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FK506 suppresses neutrophil chemoattractant production by peripheral blood mononuclear cells

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Abstract

To understand the mechanism of action of FK506 (Tacrolimus) on neutrophil chemotaxis, we examined its effect on human neutrophil chemotaxis and neutrophil chemoattractant production by peripheral blood mononuclear cells. FK506 and cyclosporin A had no direct suppressive effect on neutrophil chemotaxis induced by interleukin-8, leukotriene B_4, complement 5a (C5a), zymosan-activated serum and formyl-Met–Leu–Phe (fMLP). FK506 and cyclosporin A only slightly suppressed the chemotactic activity of platelet-activating factor (PAF). Dexamethasone did not inhibit the chemotactic activity of any chemoattractant. The supernatant of peripheral blood mononuclear cells stimulated with anti-CD3 and CD2 antibodies induced neutrophil chemotaxis. FK506 and cyclosporin A suppressed the chemotactic activity of the supernatant in parallel to the suppression of interleukin-8 production by peripheral blood mononuclear cells. Anti-interleukin-8 antibody completely suppressed the chemotactic activity of the supernatant without drugs. These studies indicate that FK506 may exert a beneficial effect on human inflammatory diseases by suppressing neutrophil chemotaxis secondary to inhibition of chemoattractant (for example, interleukin-8) production by leukocytes. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: FK506 (Tacrolimus); Neutrophil chemotaxis; Interleukin-8; Mononuclear cell peripheral blood

1. Introduction

FK506 (Tacrolimus) is a 23-membered macrolide immunosuppressant isolated from *Streptomyces tsukubaensis* (Kino et al., 1987a). Its immunosuppressive mechanism involves inhibition of calcineurin by a complex of FK506 and a 12-kDa immunophilin FKBP12, resulting in an inability to assemble the active form of the transcription factor NF-AT and subsequent down-regulation of several cells, especially T lymphocyte cytokine transcription (Jain et al., 1993; Liu et al., 1991; Kino et al., 1987b). FK506 also inhibits degranulation from mast cells, basophils and neutrophils (De Paulis et al., 1992; Forrest et al., 1991). However, the effect of FK506 on neutrophil chemotaxis has not been fully determined.

Human neutrophils play a major role in the host defense response against bacterial infection (Babior, 1978) and in the pathogenesis of various inflammatory diseases such as rheumatoid arthritis, psoriasis and asthma (Brennan et al., 1990; Biasi et al., 1998; Frangova et al., 1996). Since neutrophil chemotaxis is an early and important step in the inflammation process, the suppression of neutrophil chemotaxis should be of a great therapeutic value for such diseases. FK506 suppresses neutrophil migration to human kidney graft after transplantation and rat liver in an ischemia–reperfusion model, but the exact mechanism remains unknown (Woodle et al., 1997; Garcia-Criado et al., 1997). Although it has been reported that FK506 suppresses neutrophil chemotaxis induced by formyl-Met–Leu–Phe (fMLP) or FKBP (Burnett et al., 1994; Leiva and Lyttle, 1992), the effect of FK506 on neutrophil chemotaxis induced by another chemoattractant has not been reported.

To understand the mechanism of action of FK506 on neutrophil chemotaxis, we examined its direct effect on human neutrophil chemotaxis induced by various chemoattractants. We also examined whether FK506 can suppress neutrophil chemotaxis by inhibiting cytokine production by peripheral blood mononuclear cells.

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2. Materials and methods

2.1. Reagents

Recombinant human interleukin-8 (R&D systems), leukotriene B_4 (CASCADE), platelet-activating factor (PAF, Sigma), complement 5a (C5a, Sigma) and fMLP (Sigma) were purchased. Zymosan-activated serum was prepared by stimulating human peripheral blood serum with zymosan A (Sigma). The minimum concentrations of these chemoattractant, at which the maximum chemoattractive activities for human neutrophils were obtained, were determined in a series of preliminary experiments (data not shown). Interleukin-8 (1–100 ng/ml), leukotriene B_4 (100 pg/ml–1 μg/ml), PAF (500 pg/ml–5 μg/ml), C5a (100 pg/ml–1 μg/ml), fMLP (100 pM–1 μM) and zymosan-activated serum (3–30,000 × dilution) were tested, and the concentrations, 10 ng/ml, 1 ng/ml, 50 ng/ml, 10 ng/ml, 10 nM and 100 × dilution were selected, respectively. Monoclonal anti-human interleukin-8 antibody (genzyme) was purchased and used at 1 μg/ml as described (Zhang et al., 1995).

