The Effects of Cyclosporin A and FK-506 on the Cytokine Production of Lymphocytes in Atopic Dermatitis

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Background: It has been demonstrated that patients with atopic dermatitis(AD) show an impaired capacity of their T cells to release of interleukin-2(IL-2) in vitro and elevated serum levels of soluble IL-2 receptor(sIL-2R). Both immunosuppressive agents, cyclosporin A(CsA) and FK-506 can block early events in T lymphocyte activation and FK-506 is 10- to 100-fold more potent in the inhibition of IL-2 and other lymphokines production.

Objective: We compared the effects of CsA and FK-506 on PHA-induced lymphokine and sIL-2R production and compared the effects of CsA and FK-506 on PHA-induced IL-4 production in a high IgE group and a low IgE group in patients with AD.

Methods: A total of 32 peripheral blood samples from 17 patients with AD and 15 control groups were tested. Lymphocytes were isolated from blood samples and were cultured with PHA(positive control), PHA and CsA(10 ng/ml), PHA and FK-506 (1 ng/ml), and without stimulation(negative control). The amount of cytokines such as IL-2, IL-3, IL-4 and sIL-2R were measured using an enzyme-linked immunosorbent assay(ELISA).

Results: CsA and FK-506 inhibited significantly the production of IL-2, IL-3, IL-4 in PHA-stimulated lymphocytes of both the AD patients and the control groups. FK-506(1 ng/ml) inhibited cytokines production more significantly than CsA(10 ng/ml). However, CsA and FK-506 did not significantly inhibit the production of sIL-2R. There were no significant differences in the inhibitory effect of CsA and FK-506 on IL-4 production upon PHA-stimulation between AD patients with a high IgE level and with a low IgE level.

Conclusion: Both FK-506 and CsA inhibit lymphokine production, but not the production of sIL-2R. FK-506 inhibited more significantly lymphokine production at a 10-fold lower concentration than CsA. Our data suggest that FK-506 could be more effective in the treatment of severe AD than CsA, and both these agents show their immunosuppressive activity through the suppression of lymphokine production, not via the suppression of sIL-2R production in AD. (Ann Dermatol 8:(2)98~106, 1996).

Key Words : Atopic dermatitis, Cyclosporin A, Cytokines, FK-506

Atopic dermatitis(AD) is a chronically relapsing skin disorder that is often associated with allergic rhinitis, asthma, or a family history of atopic disease[1]. The pathogenesis has not been defined, but a variety of immunological parameters have been found to be altered in AD[2].

High levels of serum IgE to environmental and food allergens have been demonstrated in most AD patients[1,3,4]. Recently, interleukin 4(IL-4) has been shown to induce enormously the production of IgE, suggesting the possible involvement of IL-4 in the pathogenesis of AD[5,6]. Also, it has been shown that patients with AD are characterized by an impaired capacity of their T cells to release in-

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terleukin 2(IL-2) in vitro[7]. Also, elevated serum levels of soluble IL-2 receptor(sIL-2R) have been shown in AD patients[7,8,9]. Atopic asthma has been shown to be associated with activation of the IL-3, IL-4, and IL-5 in the bronchi[10].

The immunosuppressive agents cyclosporin A (CsA) and FK-506 block early events in T lymphocyte activation[11,12]. Although both are fungal metabolites, CsA is a cyclic undecapeptide and FK-506 is a macrolide. Both these agents bind to distinct families of intracellular proteins(immunophilins) termed cyclophilins and FK-506-binding proteins(FKBPs). Then, both CsA-cyclophilins and FK-506-FKBPs complexs bind to calcineurin(phosphatase 2B) and inhibit its activity. Therefore these interfere with the production of IL-2 and other lymphokines[13,14,15]. Compared with CsA, FK-506 is 10- to 100-fold more potent and the two agents can act synergistically both in vivo and in vitro[11,12,14].

