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# Effect of immunosuppressive agents FK506 and cyclosporin and steroids on the expression of IL-6 and its receptor by stimulated lymphocytes and monocytes

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**Abstract** The interaction of interleukin-6 (IL-6) with its receptor (IL- 6R) is not well understood. In the present study, we investigated the effect of different immunosuppressive agents on the expression of the couple IL-6/IL-6R on cultured lymphocytes and monocytes. IL-6 in culture supernatants from cultured monocytes were analyzed by ELISA. The expression of IL-6R was studied by flow cytometry. Dexamethazone, cyclosporin (CyA), and FK 506 at immunosuppressive concentrations induced a dose-dependent inhibition of IL-6 secretion from adherent monocytes (MO) stimulated with phytohemagglutinin (PHA). Dexamethazone was the most effective agent in inhibiting IL-6 secretion, while the inhibitory effect observed with 1 ng/ml FK 506 was comparable with that obtained with 100 ng/ml CyA. Unstimulated MO strongly expressed IL-6R (80% positive cells). Stimulation of MO with PHA resulted in a significant downregulation of IL-6R expression. Treatment of PHA-stimulated adherent MO with different concentrations of CyA and FK 506 induced a restoration of IL-6R expression. FK 506 was 100 time more effective in restoring IL-6R than CyA. This restoration of IL-6R was incomplete. FK 506, CyA, and steroids may exert their immunosuppressive effect by inhibiting IL-6 secretion and partially restoring MO IL-6R, which may be important in protecting the cell target against IL-6 autocrine stimulation.

**Key words** Monocyte · IL-6 · IL-6 receptor · Immunosuppressive drugs

## Introduction

Although a large body of information has emerged on interleukin-6 (IL-6), the interaction of this cytokine with its receptor is not well understood. The IL-6-receptor (IL-6R) is expressed by human T cells, B cells [1], and monocytes/macrophages [2]. It is also expressed by nonhematopoetic cells such as hepatocytes [2] or kidney cells [3]. IL-6R is subjected to tissue-specific regulation. It has been shown that, upon stimulation with LPS or IL-1, IL-6 secretion is increased while IL-6R expression is downregulated in monocytes [2]. Such regulation has not been observed in T lymphocytes [1] or kidney cells [3]. Recently, it has been demonstrated that the soluble form of IL-6R (sIL-6R) is secreted by stimulated peripheral blood mononuclear cells (PBMC) in culture [4]. It has also been suggested that this molecule may have a regulatory role in many normal or abnormal biologic reactions [5].

The aim of this study was to assess the effect of immunosuppressive agents FK506 and cyclosporin (CyA) on the expression of IL-6 and IL-6R in mitogen-activated lymphocytes and monocytes (MO). The way these drugs affect the expression of IL-6 and its receptor may be determining factors in their mechanism of suppression of the immune response in situations where MO activation is predominant, such as in chronic rejection.

## Materials and methods

### Reagents and antibodies

Phytohemagglutinin (PHA), concavalin A (ConA), and dexamethazone were purchased from Sigma Biochemical (St Louis, M.). CyA was kindley supplied in powder form by Sandoz Japan, and FK506 by Fujisawa Japan, and a stock solution of 2 mg/ml was prepared by dissolving the powder in absolute ethanol for CyA and absolute methanol for FK506. Final dilutions of FK506, CyA, or dexamethazone were made with culture medium. A mouse anti-human IL-6R monoclonal antibody (MT18) was a generous gift from Dr. A. Ogata (Osaka University).

### PBMC and MO preparation and culture

Heparinized venous blood was obtained from healthy donors. Mononuclear cells were separated by the Ficoll-Hypaque method (Pharmacia). PBMC were collected and washed twice with RPMI 1640 medium (Nikken, Kyoto) supplemented with penicillin/streptomycin, counted, and brought to a concentration of $2 \times 10^6$ cells/ml. MO were isolated by the adherence method as previously described [6]. PBMC plated at a concentration of $2 \times 10^6$ cells/ml in culture dishes (Falcon) were cultured in RPMI 1640 medium supplemented with 10% FBS, glutamine, hepes buffer, and antibiotics (Flow, Irvine).