2.2. Isolation of blood neutrophils

Human neutrophils were isolated from the heparinized (10 U/ml) venous blood of normal volunteers, which was diluted with Hanks' solution (Nikken Bio Medical Laboratory), using discontinuous gradients of mono–poly resolving medium (Dainippon Pharmaceutical). The purified neutrophils were washed twice and suspended at 5×10^6 cells/ml in Hanks' solution containing 0.2% bovine serum albumin (Sigma) and held on ice until use (less than 1 h).

2.3. Isolation of peripheral blood mononuclear cells

Human peripheral blood mononuclear cells were isolated from the heparinized (10 U/ml) venous blood of normal volunteers, which was diluted with RPMI-1640 (Nikken Bio Medical Laboratory), using discontinuous gradients of Ficoll-paque plus (Pharmacia Biotech). The purified peripheral blood mononuclear cells were washed three times and suspended at 3×10^6 cells/ml in RPMI-1640 containing 5% human type AB serum (ICN Biomedicals), 5×10^{-5} M 2-mercaptoethanol (Gibco BRL) and 50 IU/ml penicillin–50 μg/ml streptomycin (Gibco BRL).

2.4. Peripheral blood mononuclear cell culture supernatants

0.4 ml PBS (Nikken Bio Medical Laboratory), containing 25 μg/ml anti-mouse immunoglobulin G (IgG) Fc antibody (Chemicon), was incubated in each well of a 24-well culture plate (Sumitomo Bakelite) at 37°C for 1 h. After the wells were washed, 0.4 ml PBS containing 0.5% bovine serum albumin, 150 ng/ml anti-human CD3 antibody (OKT3, Kyowa Hakko) and 150 ng/ml anti-human CD2 antibody (Leu-5b, Kyowa Hakko) was incubated in each well at 37°C for 1 h. After a wash step, 3×10^6 cells in 2 ml RPMI-1640 containing 5% human type AB serum, 5×10^{-5} M 2-mercaptoethanol and 50 IU/ml penicillin–50 μg/ml streptomycin were incubated in each well in the presence or absence of drugs at 37°C for 24 h. FK506 was used at concentrations ranging from 10 pg/ml to 100 ng/ml and cyclosporin A in concentrations ranging from 100 pg/ml to 1 μg/ml. The supernatant fraction was obtained and stored at −80°C until use. The supernatants diluted 12 × with Hanks' solution containing 0.2% bovine serum albumin were used for neutrophil chemotaxis.

2.5. Measurement of neutrophil chemotactic activity

Chemotaxis induced by chemoattractant or peripheral blood mononuclear cell culture supernatants was evaluated using Transwell plates (Costar) containing 3-μm pore polycarbonate inserts, using a modification of a previously described method (Psychoyos et al., 1989). Briefly, 600 μl Hanks' solution containing 0.2% bovine serum albumin in the presence or absence of chemoattractant, combined with or without drugs, or peripheral blood mononuclear cell culture supernatants, was warmed in each lower well at 37°C for 30 min under the condition that the upper well was placed on the lower well. At the same time, 5×10^6 cells/ml neutrophils in Hanks' solution containing 0.2% bovine serum albumin were preincubated with or without drugs in 15 ml centrifuge tubes (IWAKI) at 37°C for 30 min. This preincubation time was determined, since the chemotactic activity induced by recombinant human interleukin-8, leukotriene B_4, PAF and fMLP was reduced in a time-dependent manner and the chemotactic activity after 4 h preincubation was reduced to 40% (data not shown). FK506, cyclosporin A and dexamethasone were used at concentrations ranging from 1 ng/ml to 1 μg/ml, as determined from previous reports (Burnett et al., 1994; Xu et al., 1992; Psychoyos et al., 1989). In the case of the PAF-induced chemotaxis experiment, FK506 was used at concentrations ranging from 10 pg/ml to 1 μg/ml. After preincubation, 5×10^5 cells were applied to the upper well and incubated at 37°C for 1 h. The total number of neutrophils that migrated into the lower well was counted with a Sysmex microcell counter CC-130A (Sysmex). The migration rate was calculated as follows: migration ratio (%) = (number of neutrophils in the lower well ÷ number of neutrophils applied in the upper well) × 100.

