FK-506 and Cyclosporin A Potentiate the IgE Antibody Production by Contact Sensitization with Hapten in Mice

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ABSTRACT

Five repeated topical applications of 2,4-dinitrofluorobenzene to the ears of BALB/c mice resulted in contact dermatitis on the ears as well as significant elevation in dinitrophenol-specific IgE antibody and total IgE in the serum. FK-506 and cyclosporin A inhibited the development of contact dermatitis in terms of skin thickness and histopathological changes of skin lesions. On the contrary, these two drugs potentiated dinitrophenol-specific and total IgE antibody production without affecting IgG and IgM levels in serum. The expression of interferon-γ mRNA in reverse transcriptase-polymerase chain reaction in the ear was inhibited by FK-506 and cyclosporin A. The expression of interleukin-4 mRNA, germline C\(\gamma\) and productive C\(\gamma\) in the auricular lymph node was not affected by these two drugs. Contrary to the above findings, the immunosuppressors, FK-506 and cyclosporin A, inhibited the production of interferon-γ and interleukin-2 by cultured Th1 cells (1E10.H2 cells) and of interleukin-4 and -5 by Th2 cells (D10.G4.1 cells) in vitro. These results indicated that FK-506 and cyclosporin A selectively inhibited the Th1 cell-mediated contact dermatitis and potentiated the Th2 cell-mediated IgE antibody production in vivo. This potentiation is probably due to the down-regulation of interferon-γ production by Th1 cells after the treatment with these drugs. However, because FK-506 and cyclosporin A inhibited the production of cytokines by both Th1 and Th2 cells in vitro and these two immunosuppressors showed higher selectivity toward inhibiting Th1 cell-mediated reactions by limitations in vivo experiments.

FK-506 and cyclosporin A are novel immunosuppressors derived from fungi. These two agents inhibit T cell proliferation, cytotoxicity and cytokine production without destroying cells (Kino et al., 1987; White, 1982). Their action is mainly based on the inhibition of calcium/calmodulin-activated protein phosphatase, calcineurin (Liu et al., 1991). Because a calcium/calmodulin-activated calcineurin function is required to activate the cytoplasmic component of the transcription NF-AT, both agents block the NF-AT activation and transcription of many cytokine genes (McCaffrey et al., 1991). This net result of the action is that these two immunosuppressors interfere with T cell activation (Dumont et al., 1990; Lin et al., 1991).

There are several different types of T cells, with a variety of functions. One group interacts with B cells or mononuclear cells to help them produce antibody or destroy intracellular pathogens. This group of cells is called Th cells. Th cells can be divided into different subsets depending on their cytokine profile. Th1 cells produce IL-2 and IFN-γ but not IL-4 and IL-5 and are chiefly responsible for the delayed-type hypersensitivity response (Cher and Mosmann, 1987; Mosmann et al., 1986). They can also help B cells to produce IgG2a but not much IgG1 and IgE, in the mouse. On the contrary, Th2 cells produce IL-4 and IL-5 but not IL-2 and IFN-γ. They are efficient helper cells for antibody production, especially IgG1 and IgE.

Regarding the production of IgE, IL-4 and IL-13 play an important role under the regulation with IFN-γ. Fuleinham et al. (1995) reported that cyclosporin A suppressed the T cell-dependent IL-4-derived IgE synthesis in human lymphocytes. In addition, some other investigators reported that suppressive effect of cyclosporin A on the production of IgE (Toorenbergen et al., 1996; Vendeville et al., 1995; Puignero et al., 1995). On the contrary, there are some reports to indicate the potentiation of IgE production by immunosuppressors including cyclosporin A and FK-506 (Bundick et al., 1995; Wheeler et al., 1995; Chang et al., 1993; Chen, 1988; Wang et al., 1993). Because IgE is the most important antibody in the development of allergic reaction and some immunosuppressors including FK-506 and cyclosporin A are applied to treat some allergic diseases, such as bronchial asthma and atopic dermatitis, it is important to clarify the

ABBREVIATIONS: DNFB, dinitrofluorobenzene; DNP, dinitrophenol; NF-AT, nuclear factor of activated T cells; IL, interleukin; IFN, interferon; Th, T helper; BSA, bovine serum albumin; ELISA, enzyme-linked immunosorbent assay; T-PBS, phosphate buffered saline containing 0.2% Tween; RT-PCR, reverse transcriptase-polymerase chain reaction; PMA, phorbol myristate acetate, CMC, carboxymethylcellulose.

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effect of FK-506 and cyclosporin A on IgE antibody production in terms of the production of cytokines. We demonstrated that contact dermatitis is mainly mediated by Th1 cells and that IgE antibody production is mediated by Th2 cells. Our study was therefore conducted to study the effect of FK-506 and cyclosporin A on Th2 cell-mediated IgE production and Th1 cell-mediated contact dermatitis by contact sensitization with DNFB in mice in vivo. In addition, we examined the effect of these drugs on the activation of Th1 and Th2 cells in vitro.