In this study, we compared the effects of CsA and FK-506 on the expression of IL-2, IL-3, IL-4, and sIL-2R, respectively. Also, we compared the effects of these agents on the expression of IL-4 between AD patients with high and low serum IgE levels.

MATERIALS AND METHODS

Blood samples

A total of 32 peripheral blood samples from 17 patients with severe AD and 15 control groups were tested. There were 8 male and 9 female patients, and the mean age was 10.6(range 2-25) years. All patients fulfilled the diagnostic criteria for severe atopic dermatitis[16], and systemic steroid therapy had been discontinued 2 months before and oral anti-histamine or topical steroid therapy 3 days before the start of the study.

We also evaluated the serum IgE level of AD patients and control groups with an enzyme-linked immunosorbent assay(ELISA).

Reagents

CsA and FK-506 were obtained from Hanmi Pharmaceutical, Seoul, Korea and Fujisawa Pharmaceutical Co. Ltd., Osaka, Japan, respectively. Both drugs were dissolved to 1 mM in ethanol and added to the culture medium.

Peripheral blood lymphocytes isolation

Lymphocytes were isolated by density centrifugation using fresh buffy coats from blood samples from the patients and control groups. Isolated lymphocytes were washed three times with RPMI-1640 and were adjusted to 2×10^6 cells/ml with RPMI-1640.

Medium

RPMI-1640, fetal calf serum(FCS) was purchased from Gibco(Grand Island, NY, U.S.A.). The complete medium comprised of RPMI-1640 supplemented with 10% heat-inactivated FCS, 2 mM L-glutamine, 50 U/mL penicillin and 50 ug/mL streptomycin.

Lymphocyte culture

The cells were cultured in the complete medium in combination with phytohemagglutinin(PHA) (2% in total medium) and CsA(10 ng/ml) or FK-506(1 ng/ml). As a positive control, the cells were cultured in the complete medium in combination with PHA(2% in total medium)(Gibco). As a negative control, the cells were cultured in the complete medium alone.

Cytokines assays

The amount of cytokines such as IL-2, IL-3, IL-4, and sIL-2R were measured using an ELISA[7,9]. Briefly, 96 wells were precoated with a murine IgG monoclonal antibody(mAb) directed against human cytokines(IL-2, IL-3, IL-4: R&D Systems, Inc.,Minneapolis, U.S.A., sIL-2R: Immunotech, Marseille, France).

At first, samples were added in the precoated wells and incubated for 2 hours at room temperature. Then each cytokine conjugate(polyclonal antibody against each cytokine, conjugated to enzyme) was added and incubated 2 hours at room temperature. Finally substrate solution was added and after 30 minutes, we determined the optical density of each well using a spectrophotometer set to 450 nm(sIL-2R: 405 nm). The inhibitory effects on the cytokine production of CsA or FK-506 were compared with the positive controls without both drugs.

Statistical analyses

The results were expressed as mean values $\pm$ SD. The statistical significance of differences was de-

termined by the Student's Paired t-Test and the Standard Two-Sample t-Test.

RESULTS

1. The effects of CsA or FK-506 on PHA-induced IL-2, IL-3, IL-4 and sIL-2R production(Table 1)(Fig. 1. A-D)

The amounts of IL-2, IL-3 and IL-4 production in PHA-stimulated lymphocytes added CsA(10 ng/ml) in vitro from 17 patients with AD were significantly decreased in comparison with the positive controls(p <0.005). Also, the amounts of IL-2, IL-3 and IL-4 in PHA-stimulated lymphocytes added FK-506(1 ng/ml) were significantly decreased in comparison with the positive controls(p <0.001). Furthermore, the production of IL-2, IL-3 and IL-4 in cultures with added FK-506 were more significantly decreased than the production of IL-2, IL-3 and IL-4 in cultures with added CsA.

There was no significant difference in sIL-2R production between the cultures with added CsA or FK-506 and the positive controls without both drugs(p >0.05).

Our results from the 15 control group without AD showed a similar effect of CsA and FK-506 on PHA-induced IL-2, IL-3, IL-4 and sIL-2R production in comparison with AD patients.

