First, innate immune responses are activated in response to infectious agents, and then acquired immune responses are induced. In BALB/c mice, both immune responses tend to induce Th2 responses. Namely, BALB/c mice produce lower IFN-γ in response to bacterial or parasite Ags at an early phase (innate immunity), and Ag-specific Th2 is preferentially developed both in vitro and in vivo (acquired immunity).

In this study, first we examined the effect of PGE₂ on IFN-γ production by Staphylococcus aureus Cowan I (SAC)-stimulated spleen cells from BALB/c, C3H/He, and C57BL/6 mice in vitro as a model of innate immune response. Next we examined the effect of a PGE₂ inhibitor on the development of Ag-specific Th1 and Th2 in vivo as a model of acquired immune response. We show evidence that PGE₂ plays an important role in the polarization to Th2 in BALB/c mice.

Materials and Methods

Mice

BALB/c, C3H/HeN, and C57BL/6 male mice, 7–8 wk old, were purchased from Seac (Ohita, Japan) and were maintained in our laboratory under a specific pathogen-free condition.

Preparation of cell

Spleen cell suspension was prepared and maintained in RPMI 1640 medium (Nissui Pharmaceutical, Tokyo, Japan) supplemented with 10 mM HEPES, pH 7.2, and 10% FBS (BioWhittaker, Walkersville, MD). For preparation of dis-herdherent cells, spleen cell suspension (1 × 10⁷/ml) was incubated in plastic culture dishes (Falcon 3002; Becton Dickinson, Franklin Lakes, NJ) at 37°C for 2 h. After incubation, nonadherent cells were discarded by gentle agitation with PBS. Adherent cells were obtained by scraping them off with a rubber policeman and using them as APCs. The purity of APCs was <5% IgM⁺ cells and <5% CD90⁺ cells, as determined by flow cytometry. Purified T cells were prepared by passing them through a nylon wool column (31). The purity of CD90⁺ cells was >90%. CD4⁺ T cells were further purified from nylon-purified T cells by magnetic cell sorting, according to the manufacturer’s procedure (Miltenyi Biotech, Bergisch-Gladbach, Germany). Depletion of B cells was performed by passing spleen cells through nylon wool columns, and spleen-adherent cells were added back as APCs.

In vitro culture of cells

In the standard culture, spleen cells (3 × 10⁶/ml) were cultured in 24-well culture plates (Falcon 3047) with or without 1 µM of indomethacin (Sigma, St. Louis, MO), 100 nM of NS-398 (Cayman Chemicals, Ann Arbor, MI), and indicated concentrations (0–100 nM) of PGE₂ (Sigma) for 12 h, and then they were stimulated with 0.05% SAC, Con A (10 µg/ml), or soluble anti-CD3 Ab (1 µg/ml) for additional 24 h. The culture supernatants were collected and used for ELISA, as described below. In the experiment to neutralize cytokines or delete NO, spleen cells were treated with indomethacin and 0 or 10 nM PGE₂ in the presence or absence of 1 µg/ml of anti-IL-4, anti-IL-10 (PharMingen, San Diego, CA), anti-TGF-β Ab (Genzyme, Cambridge, MA), or 250 µM of l-NMMA monomethyl arginine (l-NMMA; Sigma) for 12 h, and then stimulated with SAC for additional 24 h. In the experiment using fractionated cells, purified APCs (5 × 10⁶/ml) or T cells (2 × 10⁶/ml) were treated with indomethacin in the presence or absence of both 100 nM PGE₂ for 12 h, washed twice with PBS, mixed together, and then stimulated with SAC for additional 24 h. The percentages of IFN-γ production and the percentages of suppression of IFN-γ and IL-12 production were calculated as follows, and the detailed calculations are described in figure legends: % IFN-γ production = [(amounts of IFN-γ by agents or Ab-treated cells)/(amounts of IFN-γ by nontreated cells)] × 100; and IFN-γ (IL-12) suppression = [1 – (amounts of IFN-γ (IL-12) by agents and PGE₂-treated cells)/(amounts of IFN-γ (IL-12) by agent-treated cells)] × 100.

Statistics

All experiments were repeated at least three times, and some representative results are shown in tables and figures. Statistical analyses were performed between BALB/c mice and C3H/HeN or C57BL/6 mice using the Student’s t test. A confidence level of <0.05 was considered significant (33).