Materials and Methods

Animals. Female BALB/c mice (7-10 wk old) obtained from Japan SLC, Inc. (Hamamatsu, Japan), were housed in plastic cages in an air-conditioned room at 24°C, fed a standard laboratory diet and given water ad libitum. All experiments were carried out following a guideline for the care and use of experimental animals made by the Japanese Association for Laboratory Animal Science in 1987.

Reagents and antibodies. FK-506 and cyclosporin A were provided by Fujisawa Pharmaceutical Co., Ltd., Osaka, Japan and Sandou Japan Co., Ltd., Tokyo, Japan, respectively. FK-506 was suspended in 0.5% sodium carboxymethylcellulose saline solution and orally administered three times a week for 5 wk. Cyclosporin A was dissolved in myglyol and administered orally three times a week for 5 wk. DNFB was purchased from Nacalai Tesque Inc., Kyoto, Japan. Monoclonal anti-mouse IgE antibody (LO-ME-3, Serotek Co., Ltd., Oxford, UK), monoclonal anti-DNP IgE antibody (Clone SPE-7, Sigma Chemical Co., Ltd., St. Louis, MO), peroxidase-conjugated streptavidin (Dakopatts a/s, Glostrup, Denmark), (for ELISA grade, Sigma Chemical Co., Ltd., St. Louis, MO), immunoplates were coated by incubating overnight at 4°C overnight. The plates were blocked with PBS containing 1% BSA and M, immunoplates were coated with monoclonal anti-IgE, goat anti-mouse IgG or goat anti-mouse IgM antibody, respectively. The plates were blocked as described above and washed three times with T-PBS. Standard curves were generated as described above by employing standard IgE (monoclonal anti-DNP IgE, clone SPE-7, Sigma), standard mouse IgG (Miles Scientific, Napervilla, IL) and standard IgM (Miles). A total of 100 μl of serum sample was added to the each well and incubated at room temperature for 1 hr. ELISA was performed using peroxidase-conjugated anti-mouse IgE, IgG and IgM. The ELISA data compared with the standards added to each plate were analyzed out using the DELTA Soft program for the Macintosh computer. The sIgE, tIgE, tIgG and tIgM titers are expressed in μg/ml based on laboratory-generated standards and appropriate commercial standards.

Analysis of cytokine mRNA expression in mouse auricular lymph nodes and ears by RT-PCR. Changes in cervical lymph node and ear-derived cytokine mRNA levels were assessed by the RT-PCR using a thermal cycler (Bio Metra Trio-Thermoblock, Bio Metra Co., Ltd., Gottingen, Germany). Using ISOGEN, total RNA was prepared from the ears and lymph nodes of the mice 4 hr after being painted five times with either vehicle or DNFB. The amount of total RNA in each sample was measured spectrophotometrically at a wavelength of 260 nm and the quality of the RNA was checked by electrophoresis. RT-PCR was performed with following conditions: a total of 500 ng of the reverse-transcribed RNA was amplified by 30 cycles. Each well was amplified by using a thermal cycler and the density of each reaction was determined by measuring the lights per reaction using a Polaroid camera (Poraloid 665 film, Polaroid Corp., Cambridge, MA). For relative semiquantitation, the densitometry value of each cytokine was normalized to that of the housekeeping gene, β-actin, which was not affected by DNFB.
was each of which was reverse-transcribed for 60 min at 42°C using the 1st-STRANDED TM cDNA Synthesis kit (Clontech Lab., Palo Alto, CA). The cDNA samples were amplified in a total volume of 100 µl containing 0.5 mM of each 5' and 3' primers (primers sequences for germline Cε, 5' primer 1e: ACTAGAGATCC ACAACG, 3' primer Cε2: AGCGATGAAGTAGATAGC; primers sequences for productive Cε, 5' primer j4: TGGACTACTGGGTCAGGG, 3' Primer Cε2: AGCGATGAAGTAGATAGC) with the GeneAmp PCR reagents (GeneAmp PCR Reagent kit with AmpliTaq DNA Polymerase: PerkinElmer Japan Co., Ltd., Urayasu, Japan).

The mixture was underwent 30 PCR of the following cycles in a thermal cycler; 5 min denaturation at 94°C, 5 min annealing at 60°C, then 30 cycles of 1.5 min at 94°C, 1.5 min at 94°C and 1.5 min at 60°C; with a final extension of 10 min at 72°C. RT-PCR was performed on β-actin, germline Cε and productive Cε. Internal control was β-actin. Each PCR product was resolved by electrophoresis and visualized with ethidium bromide to reveal the DNA. Auricular lymph nodes were obtained 24 hr after the fifth painting with DNFB.