2.6. Detection of human interleukin-8

Secreted human interleukin-8 in peripheral blood mononuclear cell culture supernatants was measured by using an interleukin-8 enzyme-linked immunosorbent assay (ELISA) kit (Quantikine) according to the manufacturer's instructions. The standard carve was obtained at

concentrations ranging from 31.3 to 2000 pg/ml. Plates were read on SPECTRA MAX250 (Wako) at 450 nm.

2.7. Statistical analysis

Results are shown as means ± S.E.M. of three to five independent experiments and were analyzed for statistical significance by Dunnett's test for multiple comparisons.

The inhibition rate was calculated as follows: inhibition ratio (%) = (migration rate of positive control − migration rate of each sample) ÷ migration rate of positive control × 100.

3. Results

3.1. Direct effects of FK506, cyclosporin A and dexamethasone on human neutrophil chemotaxis induced by various chemoattractants

The direct effects of FK506, cyclosporin A and dexamethasone on human neutrophil chemotaxis induced by several chemoattractants, recombinant human interleukin-8, leukotriene B_4, PAF, C5a, zymosan-activated serum and fMLP, were examined. Except for PAF, no significant suppression by FK506 or cyclosporin A was observed on chemoattractant-induced chemotactic activity in concentrations up to 1 μg/ml (Table 1). Only in the case of the chemotactic activity induced by PAF did FK506 show slight suppression in a concentration dependent manner from 10 ng/ml to 1 μg/ml (percent inhibition at 1 μg/ml: 37.3 ± 5.4%). Similarly, cyclosporin A slightly suppressed the chemotactic activity induced by PAF at 1 μg/ml by 23.3 ± 3.6%. Dexamethasone did not inhibit the chemotactic activity induced by any chemoattractant at concentrations up to 1 μg/ml (Table 1).

3.2. Indirect effects of FK506 and cyclosporin A on human neutrophil chemotaxis induced by peripheral blood mononuclear cell culture supernatants

Since FK506 suppresses the migration of neutrophils in vivo (Woodle et al., 1997; Garcia-Criado et al., 1997), but not in vitro, FK506 might suppress human neutrophil chemotaxis indirectly. FK506 may inhibit the production of chemoattractant and subsequently suppress neutrophil migration in vivo. Thus, the following experiments were performed to explore this possibility. Peripheral blood mononuclear cells were stimulated with anti-CD3 and CD2 antibodies for 24 h in the presence or absence of drugs and neutrophil chemotactic activity in these supernatants was measured. The supernatant of peripheral blood mononuclear cells stimulated with anti-CD3 and CD2 antibodies induced neutrophil chemotaxis. When peripheral blood mononuclear cells were cultured with FK506, the chemotactic activity of the supernatants was reduced. The maximum suppressive effect on chemotactic activity was achieved at 1 ng/ml and no further additional effect was observed with increasing concentrations of FK506 up to 100 ng/ml (Fig. 1). Similarly, the chemotactic activity of the supernatants cultured with cyclosporin A was reduced

Table 1
Direct effects of FK506, cyclosporin A (CsA) and dexamethasone (DEX) on neutrophil chemotaxis