Results

Spleen cells from C3H/HeN, C57BL/6, and BALB/c mice have different sensitivity to PGE₂

At first, we investigated the effect of indomethacin (cyclooxygenase (COX)-1 and COX-2 inhibitor) and NS-398 (COX-2-specific inhibitor) on IFN-γ production by SAC-stimulated spleen cells from C3H/HeN, C57BL/6, and BALB/c mice in vitro. Indomethacin pretreatment increased IFN-γ production by spleen cells from all mouse strains when compared with nontreatment, but the degree of increase was different in the three strains of mouse. As shown in Fig. 1A, spleen cells from BALB/c mice showed 95% increase of IFN-γ production (2727 ± 47 to 5306 ± 106 pg/ml), while spleen cells from C3H/HeN and C57BL/6 mice showed only 30% increase (5445 ± 59 to 6916 ± 88 pg/ml and 4971 ± 194 to 6654 ± 461, respectively). These effects were not restricted to indomethacin alone. NS-398 pretreatment also increased IFN-γ production, and similar differences between BALB/c and C3H/HeN mice were observed (Fig. 1A). The amounts of IFN-γ produced are variable experiments to experiments, and BALB/c mice sometimes produce higher amount of IFN-γ than other strains do.
However, the percent increase of IFN-γ production by indomethacin or NS-398 is always higher in BALB/c mice than in other strains of mouse.

The different effects of indomethacin and NS-398 on IFN-γ production by spleen cells from three mouse strains may be due to differences in the production of PGE2 in each strain. We then examined PGE2 production by SAC-stimulated spleen cells from each mouse strain in vitro. As shown in Table I, however, there was no significant difference in PGE2 productions among three mouse strains. Indomethacin-treated spleen cells did not produce detectable amounts of PGE2 (data not shown).

Next, we investigated the effect of PGE2 on IFN-γ production by spleen cells among three mouse strains. Spleen cells from these mouse strains were preincubated with 0–100 nM of PGE2. Indomethacin was also added to inhibit the effect of endogenous PGE2, and then stimulated with SAC. The percentages of suppression of IFN-γ production were calculated and compared with cells pre-treated with indomethacin and 0 nM PGE2 and stimulated with SAC. As shown in Fig. 2A, spleen cells from BALB/c mice were

Table I. PGE2 production by spleen cells from C3H/HeN, C57BL/6, and BALB/c mice

<table>
<thead>
<tr>
<th></th>
<th>C3H/HeN (pg/ml)</th>
<th>C57BL/6 (pg/ml)</th>
<th>BALB/c (pg/ml)</th>
</tr>
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<tbody>
<tr>
<td>none</td>
<td>524 ± 4</td>
<td>6462 ± 353</td>
<td>180 ± 19</td>
</tr>
<tr>
<td>SAC</td>
<td>5716 ± 156</td>
<td>6677 ± 243</td>
<td>327 ± 12</td>
</tr>
</tbody>
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* Spleen cells (3 × 10^6/ml) from each mouse strain were cultured with or without SAC for 24 h and PGE2 in the culture supernatant was determined by EIA.
mice seems to be more markedly suppressed by endogenous PGE2 than those of other strains. We also performed the same experiments using NS-398 instead of indomethacin, and similar results were obtained (data not shown). These data indicate that spleen cells from BALB/c mice are highly sensitive to PGE2, as compared with those from C3H/HeN and C57BL/6 mice, although PGE2 is similarly produced in three mouse strains. Also, SAC-induced IFN-γ production in BALB/c mice seems to be more markedly suppressed by endogenous PGE2 than in other strains of mouse.

We also performed the same experiments using Con A and soluble anti-CD3 Ab instead of SAC. Stimulation with Con A or soluble anti-CD3 Ab produced a large amount of IFN-γ in all strains of mouse, but no significant difference was observed in the degree of indomethacin-induced increase (Fig. 1B) and PGE2-induced suppression of IFN-γ production (Fig. 2B) between C3H/HeN and BALB/c spleen cells.