2. The effects of CsA or FK-506 on PHA-induced IL-4 production between high IgE and low IgE(Table 2)(Fig. 2, A-B)

There was no significant difference in the inhibitory effect of CsA on IL-4 production of lymphocytes upon stimulation with PHA between AD patients with a high IgE level(more than 400 Iu/ml, n=9) and with a low IgE level(less than 400 Iu/ml, n=6)(p >0.05). Also, there was no significant difference in the inhibitory effect of FK-506 on IL-4 producton between AD patients with a high IgE level and with a low IgE level(p >0.05).

DISCUSSION

The pathogenesis of AD is still undefined[2]. The prominent immunologic abnormalities associated with AD are defective cell-mediated immunity and increased IgE production[1-4]. Defective cell-mediated immunity(CMI) is manifested by an increased susceptibility to severe skin infections with viruses and to chronic dermatophyte infections; a reduced capacity to manifest delayed-type hypersensitivity and a low PHA-stimulated lymphocyte transformation[1,3,4]. Several investigators have reported that AD patients have a decreased proportion of circulating CD3+ T cells and CD8+ suppressor T cells and, therefore, a selective increase in the ratio of CD4+ cells to CD8+ cells[1,2].

In mice, CD4+ T cells have been classified: TH1 cells, which produce IL-2, IFN-γ, TNF-β; TH2 cells, which produce IL-4, IL-5, IL-6, and IL-10[1,17]. Several investigators have reported that allergen-specific T cell clones from AD patients produced increased IL-4 and IL-5 in combination with no or low levels of IFN-γ[18,19]. Also, it has been seen that atopic asthma is associated with activation of the IL-3, IL-4 and IL-5 in the bronchi[10]. Therefore, the predominant CD4 subset in AD is a TH2-like subset but not TH1-like subset that secrete IF-γ. IL-4 is a potent inducer of IgE production, and IFN-γ inhibits IL-4-induced

Table 1. Comparison of PHA-induced IL-2, IL-3, IL-4 and sIL-2R production between positive controls and lymphocytes added CsA or FK-506 in 17 patients with AD(Mean±SD)

	Pos. C. §	CsA	FK-506
IL-2(pg/ml)	999.67±192.88	438.40±135.12*	195.03±115.96**
IL-3(pg/ml)	1349.07±211.81	1161.39±183.81*	688.53± 86.75**
IL-4(pg/ml)	140.46± 32.15	107.53± 25.30*	72.03± 16.97**
sIL-2R(pM)	226.88± 43.73	207.06± 37.07	230.06± 38.78

§: positive controls indicate PHA-stimulated lymphocytes.

*, ** : statistically significant in difference from positive controls(Student's Paired t-Test, *p <0.005, **p <0.001)