Drug	Concentration (ng/ml)	Chemotactic activity (%)					
		IL-8	LTB_4	C5a	PAF	fMLP	ZAS
(−)		11.7 ± 0.6	8.6 ± 1.7	11.9 ± 2.2	10.1 ± 0.8	9.5 ± 2.2	12.1 ± 2.8
(+)		76.0 ± 4.9	81.0 ± 1.4	79.6 ± 5.3	66.0 ± 1.4	59.4 ± 7.9	75.9 ± 1.7
FK506	0.01	–	–	–	63.7 ± 4.2	–	–
	0.1	–	–	–	65.0 ± 3.6	–	–
	1	77.8 ± 5.3	80.3 ± 3.1	79.2 ± 4.6	56.8 ± 3.2	57.0 ± 7.0	78.8 ± 2.0
	10	77.6 ± 5.3	82.7 ± 1.5	78.8 ± 5.5	51.2 ± 1.8[a]	56.5 ± 7.1	78.4 ± 2.0
	100	76.8 ± 5.7	81.1 ± 1.5	77.5 ± 4.6	49.0 ± 1.9[b]	55.3 ± 6.6	80.1 ± 0.1
	1000	71.6 ± 4.9	79.7 ± 2.4	78.8 ± 5.3	44.8 ± 2.5[b]	57.0 ± 8.5	75.4 ± 2.8
CsA	1	79.2 ± 3.3	80.7 ± 2.1	77.9 ± 5.4	68.0 ± 1.6	57.2 ± 7.7	77.2 ± 2.4
	10	78.8 ± 5.8	82.6 ± 3.6	78.4 ± 4.9	64.2 ± 3.4	55.2 ± 7.3	77.1 ± 1.3
	100	77.0 ± 5.9	82.3 ± 2.6	79.6 ± 5.4	61.1 ± 2.0	55.6 ± 6.1	77.0 ± 1.6
	1000	74.1 ± 4.8	83.1 ± 0.3	81.0 ± 5.2	52.8 ± 2.1[a]	51.6 ± 8.4	74.7 ± 1.2
DEX	1	79.4 ± 3.3	80.7 ± 2.8	80.9 ± 5.6	64.9 ± 3.6	54.9 ± 7.9	78.0 ± 1.6
	10	79.1 ± 4.6	79.8 ± 2.3	77.6 ± 5.0	60.7 ± 4.2	53.4 ± 7.6	75.8 ± 0.2
	100	79.2 ± 3.9	79.5 ± 2.0	82.3 ± 3.4	60.7 ± 3.5	52.2 ± 9.4	76.4 ± 0.9
	1000	80.5 ± 5.7	79.8 ± 3.2	78.6 ± 5.4	56.0 ± 3.9	54.2 ± 6.5	75.4 ± 0.9

Chemotactic activity was induced by recombinant human interleukin-8 (IL-8) 10 ng/ml, leukotriene B_4 (LTB_4) 1 ng/ml, C5a 10 ng/ml, PAF 50 ng/ml, fMLP 10 nM and zymosan-activated serum (ZAS) diluted 100×. (−) indicates that chemotactic activity was induced by medium as negative control and (+) indicates that chemotactic activity was induced by the chemoattractants without drugs as positive control. Results are shown as means ± S.E.M. of three to five independent experiments and analyzed for statistical significance by Dunnett's test for multiple comparisons.

[a] $P < 0.05$ vs. positive control.

[b] $P < 0.01$ vs. positive control.