**PGE2-mediated suppression of IFN-γ production in BALB/c mice is not due to other suppressive cytokines or NO**

Some other molecules such as IL-4, IL-10, and TGF-β and NO are known to exhibit an inhibitory effect on IFN-γ production the same as PGE2 (34–36). Moreover, since it has been reported that PGE2 enhances IL-10 production in activated macrophages (28), we examined whether the higher sensitivity of BALB/c spleen cells to PGE2 is mediated by other inhibitory cytokines and NO. Spleen cells from C3H/HeN and BALB/c mice were pretreated with indomethacin and 10 nM of PGE2 in the presence or absence of anti-IL-4, anti-IL-10, anti-TGF-β Ab, or L-NMMA, an inhibitor of NO synthesis, and then stimulated with SAC. The percentages of suppression of IFN-γ production were calculated and compared with cells pretreated with indomethacin and 0 nM of PGE2 in the presence or absence of Ab or L-NMMA and stimulated with SAC. As shown in Fig. 3, neutralization of inhibitory cytokines or inhibition of NO production has no effect on reversing the higher suppression of IFN-γ production by PGE2 in spleen cells from BALB/c mice. We also examined the amount of IL-4, IL-10, and NO production by PGE2-treated and nontreated spleen cells. IL-4 and NO were not detected in culture supernatants of treated or nontreated spleen cells after stimulation with SAC (IL-4:ELISA, NO:Griess method, data not shown). SAC-induced IL-10 production was 20% higher in nontreated cells than in PGE2-treated cells (data not shown). These results indicate that the higher sensitivity to the suppressive effect of PGE2 in BALB/c mice is not due to other suppressive molecules, and that PGE2 directly acts on spleen cells from BALB/c mice.

**Higher sensitivity of IFN-γ production to the suppressive effect of PGE2 in BALB/c mice is mediated by APCs**

PGE2 affects various cells such as T cells, B cells, and APCs. We investigated cell types responsible for the higher sensitivity to the suppressive effect of PGE2 in BALB/c spleen cells. B cell depletion from spleen cells had no effect on IFN-γ production (2082 ± 119 pg/ml spleen cells to 1875 ± 16.3 pg/ml B cell-depleted spleen cells in BALB/c mice). Furthermore, B cell-depleted spleen cells show similar indomethacin-induced enhancement (115.5% increase in spleen cells to 106.6% increase in B cell-depleted spleen cells in C3H/HeN mice, and 198.5% increase in spleen cells to 183.6% increase in B cell-depleted spleen cells in BALB/c mice) and PGE2-induced suppression of IFN-γ production (35% suppression in spleen cells to 33.3% suppression in B cell-depleted spleen cells in C3H/HeN mice, and 69.8% suppression in spleen cells to 62.4% suppression in B cell-depleted spleen cells in BALB/c mice). These results suggest that B cells play an important role in the IFN-γ production by spleen cells with SAC stimulation.

It has been reported that PGE2 suppresses IFN-γ production of T cells via the down-regulation of IL-12R on T cells and also suppresses IL-12 production of APCs (27, 28). We therefore examined whether the higher sensitivity to the suppressive effect of PGE2 in BALB/c spleen cells was due to T cells or APCs. As shown in Fig. 4, when both T cells and APCs were pretreated with 100 nM of PGE2, IFN-γ production was more clearly suppressed in spleen cells from BALB/c mice than those from C3H/HeN mice. When T cells were pretreated with PGE2 and mixed with nontreated APCs, there was no difference in the suppression of IFN-γ production between two mouse strains. However, when APCs...
were pretreated with PGE₂, IFN-γ production by T cells was suppressed in BALB/c mice, but not in C3H/HeN mice.

We also investigated the effect of exogenous addition of IL-12 in whole spleen cell cultures. Spleen cells from C3H/HeN and BALB/c mice were pretreated with indomethacin, IL-12, and 1 or 10 nM of PGE₂, and then stimulated with SAC. The percentages of suppression of IFN-γ production were calculated and compared with spleen cells pretreated with indomethacin, IL-12, and 0 nM of PGE₂, and stimulated with SAC. As shown in Fig. 5, the exogenous addition of IL-12 reversed the higher suppression of IFN-γ production by PGE₂ in spleen cells from BALB/c mice, and the percentages of suppression of IFN-γ production in the presence of IL-12 were the same in C3H/HeN and BALB/c mice. We also assessed the capacity of IFN-γ production by purified T cells stimulated with plate-coated anti-CD3 Ab and IL-12 in the presence of PGE₂, but no differences were observed in the suppression of IFN-γ production by T cells from C3H/HeN and BALB/c mice (19.6% suppression in C3H/HeN mice and 21.8% suppression in BALB/c mice). These data indicate that the capacity of IFN-γ production by T cells is down-regulated by PGE₂, but the higher suppressive effect of PGE₂ on IFN-γ production in BALB/c mice is not directed to T cells.