### Cytokine assay

Adherent monocytes were treated for 48 h with PHA 10 μg/ml alone or PHA with different concentrations of CyA, FK506, or dexamethazone (Sigma, St Louis) ($10^{-5}$, $10^{-6}$, $10^{-7}$, $10^{-8}$, $10^{-9}$, $10^{-10}$ *M*). At the end of the incubation time, supernatants were harvested and stored at − 20 °C until assayed for IL-6. IL-6 was determined using an ELISA method (Tosoh, Kanazawa).

### Flow cytometry for the assessment of IL-6R

Cells were treated with PHA 10 μg/ml alone or with different concentrations of CyA or FK506 (100, 10, 1, 0.1 ng/ml) for 72 h. Untreated cells served as controls. At the end of the incubation time, cells were washed twice with PBS. Approximately $10^5$ cells suspended in PBS and 0.5% BASA were allowed to react with mouse anti-human IL-6R mAb (MT18) for 45 min at 4 °C. Cells incubated with irrelevant mouse antibody served as negative controls. The cells were washed twice with PBS, then treated for 30 min at 4 °C with a second antibody – goat $F(ab')_2$ anti-mouse immunoglobulin-PE conjugated mAb (Tago). The expression of IL-6R was assessed by FACS SCAN (Becton Dickinson). A gate on the two major cell populations (lymphocytes and monocytes) was created and each population was studied for the expression of IL-6R.

## Results

### Dexamethazone, CyA, and FK506 inhibited the secretion of IL-6 from cultured human adherent MO

Since IL-6 is mainly secreted by monocytes and macrophages, we investigated the effect of dexamethazone CyA,

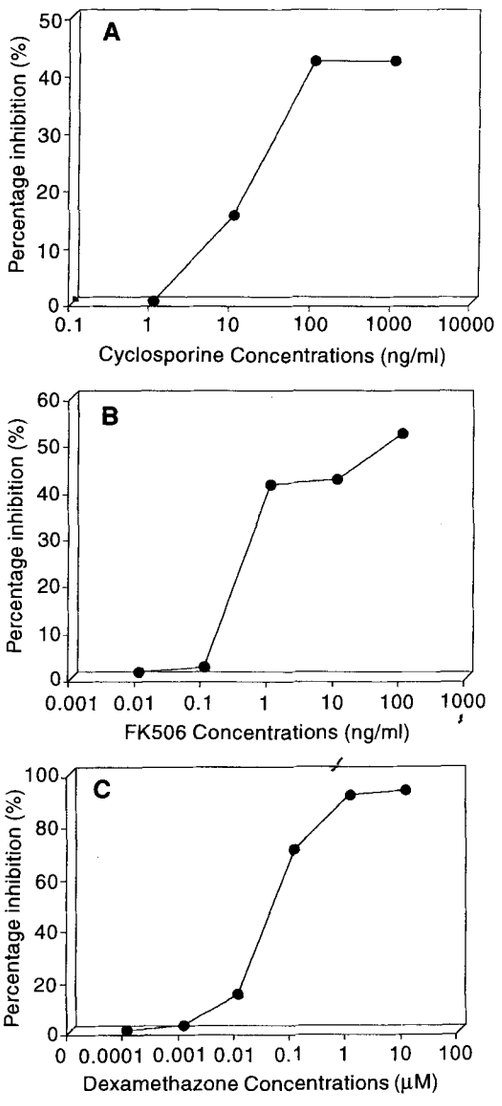


**Fig. 1 A–C** The effect of FK506, cyclosporin, and steroids on the secretion of IL-6 from cultured adherent monocytes stimulated with PHA