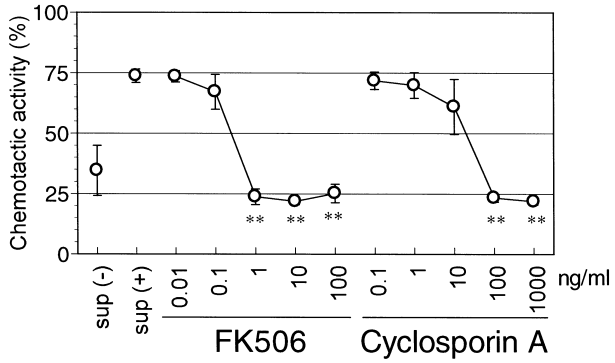

Fig. 1. Indirect effects of FK506 and cyclosporin A on human neutrophil chemotaxis induced by peripheral blood mononuclear cell culture supernatants. Peripheral blood mononuclear cells were stimulated with anti-CD3 and CD2 antibodies in the presence or absence of drugs for 24 h. Sup (−) refers to the supernatant of unstimulated peripheral blood mononuclear cells as a negative control and sup (+) refers to the supernatant of peripheral blood mononuclear cells stimulated with anti-CD3 and CD2 antibodies in the absence of drugs as a positive control. Results are shown as means ± S.E.M. of three independent experiments and analyzed for statistical significance by Dunnett's test for multiple comparisons (*: $P < 0.05$, **: $P < 0.01$ vs. positive control).

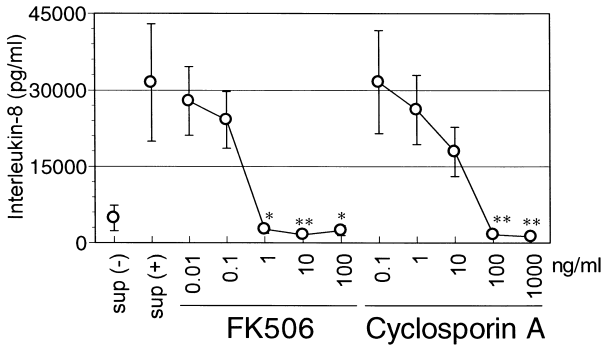

Fig. 2. Effects of FK506 and cyclosporin A on interleukin-8 production by peripheral blood mononuclear cells. Interleukin-8 in peripheral blood mononuclear cell culture supernatants was measured by ELISA. Sup (−) refers to the supernatant of unstimulated peripheral blood mononuclear cells as a negative control and sup (+) refers to the supernatant of peripheral blood mononuclear cells stimulated with anti-CD3 and CD2 antibodies in the absence of drugs as a positive control. Results are shown as means ± S.E.M. of three independent experiments and analyzed for statistical significance by Dunnett's test for multiple comparisons (*: $P < 0.05$, **: $P < 0.01$ vs. positive control).

at concentrations ranging from 100 ng/ml to 1 μg/ml (Fig. 1).

These results suggest that FK506 and cyclosporin A can suppress human neutrophil chemotaxis by inhibiting chemoattractant production by peripheral blood mononuclear cells. FK506 was about 100× more potent than cyclosporin A in suppressing chemoattractant production by peripheral blood mononuclear cells.

3.3. Effects of FK506 and cyclosporin A on neutrophil chemoattractant production

Since interleukin-8 as a representative chemoattractive cytokine, is a major neutrophil chemoattractant (Broaddus et al., 1992), we measured interleukin-8, in peripheral blood mononuclear cell culture supernatants stimulated with anti-CD3 and CD2 antibodies. There was more than 30 ng/ml interleukin-8 in the supernatant that did not include drugs (Fig. 2), an amount sufficient for neutrophil chemotaxis, because recombinant human interleukin-8 achieved maximum chemotactic activity at 10 ng/ml (Fig. 3). As shown in Fig. 2, FK506 suppressed interleukin-8 production by peripheral blood mononuclear cells. The maximum effect was achieved at 1 ng/ml and no further additional effect was observed with increasing concentrations of FK506 up to 100 ng/ml. Cyclosporin A also suppressed interleukin-8 production at concentrations ranging from 100 ng/ml to 1 μg/ml (Fig. 2). FK506 was about 100× more potent than cyclosporin A in suppressing interleukin-8 production by peripheral blood mononuclear cells. The suppressive effects of FK506 and cyclosporin A on interleukin-8 production appeared to be parallel to those on chemotactic activity in the peripheral blood mononuclear cell culture supernatants (Figs. 1 and 2).