IL-12 appears to play a control in the production of IFN-γ by the splenic T and NK cells, because the neutralization of IL-12 by Ab reduces IFN-γ production by more than 90% (4064 ± 229 pg/ml by nontreatment to 213 ± 68 pg/ml by anti-IL-12 Ab treatment). We investigated IL-12 production by spleen cells from C3H/HeN and BALB/c mice. Pretreatment of spleen cells with indomethacin increased IL-12p70 production the same as IFN-γ production. As shown in Fig. 6A, there is significant difference between the degree of increase: 8% increase in C3H/HeN mice (170 ± 5 to 182 ± 3 pg/ml) and 51% increase in BALB/c mice (89 ± 2 to 135 ± 5 pg/ml). IL-12p40 production was also increased by the pretreatment with indomethacin in both mouse strains, but the degree of increase was not significantly different. Spleen cells from C3H/HeN and BALB/c mice showed about 21% and 19% increase, respectively (2602 ± 2 to 3158 ± 6 pg/ml in C3H/HeN mice, and 1870 ± 6 to 2024 ± 6 pg/ml in BALB/c mice).

Next, we assessed the sensitivity of IL-12 production to PGE₂. Spleen cells from C3H/HeN and BALB/c mice were preincubated with indomethacin and 0–100 nM of PGE₂, and then stimulated with SAC. The percentage suppression of IFN-γ (Fig. 6B), IL-12 (Fig. 6C), and IFN-γ (Fig. 6D) production were calculated as follows: % suppression = [(amounts of cytokines by indomethacin-treated cells)/ (amounts of cytokines by indomethacin and PGE₂-treated cells)] × 100. * Significant different from other strain.
FIGURE 7. BALB/c spleen cells have more PGE₂ binding sites than C3H/HeN. Ten million spleen cells from each mouse strain were incubated with various concentrations of [³H]PGE₂ ranging from 0.25 to 10 nM in the presence or absence of 1000-fold amount of unlabeled PGE₂ at 15°C for 45 min. After washing, cell-bound radioactivities were counted. A. Saturation curve for [³H]PGE₂ binding of spleen cells. B. Scatchard plot analysis.

Cox-2-specific inhibitor, NS-398, serves as enhancer for development of Th1 in BALB/c mice in vivo

Because our in vitro experiments indicated that PGE₂ played an important role for IFN-γ and IL-12 production in BALB/c spleen cells, we investigated the effect of NS-398 and PGE₂ on IFN-γ production in vivo. BALB/c mice were administered with PBS, PGE₂, or NS-398 by i.p. injection during the immunization of KLH. Spleen cells from immunized mice were then stimulated with KLH in vitro, and Ag-specific IFN-γ and IL-4 productions were examined. Spleen cells from PGE₂-administered mice produced IFN-γ about 67% lower than those from control mice, while IL-4 production was the same between PGE₂-administered and PBS-administered mice (data not shown). In contrast with the administration of PGE₂, spleen cells from NS-398-administered BALB/c mice produced IFN-γ about 2-fold higher than control mice did (Fig. 8A). However, as in the case of PGE₂-administered mice, IL-4 production was the same as compared with control. Administration of IL-12 is a major and the most effective Th1-activating method. We then compared NS-398-administered mice with IL-12-administered mice from the standpoint of the efficiency of Ag-specific IFN-γ production. As shown in Fig. 8A, spleen cells from NS-398-administered BALB/c mice produced higher amounts of Ag-specific IFN-γ similar to IL-12 administration. However, NS-398 administration had no effect on Ag-specific IL-4 production. The enhanced production of Ag-specific IFN-γ in NS-398-administered mice was due to activated Th1, because CD4⁺ T cell fraction produced increased amount of IFN-γ (Fig. 8B). We also tried the same experiment using indomethacin, but an enhanced development of Th1 was not observed in indomethacin-administered mice (data not shown). From these data, we suggest that the modulation of PGE₂ plays a pivotal role in Th1/Th2 balance in vivo, and the administration of NS-398, like IL-12 administration in BALB/c mice, is useful as an enhancer of IFN-γ production.

IL-10 also shows suppressive effect on IFN-γ production of BALB/c spleen cells, but keeps balance between production and sensitivity

Because IL-10 is a representative cytokine for suppressing IFN-γ and IL-12 production (27, 28, 30), we examined whether IL-10 had similar effect on IFN-γ production between C3H/HeN and BALB/c spleen cells. Anti-IL-10 Ab pretreatment increased IFN-γ production, but the degree of increase was similar between C3H/HeN and BALB/c spleen cells, which is different from the result of indomethacin treatment (Fig. 9). However, IL-10 production by SAC-stimulated spleen cells from BALB/c mice was about one-tenth of that from C3H/HeN mice (Table III). From these results, we suggest that BALB/c spleen cells have higher sensitivity to IL-10, just like PGE₂, but “the balance between production of and