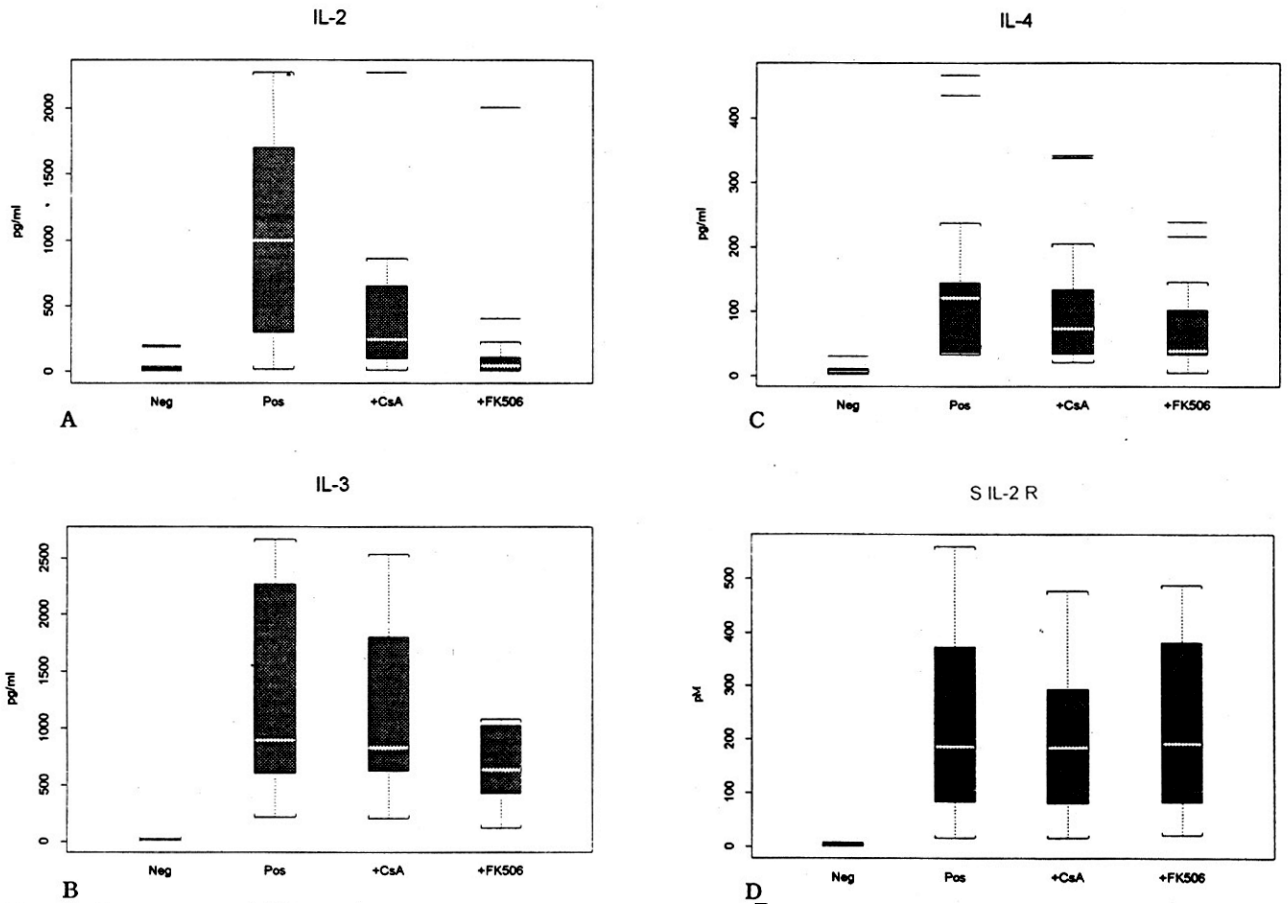


Fig. 1. Comparison of PHA-induced IL-2(A), IL-3(B), IL-(4) and sIL-2R(D) production between positive controls and lymphocytes added CsA(10 ng/ml) or FK-506(1 ng/ml) in 17 patients with AD. Neg(negative controls) indicate non-stimulated lymphocytes. Pos(positive controls) indicate PHA-stimalated lymphocytes. Statistcal evalutions were determined by Student's Paired t-Test(**p<0.005, **p<0.001, significant).

IgE synthesis in vitro. Therefore, the increased IgE production in AD may be influenced not only by IL-4 production but also by the simultaneous suppression of IFN-γ[1,4,17]. AD patients release less IL-1, TNF and IL-2, possibly due to the selective inhibitory effect on CD4+ T cells of prostaglandin E overproduction by AD monocytes[7].