3.4. Effect of anti-interleukin-8 antibody on human neutrophil chemotaxis induced by peripheral blood mononuclear cell culture supernatants

To determine whether interleukin-8 was the major chemoattractant in peripheral blood mononuclear cell culture supernatants, anti-human interleukin-8 antibody was added to the supernatants and neutrophil chemotactic activitiy was assessed. The concentration of anti-interleukin-8 antibody used was enough to neutralize the chemotactic activity of 10 ng/ml recombinant human interleukin-8 (Fig. 3). When anti-interleukin-8 antibody was added to the supernatant cultured without drugs, the chemotactic activity was dramatically reduced from 51.4 ± 11.3% to 19.4 ± 3.6% (Fig. 4). Since the chemotactic activity in the

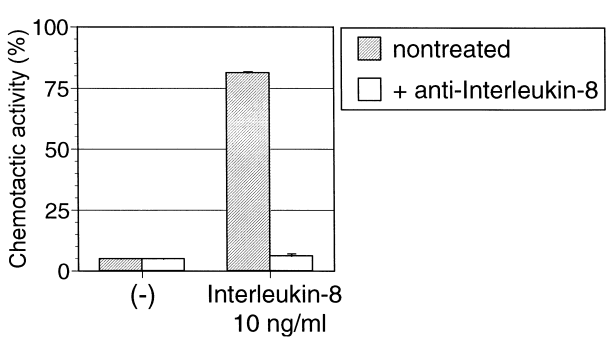

Fig. 3. Effect of anti-interleukin-8 antibody on human neutrophil chemotaxis induced by recombinant human interleukin-8. 1 μg/ml anti-interleukin-8 antibody was added to the chemotactic activity of 10 ng/ml recombinant human interleukin-8. The symbol (−) refers to the medium as a negative control. Results are shown as means ± S.E.M. of three independent experiments.

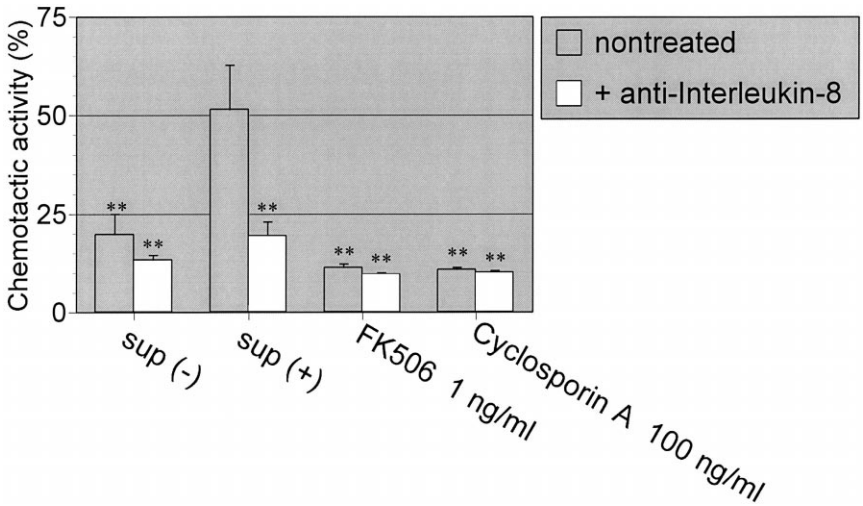

Fig. 4. Effect of anti-interleukin-8 antibody on human neutrophil chemotaxis induced by peripheral blood mononuclear cell culture supernatants. 1 μg/ml anti-interleukin-8 antibody was added to the peripheral blood mononuclear cell supernatants cultured with or without drugs and the chemotactic activity of these supernatants was measured. Sup (−) refers to the supernatant of unstimulated peripheral blood mononuclear cells as a negative control and sup (+) refers to the supernatant of peripheral blood mononuclear cells stimulated with anti-CD3 and CD2 antibodies in the absence of drugs as a positive control. Results are shown as means ± S.E.M. of three independent experiments and analyzed for statistical significance by Dunnett's test for multiple comparisons (*: $P < 0.05$, **: $P < 0.01$ vs. positive control).

supernatants cultured with 1 ng/ml FK506 or 100 ng/ml cyclosporin A was not affected by anti-interleukin-8 antibody, these drugs completely suppressed interleukin-8 production by peripheral blood mononuclear cells (Fig. 4).