Table II. Both T cells and APCs of BALB/c mice have higher PGE₂ binding capacity than those of C3H/HeN mice

<table>
<thead>
<tr>
<th>Cells</th>
<th>Expt. 1 C3H/HeN</th>
<th>Expt. 1 BALB/c</th>
<th>Expt. 2 C3H/HeN</th>
<th>Expt. 2 BALB/c</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spleen cells (whole)</td>
<td>1.11</td>
<td>1.79</td>
<td>1.57</td>
<td>2.15</td>
</tr>
<tr>
<td>Dish adherent cells</td>
<td>1.03</td>
<td>3.05</td>
<td>0.83</td>
<td>2.03</td>
</tr>
<tr>
<td>Nylon purified T cells</td>
<td>0.68</td>
<td>1.75</td>
<td>1.59</td>
<td>2.49</td>
</tr>
</tbody>
</table>

* Spleen cells, T cells, and APCs (10⁷) from each mouse strain were incubated with 5 nM [³H]PGE₂ in the presence or absence of 1000-fold amount of unlabeled PGE₂ at 15°C for 45 min. After washing, cell-bound radioactivities were counted.

12p70 (Fig. 6C), and IL-12p40 (Fig. 6D) production was calculated and compared with cells pretreated with indomethacin and 0 nM PGE₂ and stimulated with SAC. Bioactive IL-12p70 production by BALB/c spleen cells was highly sensitive to the suppressive effect of PGE₂, which corresponds to that of IFN-γ. There was no significant difference in the suppression of IL-12p40 production between two mouse strains. These data indicate that the higher sensitivity of IFN-γ production to the suppressive effect of PGE₂ in BALB/c mice is mediated by APCs; especially bioactive IL-12 production by APCs plays a key role in BALB/c mice.

BALB/c spleen cells have more PGE₂ binding sites than C3H/HeN and C57BL/6 spleen cells

Because spleen cells from BALB/c mice showed a higher sensitivity to the suppressive effect of PGE₂ on IFN-γ and IL-12p70 production, we investigated the binding sites of [³H]PGE₂ on spleen cells from three mouse strains. Fig. 7A showed dose-response curves of the binding of [³H]PGE₂ on spleen cells from C3H/HeN and BALB/c mice. From Scatchard plot analysis, the binding sites of [³H]PGE₂ on spleen cells from C3H/HeN and BALB/c mice were estimated as 131 and 201 molecules per cell, respectively. The Kd values were estimated as 1.40×10⁻⁹ M in C3H/HeN mice and 1.56×10⁻⁹ M in BALB/c mice, respectively (Fig. 7B). The [³H]PGE₂ binding to spleen cells from C57BL/6 mice was similar to that from C3H/HeN mice, and the binding sites were 144 molecules per spleen cells and the Kd value was 1.37×10⁻⁹ M (Scatchard analysis not shown). We also assessed the binding capacities of [³H]PGE₂ on purified T cells and APCs (Table II). The specific binding of PGE₂ to APCs was about 3-fold higher in BALB/c mice than in C3H/HeN mice. The binding of PGE₂ to T cells was also about 2-fold higher in BALB/c mice. These data indicate that spleen cells from BALB/c mice, especially APCs, have more binding sites than ones from other strains, but the binding affinity is almost similar among three mouse strains.
sensitivity to IL-10" is maintained in both mouse strains concerning the suppression of IFN-\(\gamma\) production, which are different from that of PGE\(_2\).

**Discussion**

Immune responses of BALB/c mice have been mysterious for a long time. Many reports have indicated that Th2 responses are preferentially induced by infections or immunizations in BALB/c mice (9–23). The mechanisms of this phenomenon are thought to be due to a higher production of IL-4 or lower productions of IL-12 and IFN-\(\gamma\) in BALB/c mice than other mouse strains. This hypothesis is based on evidence that the immune response in BALB/c mice can be shifted to Th1 by the neutralization of IL-4 or the administration of IL-12 at the initial phase of infections or immunizations the same as C3H/HeN and C57BL/6 mice. There is a report that T cells bearing V\(\alpha\)8V\(\beta\)4 TCR rapidly produce IL-4 by the stimulation with Leishmania homologue of receptors for activated C kinase of L. major in BALB/c mice, and this early burst of IL-4 plays an essential role for instructing the subsequent Th2 differentiation and consequently becomes susceptible to L. major infection (11). However, the preferential activation of Th2 and the failure of Th1 activation in BALB/c mice are induced not only in L. major infection but also in infections of several pathogens, and immunizations with CFA as an adjuvant also fail to induce Th1 responses in BALB/c mice (13–23). Moreover, there is a report that in the infection of Nippostrongylus braziliensis, which generally induces strong Th2 responses, BALB/c mice show a more excessive Th2 response than C57BL/6 mice do (37). From these findings, we considered that the preferential activation of Th2 in BALB/c mice was not only due to the activation of V\(\alpha\)8V\(\beta\)4 T cells.