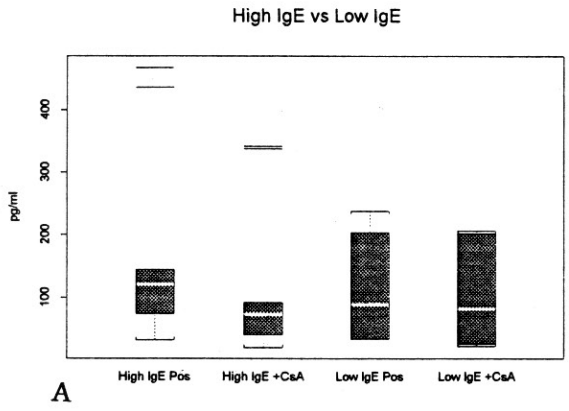
CsA, a cyclic undecapeptide, has been found to be effective in various dermatoses, including AD[20-23]. However, its use is restricted to severely affected patients because of drug-induced hypertension and nephrotoxicity[23]. FK-506, a macrolide antibi-

Table 2. The effects of CsA or FK-506 on IL-4 production between a high serum IgE(n=9) and low serum IgE(n=6)(Mean±SD)

	Pos. C.	CsA	FK-506
High IgE[a]	114.58±37.72	103.37±36.05[+]	68.04±23.41[+]
Low IgE[b]	169.55±54.91	119.99±42.28	76.86±28.92

a: more than 400 IU/ml, b: less than 400 IU/ml

+: statistically not significant in difference between AD patients with a high serum IgE level and low serum IgE level (Standard Two-Sample t-Test, [+]P >0.05)



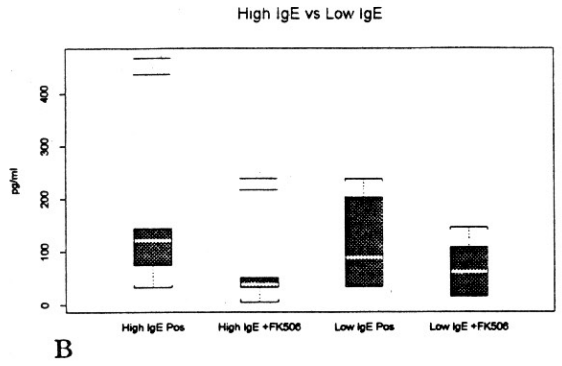


Fig. 2. The effects of CsA(A) or FK-506(B) on PHA-induced IL-4 production between AD patients with a high serum IgE level (more than 400 IU/ml, n=9) and with a low serum IgE level(less than 400 IU/ml, n=6). Statistical evaluations were determined by Standard Two-Sample t-Test(p>0.05, not significant).

otic, has been shown to be 10- to 100-fold more potent than CsA in its inhibitory action on T lymphocyte responses[11,12,14]. The immunosuppressive agents CsA and FK-506 have been shown to interfere with the transcriptional activation of early T cell activation genes such as IL-2, IL-3 and IL-4 in activated human peripheral blood T cells[12,24-30].

In this study, we investigated the effects of both drugs on the expression of cytokines such as IL-2, IL-3, IL-4 and sIL-2R in vitro in patients with AD. We showed that CsA and FK-506 inhibited significantly the production of each IL-2, IL-3, IL-4 in PHA stimulated lymphocytes in vitro in patients with AD. Furthermore, we showed that FK-506(1 ng/ml) inhibited more significantly cytokines production at a 10-fold lower concentration than CsA(10 ng/ml). Our results suggested that CsA and FK-506 selectively suppress the activation of CD4+ cells by inhibiting the expression of early T cell - activating genes encoding the cytokines such as IL-2, IL-3 and IL-4. Because AD patients appear to have decreased numbers of CD8+ T cells, the use of both drugs that suppress the activation of CD4+ cells may restore the T cell functional balance in AD and may result in a beneficial clinical effect[22]. Increased serum levels of sIL-2R have been shown in AD patients and has been shown a significant correlation with IgE levels and body surface involvement[7,8,9]. As well as being an early sign of T cell activation, sIL-2R may have an immunoregulatory role with inhibitory effects on T cell activation by binding IL-2[9]. Increased sIL-2R levels may reflect activation of immunopathogenic mechanisms contributing to the exacerbation of AD. Therefore, sIL-2R levels may be a helpful tool for assessing responses to new treatments. We showed that CsA and FK-506 could not significantly inhibit sIL-2R production in PHA-stimulated lymphocytes in vitro in AD patients. This result may indicate that sIL-2R production in PHA-stimulated lymphocytes is probably via post-transcriptional activation pathway[24,31,32]. as has been reported for CD28-activated cells[33].