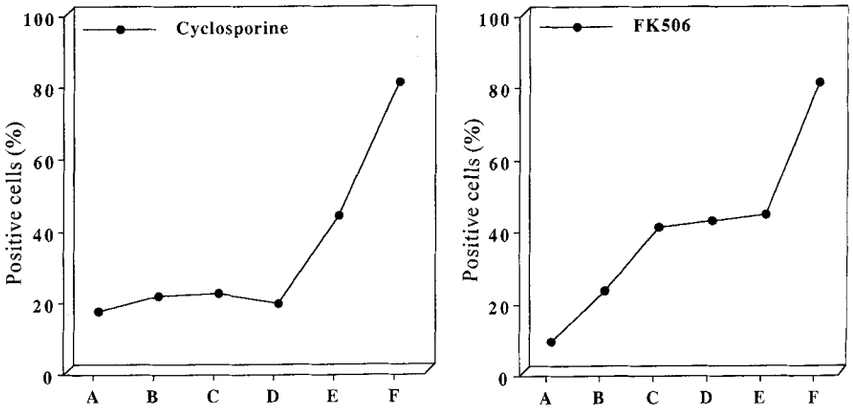


**Fig. 2** The effect of KF 506 and cyclosporin A on the expression of IL-6R in PHA-stimulated monocytes (MO). IL-6R expression was assessed by flow cytometry. *A* PHA-treated MO; *B* PHA-treated MO + FK/CyA 0.1 ng/ml; *C* PHA-treated MO + FK/CyA 1 ng/ml; *D* PHA-treated MO + FK/CyA 10 ng/ml; *E* PHA-treated MO + FK/CyA 100 ng/ml; *F* Unstimulated MO

and FK 506 on the production of this cytokine in cultured adherent MO enriched from PBMC. PHA stimulation of cultured MO induced a dramatic increase in IL-6 secretion (61.29 ng/ml vs. 0.2 ng/ml, 300-fold increase). Figure 1 shows that, at concentrations known to inhibit the immune response in vitro, FK 506 and CyA inhibited the release of IL-6 from PHA-activated monocytes in a dose-dependent manner. FK 506 concentrations of 1 ng/ml induced 41% inhibition of IL-6 secretion. Only a slight inhibitory effect was observed when concentrations were increased to 10, 100, or 1000 ng/ml. At similar concentrations, CyA was less effective than FK 506 in inhibiting IL-6 secretion. The inhibitory effect observed with 100 ng/ml CyA was comparable with that observed with 1 ng/ml FK 506 (Fig. 1 A and B). However, both drugs were far less effective than dexamethazone, which at $10^{-7}$ *M* induced 70% inhibition of IL-6 release from adherent monocytes (Fig. 1 C).

Expression of IL-6R by monocytes and lymphocytes and its regulation by FK 506 and CyA

We assessed the expression of IL-6R in stimulated and unstimulated lymphocytes or monocytes, using specific mAb against IL-6R. The lymphocyte population expressed IL-6R (50% positive cells vs. 8% in negative controls) and this expression was only slightly increased by PHA activation (data not shown). FK 506 and CyA had no effect on the expression of IL-6R in gated lymphocytes (data not shown). Figure 2 shows that unstimulated monocytes (F) expressed high levels of IL-6R (80% positive cells). A downregulation of the expression of IL-6R was observed on PHA stimulation (A), (less than 20% positive cells). Treatment of PHA-activated monocytes with increasing concentrations of CyA restored the expression of this receptor on monocytes. The maximal upregulatory effect on IL-6R expression was observed with 100 ng/ml CyA, whereas only 1 or 10 ng/ml FK 506 were sufficient to reach the maximum upregulatory effect. However, this restoration was not complete even at 1000 ng/ml CyA or FK 506. At the same concentrations, FK 506 was 100 times more effective in restoring IL-6R than CyA.

## Discussion

In noninflammatory conditions, monocytes were not activated and expressed high levels of IL-6R. This allowed them to play a major role in binding IL-6. During monocyte activation, IL-6 was secreted abundantly and its receptor was lost, therefore, target cells were more exposed to IL-6 and its inflammatory effects. Regulation of IL-6R upon stimulation is a tissue-specific event. We have demonstrated, for instance, that unstimulated kidney epithelial cells express IL-6R, but upon stimulation by LPS or IL-1$\beta$, the expression of this receptor is increased [4]. The differential regulation of IL-6R between monocytes and targets cells may have some pathologic implications. During noninflammatory homeostasis, monocytes bind IL-6 on their surface, thereby protecting other target cells from IL-6 activity. When an inflammatory situation occurs, the target for IL-6 is shifted from the monocyte to target cells (kidney cells for instance).

FK 506, CyA, and steroids may exert their immunosuppressive effect by inhibiting IL-6 secretion and partially restoring monocyte IL-6R, which may be important in protecting cell targets against IL-6 autocrine stimulation. We demonstrated recently that sIL-6R at concentrations over 0.5 μg/ml exerted an immunosuppressive

effect on the T lymphocyte proliferative response (data not shown). These results suggest that the expression and the release of IL-6R might be linked to regulatory functions of the immune response during the process of immunosuppression.

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