**Cytokine production from cultured murine T cell clones stimulated with chemicals.** The Th1 cell clone 1E10.H2 was kindly donated by Prof. H. Oomori (Okayama University, Okayama, Japan). This clone is specific for keyhole limpets hemocyanin in the context of I-As. The Th2 clone D10.G4.1 (Dainippon Seiyaku, Osaka, Japan) is specific for conalbumin in the context of I-A k. These two T cell clones were stimulated every week with antigen and mitomycin C-treated syngeneic splenocytes as antigen-presenting cells. The supernatant containing α-methyl-d-mannoside from rat spleen cells that had been stimulated with concanavalin A for 48 hr was added at a ratio of 10% as a source of lymphokines, to complete medium during cell culture. Cells of each T cell clone were studied after stimulation with antigen and mitomycin C treated splenocytes for 7 to 14 days. Cells were collected by centrifugation and resuspended in RPMI 1640 medium containing 10% fetal calf serum to a density of 3 × 10^6 cells/ml. Each cell was stimulated with 10 ng/ml PMA, 0.6 µg/ml calcium ionophore A23187 and 10 µg/ml concanavalin A for 24 hr. The amount of cytokine in the supernatant was measured by enzyme-linked immunonoassay. At least four doses of drugs were tested and the IC_{50} value with 95% confidence limits were calculated by linear regression.

**Statistics.** Results are expressed as the mean ± S.E.M. Data were evaluated by either Student’s or Welch’s t test after examining the variances using the F test. P < .05 was considered to be statistically significant.

**Results**

**Effect of FK-506 and cyclosporin A on Th1 cell-mediated contact dermatitis.** Repeated topical application (painting) of DNFB on the ear skin provoked typical contact dermatitis in mice. Figure 1 shows the time course for the effect of FK-506 and cyclosporin A on the changes in ear thickness due to dermatitis. The thickness increased in proportion to the increase in the number of exposure to DNFB. The ear thickness at time zero was 21.68 ± 0.18 × 10^{-3} mm. Contact dermatisis was detected 24 hr after the second, third, fourth and fifth painting with DNFB. The increase in ear thickness at 24 hr after each painting with DNFB was suppressed by FK-506 and cyclosporin A. Each drug at a high dose suppressed the increment of the ear almost 50%. Figure 2 shows the effect of FK-506 and cyclosporin A on the histopathological changes of the mice skin lesions 24 hr after the fifth painting with DNFB. Marked infiltration of inflammatory cells such as neutrophils, eosinophils and monocytes and hypertrophy of the epidermis was evident in the control group. FK-506 and cyclosporin A suppressed the infiltration of inflammatory cells and hypertrophy of the epidermis.

**Discussion**

In our study, we demonstrated that the novel immunosuppressors, FK-506 and cyclosporin A, inhibited contact derma-
Fig. 2. Histopathological pictures of mouse skin lesions 24 hr after the fifth painting with DNFB. A, Vehicle; B, control; C, FK-506 (5 mg/kg); D, Cyclosporin A (50 mg/kg).
titis and potentiated IgE antibody formation caused by contact sensitization with DNFB in mice. Our results also indicate that two drugs suppress the expression of IFN-γ mRNA in the skin lesion. Because the expression of IL-4, germline Ce and productive Ce mRNAs in the lymph node was not affected by the administration of the two drugs, they did not affect IL-4-induced IgE class switching. Contrary to above in vivo experiments, in vitro experiments indicated that cytokine production is inhibited in both cultured Th1 and Th2 cells. Two immunosuppressors, therefore, showed different action on the function of Th2 cells in vitro and in vivo.

The immune system is regulated by various feedback mechanisms that control the magnitude, type and specificity of the immunological reactions. In the present study, the production of IgE was significantly enhanced by FK-506 and cyclosporin A. These drugs did not affect the simultaneous production of IgG and IgM antibodies. Because the expression of IFN-γ and contact dermatitis are suppressed by FK-506 and cyclosporin A, these data suggest the inhibition of mainly Th1 cells activity in the ears. The suppressed IFN-γ mRNA expression may also affect the production of IgE antibody. Many investigators suggest an important role of the immunological balance of IL-4 and IFN-γ in IgE production in mice and human (Parronchi et al., 1992; Finkelman et al.,