4. Discussion

In this study, we initially examined the direct effect of FK506 on human neutrophil chemotaxis induced by the chemoattractants, recombinant human interleukin-8, leukotriene B_4, PAF, C5a, zymosan-activated serum and fMLP. The effects of two other well known immunosuppressive agents, cyclosporin A and dexamethasone, were also examined in a similar manner. Except for PAF, no significant suppressive effects of FK506 or cyclosporin A were observed. Only in the case of the chemotactic activity induced by PAF, did FK506 and cyclosporin A have a concentration dependent suppressive effect, although the effects were small.

Since FK506 did not show any inhibitory effect in a rabbit PAF receptor binding assay up to 100 μM (about 82 μg/ml), it is unlikely that FK506 directly interacted with the PAF receptor (data not shown). FK506 was about 100× more potent than cyclosporin A in suppressing PAF-induced neutrophil chemotaxis. FK506 is about 100× more potent than cyclosporin A in suppressing cytokine production from T lymphocytes by inhibiting the calcineurin-dependent pathway (Kino et al., 1987b; Liu et al., 1991), suggesting that the calcineurin-dependent pathway may be partially involved in PAF-induced neutrophil activation. PAF activates several signalling pathways, such as protein kinase C, phospholipase C and phospholipase A in neutrophils (Izumi and Shimizu, 1995; Bozza et al., 1996; Barnette et al., 1994). The calcineurin-dependent pathway is downstream of the phospholipase C pathway and FK506 suppresses cytokine production from T lymphocytes by inhibiting this pathway (Brizuela et al., 1995). These results suggested that FK506 and cyclosporin A might suppress neutrophil chemotaxis by inhibiting the phospholipase C pathway, although the contribution might be small in PAF-induced chemotaxis. Taken together, we conclude that FK506 and cyclosporin A have only marginal direct effects on human neutrophil chemotaxis.

In contrast to our results, Burnett et al. (1994) have reported that FK506 directly suppresses neutrophil chemotaxis induced by fMLP. Their preincubation time of neutrophils with FK506 was 2 h and our time was 30 min, which may explain the difference. However, when we tested the effect of FK506 with preincubation for 2 h and 4 h, FK506 again did not suppress neutrophil chemotaxis induced by fMLP (data not shown). This discrepancy may be due to other differences in the methods used, especially in the method of assessing neutrophil chemotaxis.

Dexamethasone showed no direct effect on neutrophil chemotaxis induced by any of the chemoattractants tested. Similar results have been reported previously. For example, dexamethasone did not suppress neutrophil chemotaxis induced by leukotriene B_4 or PAF (Psychoyos et al., 1989; Kurihara et al., 1989), and only slightly suppressed neutrophil chemotaxis induced by fMLP or zymosan-activated serum at high concentrations in vitro (Lomas et al., 1991). In contrast, it has been reported that dexamethasone suppresses neutrophil chemotaxis in vivo, for example, interleukin-8 or interleukin-1 induced neutrophil infiltration

into mouse air pouch (Perretti and Flower, 1993; Perretti et al., 1994). Dexamethasone inhibits interleukin-8 production by macrophages, leading to suppression of neutrophil chemotaxis (Lin et al., 1994), suggesting that suppression may be secondary to inhibition of chemoattractant production, in a similar manner to FK506 and cyclosporin A, as discussed below.

Since FK506 suppresses the migration of neutrophils in vivo (Woodle et al., 1997; Garcia-Criado et al., 1997), but not in vitro, we hypothesized that FK506 inhibits neutrophil migration in vivo by suppressing cytokine production by leukocytes, especially T lymphocytes or monocytes. We examined the neutrophil chemotactic activity in the supernatants of peripheral blood mononuclear cells stimulated with anti-CD3 and CD2 antibodies in the presence of FK506 or cyclosporin A. When peripheral blood mononuclear cells were stimulated in the presence of FK506 or cyclosporin A, chemotactic activity was reduced in a concentration-dependent manner. These results indicate that FK506 inhibits chemoattractant production by peripheral blood mononuclear cells stimulated with anti-CD3 and CD2 antibodies.