Another mechanism of Th2 polarization in BALB/c mice may be the reduced response of naive Th to IL-12 (23). When naive Th is exposed to IL-4, they lose IL-12 R\(\beta\)2 chain and subsequently differentiate to Th2 (38–41). However, the down-regulation of IL-12 R\(\beta\)2 expression is recovered by the addition of IL-12 itself or IFN-\(\gamma\) (12). Thus, IL-12 and IFN-\(\gamma\) are important to the maintenance of IL-12 responsiveness and the suppression of Th2 differentiation. Accordingly, we consider that the lower production of a report that T cells bearing V\(\alpha\)8 V\(\beta\)4 TCR rapidly produce IL-4 by the stimulation with Leishmania homologue of receptors for activated C kinase of L. major in BALB/c mice, and this early burst of IL-4 plays an essential role for instructing the subsequent Th2 differentiation and consequently becomes susceptible to L. major infection (11). However, the preferential activation of Th2 and the failure of Th1 activation in BALB/c mice are induced not only in L. major infection but also in infections of several pathogens, and immunizations with CFA as an adjuvant also fail to induce Th1 responses in BALB/c mice (13–23). Moreover, there is a report that in the infection of Nippostrongylus braziliensis, which generally induces strong Th2 responses, BALB/c mice show a more excessive Th2 response than C57BL/6 mice do (37). From these findings, we considered that the preferential activation of Th2 in BALB/c mice was not only due to the activation of V\(\alpha\)8V\(\beta\)4 T cells.

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**FIGURE 8.** NS-398 serves as an enhancer for Th1 development in BALB/c mice in vivo. A, BALB/c mice were immunized with 50 µg of KLH in CFA by i.m. injection twice at a 7-day interval. PBS, IL-12 (250 ng), or NS-398 (5 or 50 nmol) was administered by i.p. injection into mice 3 h before and 1 and 2 days after the first immunization. Fourteen days later, spleen cell suspensions (5 × 10⁶/ml) were prepared and cultured with KLH for 48 h, and IFN-\(\gamma\) and IL-4 in the culture supernatant were determined by ELISA. B, Purified CD4⁰ T cells (1 × 10⁶/ml) from immunized mice were cultured with KLH in the presence of APCs (5 × 10⁶/ml) from nonimmunized mice for 48 h, and IFN-\(\gamma\) in the culture supernatant was determined by ELISA. *, Significantly enhanced from PBS group.

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**FIGURE 9.** Effect of IL-10 depletion on IFN-\(\gamma\) production by spleen cells from C3H/HeN and BALB/c mice. Spleen cells (3 × 10⁶/ml) from each mouse strain were treated with indomethacin (INDO) or anti-IL-10 Ab for 12 h, then stimulated with SAC for additional 24 h, and IFN-\(\gamma\) in the culture supernatant was determined by ELISA. The results are expressed as percentage of IFN-\(\gamma\) production, which was calculated as follows. % IFN-\(\gamma\) production = [amounts of IFN-\(\gamma\) by indomethacin or anti-IL-10 Ab-treated cells] / [amounts of IFN-\(\gamma\) by nontreated cells] × 100. The amounts of IFN-\(\gamma\) by agent- or Ab-nontreated cells stimulated with SAC are used as 100% control. *, Significantly different from other strain.

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**Table III.** SAC-stimulated IL-10 production is lower in BALB/c mice than C3H/HeN mice

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<thead>
<tr>
<th></th>
<th>C3H/HeN</th>
<th>BALB/c</th>
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</thead>
<tbody>
<tr>
<td>IL-10 production (pg/ml)</td>
<td>22.3 ± 11</td>
<td>911 ± 86</td>
</tr>
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* Spleen cells (3 × 10⁶/ml) from each mouse strain were stimulated with SAC for 24 h, and IL-10 in the culture supernatant was determined by ELISA. ND, not detected.

b Significantly lower than that of other strain.
IL-12 and IFN-γ is important in Th2 activation in BALB/c mice in the same way as early and overproduction of IL-4. In this study, we investigated the immunological functions of BALB/c mice from the standpoint of the modulation of IL-12 and IFN-γ production.