As IL-4 has been shown to induce enormously the production of IgE in the pathogenesis of AD[5,6], we wanted to investigate the differences in the effects of CsA and FK-506 on IL-4 production between a high IgE and a low IgE group in AD patients. In our study, CsA and FK-506 significantly inhibited IL-4 procuction in PHA-stimulated lymphocytes as reported in recent studies[26-28]. However, there was no significant difference in the inhibitory effect of both drugs on IL-4 production between AD patients with a high IgE level and with a low IgE level. These results suggest that the extent of the inhibitory effect of both drugs on IL-4 procuction is not related to the serum IgE level.

There has been controversy over the mean IgE values of AD patients in Korea as reported with 293.4 IU/ml by Cheon[34] or 185 IU/ml by Kang[35]. In this study, we selected clinically severe AD patients and their serum IgE levels were relatively

high. Therefore, serum IgE levels were classified into a high and low groups on the basis of 400 IU/ml.

CsA and FK-506 could be suspected to affect IgE production through interfereing IL-4 production in AD patients. Therefore, in this study, we wanted to investigate the effects of CsA and FK-506 on the expression of IgE level in supernatant with a paper radioimmunosorbent test(PRIST) method. A minimal unit of IgE measure with a PRIST method is 5 IU and most of the amount of IgE isolated from supernatant in this study were below 0.25 IU. Therefore, we could not evaluate the effect of CsA and FK-506 on the expression of IgE level in vitro. More sensitive IgE measuring techniques such as a overnight PRIST using RAST anti-IgE trace method than the usual PRIST will be necessary for the evaluation of IgE level in supernatant.

CsA has been used for the prevention and treatment of organ transplant rejection, including the kidney, liver, and heart[36,37,38]. Also, it has been shown to reduce the severity of graft-versus-host reactions in patients undergoing bone marrow transplants[39]. Recently, its reports have shown benificial results in autoimmune medical disorders including ulcerative colitis[40], Crohn's disease[41], primary biliary cirrhosis[42], uveitis[43], pulmonary sarcoidosis[44], type I diabetes mellitus[45], aplastic anemia[46], myasthenia gravis[47], Goodpasture's syndrome[47], rheumatoid arthritis[47], multiple sclerosis[48], Grave's disease[49]. Also, it has shown a potential usefulness for dermatologic conditions of presumed autoimmune T cell mediated pathogenesis. These include: psoriasis[50,51], pemphigus and pemphigoid[52], dermatomyositis and polymyositis[53,54], systemic lupus erythematosus[55], cutaneous T cell lymphoma[56], Behçet's disease[57], alopecia[58], ichthyosis[59], atopic dermatitis[20,21,22].

FK-506 is a new immunosuppressive agent that is more potent than CsA and can act synergistically with CsA[11,12,14]. It has been reported that FK-506 prevented allograft rejection in rats[60], dogs[61], monkeys, and baboons[62]. In humans, the immunosuppressive activity of FK-506 for liver, kidney, and pancreas transplantation has been reported[63]. Also, It has been reported that FK-506 was effective in treating patients with severe recalcitrant psoriasis[64]. Topical FK-506 has recently been shown to have a suppressive effect of contact dermatitis in the guinea pig[65] and to have a therapeutic effect in AD patients[66]. Therefore, it may have potential clinical applications in human skin diseases.

In conclusion, we demonstrated that FK-506 more significantly inhibited cytokines such as IL-2, IL-3 and IL-4 production in vitro than CsA in AD patients. Furthermore, the inhibition of IL-4 production by FK-506 suggests that FK-506 can inhibit IgE production. Our data suggest that FK-506 could be more effective in the treatment of severe AD than CsA, and both these agents show their immunosuppressive activity through the suppression of lymphokine production, not via the suppression of sIL-2R production in AD.

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