Since interleukin-8 as a representative chemoattractive cytokine, is a major neutrophil chemoattractant (Broaddus et al., 1992), we measured interleukin-8, in peripheral blood mononuclear cell culture supernatants stimulated with anti-CD3 and CD2 antibodies. In accord with our expectations, there was sufficient interleukin-8 for neutrophil chemotaxis in the supernatant without drugs. FK506 and cyclosporin A completely suppressed interleukin-8 production by peripheral blood mononuclear cells in parallel to the suppression of chemotactic activity in the supernatants. Furthermore, neutrophil chemotaxis of the supernatant without drugs was completely suppressed by anti-interleukin-8 antibody. These results indicate that interleukin-8 is a major chemoattractant in peripheral blood mononuclear cell culture supernatant and that FK506 and cyclosporin A can inhibit the production of neutrophil chemoattractant, such as interleukin-8, by leukocytes. Interleukin-8 is produced by various kinds of cells such as macrophages, neutrophils, T lymphocytes, endothelial cells and keratinocytes (Lin et al., 1994; Yoshimura et al., 1987; Okamoto et al., 1994; Karlsson and Nassberger, 1997; Kaplan et al., 1995). In peripheral blood mononuclear cells, it is most likely that interleukin-8 is secreted by monocytes or T lymphocytes and FK506 may act on these types of cells.

Interleukin-8 is produced in response to various stimulants such as calcium ionophore, lipopolysaccharide, interleukin-1β and tumor necrosis factor-α (TNF-α) (Karlsson and Nassberger, 1997; Kohyama et al., 1999). CD3 stimulation activates the calcineurin-dependent signalling pathway, such as with calcium ionophore (Liu et al., 1991). It has been reported that FK506 directly suppresses interleukin-8 production from eosinophils stimulated with calcium ionophore, endothelial cells stimulated with histamine and Jurkat cells stimulated with phorbol ester plus calcium ionophore (Okamoto et al., 1994; Boss et al., 1998; Kohyama et al., 1999). FK506 did not directly suppress interleukin-8 production by endothelial cells stimulated with lipopolysaccharide, interleukin-1β or TNF-α, and keratinocytes stimulated with TNF-α (Kaplan et al., 1995; Karlsson and Nassberger, 1997). These studies suggest that FK506 directly suppresses interleukin-8 production induced via the calcineurin-dependent signalling pathway but does not suppress production induced via other pathways, activated by lipopolysaccharide, interleukin-1β or TNF-α.

Neutrophils play a major role in the pathogenesis of various diseases such as rheumatoid arthritis, psoriasis and asthma (Brennan et al., 1990; Biasi et al., 1998; Frangova et al., 1996). In animal models, inhibition of interleukin-8 by anti-interleukin-8 antibody suppresses neutrophil migration and resulted in reduced tissue damage from lung reperfusion injury, endotoxin-induced pleurisy and delayed-type hypersensitivity reaction in rabbits and lung inflammatory reaction in rats (Sekido et al., 1993; Broaddus et al., 1994; Larsen et al., 1995; Mulligan et al., 1993). In humans, FK506 suppresses interleukin-8 production in human inflammatory bowel disease and psoriasis patients (Van Hogezand and Verspaget, 1996; Lemster et al., 1995). In addition, it has been reported that FK506 suppresses interleukin-8 receptor expression of keratinocytes (Schulz et al., 1993), suggesting that FK506 may have some effect on interleukin-8 receptor expression as well as interleukin-8 production. In conclusion, FK506 may exert a beneficial effect on such inflammatory diseases by suppressing neutrophil chemotaxis, mostly by an indirect effect, such as inhibition of chemoattractive cytokine production or cytokine receptor expression, but not by a direct effect on neutrophil chemotaxis itself.

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