Many effector molecules have been reported to suppress the production of IL-12 and IFN-γ, such as IL-4, IL-10, TGF-β, NO, PGs, and corticosteroids (24–27, 34, 35). Especially, PGE2 is one of the most important factors for Th2 responses (25, 26, 28, 29). However, to our knowledge, there has been no report concerning PGE2 as the most important factors for Th2 responses (25, 26, 28, 29). How-ever, to our knowledge, there has been no report concerning PGE2 and Th2 responses in BALB/c mice, because no significant difference was observed in the amount of PGE2 produced in BALB/c and other mouse strains. We also found no significant difference in the amount of PGE2 produced from spleen cells stimulated with SAC among these three mouse strains (Table I). However, we did find that the inhibition of PGE2 synthesis by indomethacin resulted in a marked production of IL-12p70 and IFN-γ in BALB/c mice, but not in C57BL/6 and C3H/HeN mice, suggesting that BALB/c mice are highly sensitive to the suppressive effect of PGE2 (Figs. 1A and 2A). The enhancement of IFN-γ production in BALB/c mice was also found by the pretreatment with COX-2-specific in-hibitor, NS-398 (Fig. 1A). This result indicates that the COX-2-mediated PGE2 suppresses IFN-γ more effectively in BALB/c mice than the constitutive PGE2 production mediated by COX-1 does.

The target cells of the suppressive effect of PGE2 seem to be APCs in BALB/c mice (Fig. 4). PGE2 also affected IFN-γ production by T and NK cells (25, 27). However, in our experimental system, the pretreatment of T and NK cells with PGE2 did not show any significant difference in the suppression of IFN-γ production between C3H/HeN and BALB/c mice, and an exogenous addition of IL-12 reversed the suppression of IFN-γ production by PGE2 in only BALB/c mice (Fig. 5). Moreover, when purified T cells were stimulated with plate-coated anti-CD3 Ab and IL-12 in the presence of PGE2, the degree of suppression of IFN-γ production was the same between C3H/HeN and BALB/c mice. These data suggest that the susceptibility of IFN-γ-producing T cells to PGE2 is the same between BALB/c and C3H/HeN mice. On the other hand, IL-12p70 production by APCs, which corresponds to IFN-γ production, is more markedly suppressed by PGE2 in BALB/c mice than in C3H/HeN mice (Fig. 6). We also found that spleen cells, T cells, and APCs from BALB/c mice had more PGE2 binding sites than those of C3H/HeN mice (Fig. 7 and Table II). These data indicate that the higher sensitivity of IFN-γ production by T and NK cells to PGE2 in BALB/c mice is caused by higher sensitivity of IL-12 production by APCs to PGE2.

When spleen cells were stimulated with Con A or anti-CD3 Ab instead of SAC, there was no significant difference in PGE2-induced suppression of IFN-γ production and indomethacin-induced enhancement of IFN-γ production between spleen cells from BALB/c and C3H/HeN mice (Figs. 1B and 2B). T cell mitogen also stimulates APCs to produce IL-12 via interaction with T and APCs (42). However, SAC and other bacterial Ags are known to stimulate directly APCs, and spleen cells stimulated with Con A produced only 80% lower PGE2 than SAC stimulation (data not shown). Thus, we consider that this direct APC stimulation is more sensitive to PGE2 than indirect APC stimulation in our experimental system. In BALB/c mice, the failure of Th1 activation is generally observed in intracellular parasites or bacteria infections and immunizations with CFA, which contains dead mycobacterium. APCs seem to be directly stimulated in vivo by these infections and immunizations. We consider that IL-12 is not fully produced by the direct stimulation of APCs, and Th2 are preferentially dif-ferentiated in BALB/c mice. A similar result has been reported regarding bacillus Calmette-Guérin infection, that is, the failure of Th1 activation in BALB/c mice is due to the failure of IL-12 production by macrophages, while the capacity of IFN-γ production is the same in BALB/c and B10D6 mice (43).

Several reports suggest that macrophages and dendritic cells play an important role for the determination of Th1/Th2 differentiation. Corticosteroids-treated macrophages preferentially stimulate Th2 by inhibiting IL-12 production, and human dendritic cells that are differentiated by GM-CSF and IL-4 in the presence of PGE2 produce no IL-12 and preferentially stimulate Th to produce type II cytokines (44–46). There is also a report that the amount of IL-12 and PGE2 derived from APCs determines the IFN-γ level of human Th cells (30). In this way, the character of APC seems to play an important role for the determination of Th1/Th2 responses. Thus, APCs from BALB/c mice have tendency of higher sensitivity to PGE2 and preferentially activate Th2 via insufficient production of IL-12.