TABLE 1

<table>
<thead>
<tr>
<th>IC50 (nM)</th>
<th>IFN-γ</th>
<th>IL-2</th>
<th>IL-4</th>
<th>IL-5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Th1 cells (1E10.H2)</td>
<td>0.62</td>
<td>0.25</td>
<td>1.54</td>
<td>2.64</td>
</tr>
<tr>
<td>(0.55–0.69)</td>
<td>(0.22–0.28)</td>
<td>(1.36–1.72)</td>
<td>(2.30–2.98)</td>
<td></td>
</tr>
<tr>
<td>Th2 cells (D10.04.1)</td>
<td>0.10</td>
<td>0.06</td>
<td>0.20</td>
<td>0.22</td>
</tr>
<tr>
<td>(0.09–0.11)</td>
<td>(0.05–0.07)</td>
<td>(0.18–0.22)</td>
<td>(0.19–0.25)</td>
<td></td>
</tr>
</tbody>
</table>

Each value is the mean of four experiments. Each of Th1 cell clone (1E10.2H) and Th2 cell clone (D10.04.1) was stimulated by incubation with phorbol myristate acetate (10 ng/ml), calcium ionophore A23187 (0.6 μg/ml) and concanavalin A (10 μg/ml) for 24 hr. IC50 values with 95% confidence limits were calculated by linear regression.
that the down-regulation of IFN-γ suppresses Th1 activity. Some clinical studies suggest that the potentiating mechanism of IgE production may relate to the IL-4. Further experiments will be necessary to elucidate the potentiation of the IgE production and down-regulation of IL-4 level (Mukoyama et al., 1995; Kou et al., 1994). The precise mechanism of high IgE production by the down-regulation of IFN-γ remains still obscure. Further experiments will be necessary to elucidate the relationship between the potentiation of the IgE production and down-regulation of IFN-γ production in vivo not in vitro. Moreover, recently, Yamashita et al. (1996) reported that FK-506 inhibited primary IgE production but not secondary IgE production in mice. They pointed out the role of non-T cell-derived IL-4 in the secondary IgE production. In our experiments, FK-506 and cyclosporin A were administered immediately after the primary immunization. The difference between their data and our results may be based upon the different immunizing procedure, antigen and administration timing. We are now examining the role of IL-4 in the onset of this dermatitis and IgE production. The role of non-T cell-derived IL-4 in the present system will be elucidated in the near feature. In addition, some recent investigations indicated that IgE antibody response can be introduced by the IL-4 independent mechanism (Morawetz et al., 1996, Smiley et al., 1997). These evidence suggest the possibility of another mechanism of IFN-γ on the suppression of IgE response without affecting IL-4. Further experiments will be necessary to elucidate the role of IFN-γ in IL-4 independent IgE antibody response.

Contrary to the above in vivo results, FK-506 and cyclosporin A suppressed the production of cytokines in Th1 or Th2 cells. The activation of STAT families and transcription factors such as Fyn and Lck in Th1 and Th2 cells, may participate on the activation of T cells in a different mechanism (Tamura et al., 1993; Gajewski et al., 1990). However, the activation of calcineurin is the common pathway to activate each subset of T cells (Lin et al., 1991). Our results confirmed the inhibitory effect of FK-506 or cyclosporin A on the activation of both T1 and Th2 cells in vitro. These drugs tended to favor Th1 over Th2 cells. Similar results regarding selective inhibition of Th1 cells by these two immunosuppressors in vitro were reported by Naora and Young (1994), Schmidt et al. (1994), Van Wauwe et al. (1995) and McHugh et al. (1995). These results, taken together with our in vitro and in vivo data indicate that FK-506 and cyclosporin A have a selective inhibitory action on Th1 cells, especially in vivo. FK-506 and cyclosporin A are used to prevent or treat organ allograft rejection. These two drugs are also effective for treating patients with chronic severe allergic diseases such as bronchial asthma and atopic dermatitis (Sepp and Fritsch, 1993; Alexander et al., 1992). IgE antibody may play an important role in the development of these conditions. It is important, therefore, to monitor the serum level of IgE antibody during drug therapy.

As for IgE antibody production by repeated application of DNFB on the mice skin, some investigators reported a failure of the elevation of serum IgE level by topical application of DNFB (Dearman and Kimber, 1991, Dearman et al. 1996). The reasons of these inconsistencies may be resulted from the difference of experimental conditions including animal strain, the sensitivity to identify the level of serum IgE and other experimental protocols.

In conclusion, these results suggest that five exposures to the hapten caused contact dermatitis in the skin and IgE production in the serum. RT-PCR studies indicated that contact dermatitis was induced by the activation of Th1 cells and that IgE production was initiated by Th2 cell activation. The novel immunosuppressants, FK-506 and cyclosporin A, inhibited the Th1 cell-dependent reaction but enhanced the Th2 cell-dependent reaction in vivo. On the contrary, these two agents inhibited the activation of Th1 and Th2 cells in vitro. This evidence suggests that FK-506 and cyclosporin A potentiate IgE antibody production in vivo due to a relatively selective inhibitory action upon Th1 cells.

References


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