The major inhibitory molecules, such as IL-4, IL-10, and TGF-β and NO, inhibit IFN-γ production the same as PGE2 (34–36). Moreover, it has been reported that PGE2 enhances IL-10 production in activated macrophages (28, 48). IL-10 itself is not a direct Th2 inducer, but is known as a strong suppressor of IFN-γ production, while IL-4 and NO are suppressors for Th1 differentiation (35, 36, 47). We assessed the role of other inhibitory molecules in this system, but anti-IL-4, anti-IL-10, and anti-TGF-β Abs and 1-NNMA did not recover the suppressed IFN-γ production by PGE2 in BALB/c and C3H/HeN mice (Fig. 3). These results suggest that the suppressive activity of PGE2 is not mediated by other inhibitory molecules.

We obtained interesting findings regarding IL-10. BALB/c mice showed high sensitivity to IL-10 in the same way as PGE2. However, different from PGE2, the amount of IL-10 was 90% lower in BALB/c mice than in C3H/HeN mice, and the enhancement of IFN-γ production by the neutralization of IL-10 was similar between two mouse strains. From these results, we suggest that “the balance between production of and sensitivity to IL-10” is maintained in both mouse strains, but not of PGE2 (Fig. 9 and Table III). We did not investigate whether these balances were maintained or impaired in other suppressive molecules (IL-4, TGF-β, and NO) and type 1 cytokines (IL-12 and IFN-γ), and there is a possibility that “the balance between production of and sensitivity to” is impaired in some cytokines in the same way as PGE2 in some immunological diseases. One suggestion that we want to emphasize in this study is that “the balance between production of and sensitivity to,” rather than the amount of production, is one of the most important factors in determining the level of immune responses.

We obtained marvelous results that the administrations of NS-398 in vivo induced the activation of Th1 the same as the administration of IL-12. These results further indicate that PGE2 works as a Th1 suppressor in BALB/c mice (Fig. 8). However, the administration of indomethacin instead of NS-398 did not enhance Ag-specific IFN-γ production (data not shown). We suspect that indomethacin inhibits not only COX-2, but also COX-1, and a constitutive production of PGE2 by COX-1 may need to maintain the activation of several cell types, because PGE2 produced by COX-1 serves to maintain the homeostasis and is produced by a wide variety of cell types to regulate a broad range of physiological activities. However, in vitro system, we handled only immune systems that are easily affected by indomethacin, and different from in vivo system. We also investigated the effect of PGE2.
in vivo. When PGE₂ is administered at the initial phase of immunization, Ag-specific IFN-γ production was about 67% lower than that of the control (data not shown). These results indicate that Th1 development can be regulated by the modulation of PGE₂ production.

Another arachidonic acid derivatives, such as leukotriene (LT), also may play some roles in the regulation of IFN-γ production. Cyclooxygenase and lipoxygenase catalyze arachidonic acid to synthesize PGs or LTs, respectively. There is a possibility that COX inhibitors increase LT synthesis because COX inhibitors shut off the stream of arachidonic acid to PGs and shift to the lipoxygenase stream. It is reported that LT₄ up-regulates IL-1, IL-2, and IFN-γ production and enhances NK cell activity (49). These effects of LT₄ are just opposite of that of PGE₂. We did not examine the LT₄ production by spleen cells treated with COX inhibitor or PGE₂. However, it is interesting to investigate the relationship between Th1/Th2 development and LT₄, and cross-regulation of PGE₂ and LT₄ by APCs.

In conclusion, we did find that IFN-γ and IL-12 productions of BALB/c mice were highly sensitive to the suppressive effect of PGE₂ as compared with C3H/He and C57BL/6 mice, while the production of PGE₂ was the same among three strains of mouse. The main target of PGE₂-induced suppression seems to be on APCs in BALB/c mice. BALB/c spleen cells have more PGE₂ binding sites than those of other mouse strains. These results suggest that PGE₂ plays an important role to polarize Th2 type response in BALB/c mice. As discussed above, we want to emphasize that “the balance between production of and sensitivity to” is an important factor for the determination of the level of immune responses. The disturbance of “the balance between production of and sensitivity to” in some cytokines should be investigated in some immunological diseases.

References


