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**IN VITRO IMMUNOSUPPRESSIVE EFFECTS OF FR 900506  
ON HUMAN T LYMPHOCYTE ALLOACTIVATION**

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**Abstract** FR 900506 (FR) is a new immunosuppressive drug which prolongs allograft survival. Our studies have compared the *in vitro* inhibitory effects of FR and Cyclosporine (CsA) on human lymphocyte proliferation. Considerably lower doses of FR were required to induce inhibition of lymphocyte proliferation induced by concanavalin A (ConA) stimulation or in primary mixed lymphocyte reactions (MLR). Similar differences between FR and CsA were observed with the secondary stimulation of alloactivated T cells generated in MLR or propagated from liver transplant biopsies. These observations provide further evidence that FR is about 500 fold more potent than CsA and may be a useful immunosuppressive agent in organ transplantation.

The new immunosuppressive drug, FR 900506, (FR) greatly prolongs heart, kidney, and liver allograft survival in rats or dogs (1-3). In vitro, FR inhibits the murine mixed leukocyte reaction (MLR) and the generation of human cytotoxic cells (1). Its immunosuppressive effect seems to be mediated through inhibition of Interleukin-2 (IL-2) release and there is also a diminished appearance of IL-2 receptors on activated lymphocytes (1). We describe here a comparison of the in vitro immunosuppressive effects of FR and Cyclosporine (CsA) on human lymphocytes.

## **MATERIALS AND METHODS**

### **Concanavalin A (ConA) Induced Activation**

Human peripheral blood lymphocytes (PBL) were isolated from heparinized blood by Ficoll-Hypaque density gradient centrifugation. Using 96-well round-bottom microplates, these cells ( $10^5$ /well) were incubated at  $37^{\circ}\text{C}$  in 200  $\mu\text{l}$  of tissue culture medium (TCM) supplemented with 10% human serum in the presence of Con-A (10  $\mu\text{g}/\text{ml}$ ) for 72 hours. During the final 20 hours of incubation, each culture was labeled with one  $\mu\text{Ci}$  of  $^3\text{H}$ -thymidine. The cultures were harvested and counted in liquid scintillation counter as previously described (4).

### **MLR-Induced Activation**

Unidirectional human MLR cultures were set up with  $10^5$  responder and  $10^5$  irradiated (2000 R) stimulator cells in a volume of 200  $\mu\text{l}$  TCM supplemented with 10% human

serum for 6 days. Proliferation was assessed by the degree of  $^3\text{H}$ -thymidine incorporation during the final 20 hours of incubation (4).

### **PLT Testing of Alloreactive T Cells**

Alloreactive human T cell clones were generated from in vitro MLR cultures as previously described (4). Alloreactive lymphocyte cultures were also propagated from human liver core biopsies in the presence of interleukin 2 (IL-2) (2). Secondary proliferation of alloreactive cells was assessed in a three-day primed lymphocyte test (PLT), whereby  $10^4$  responder cells were incubated with  $10^5$  irradiated (2000 R) stimulator cells (4). Lymphocyte proliferation was assessed by  $^3\text{H}$ -thymidine incorporation as described above.

### **Drug Sources**

FR was kindly supplied by the Fujisawa Corporation, Osaka, Japan as a crystalline powder. It was dissolved in methanol and kept in  $-4^\circ\text{C}$ . CsA was obtained from Sandoz. It was dissolved in ethanol and kept at  $-4^\circ\text{C}$ .

### **Dose Effects of FR and CsA on Lymphocyte Proliferation**

The immunosuppressive effect of FR and CsA on the Con-A-induced response, MLR reactivity and PLT response of alloreactive lymphocytes was measured at different concentrations of drugs ranging from 0.06 - 500 ng/ml.

The results were expressed as percent inhibition using the formula:

$$\% \text{ inhibition} = \left( 1 - \frac{\text{cpm with drug}}{\text{cpm without drug}} \right) \times 100$$

## RESULTS

Studies on Con-A-induced proliferation showed that FR had a much higher inhibitory effect than CsA. This is illustrated in Figure 1, which shows that significant inhibition was observed at concentrations of FR that were 500 to 1000 fold lower than those of CsA.

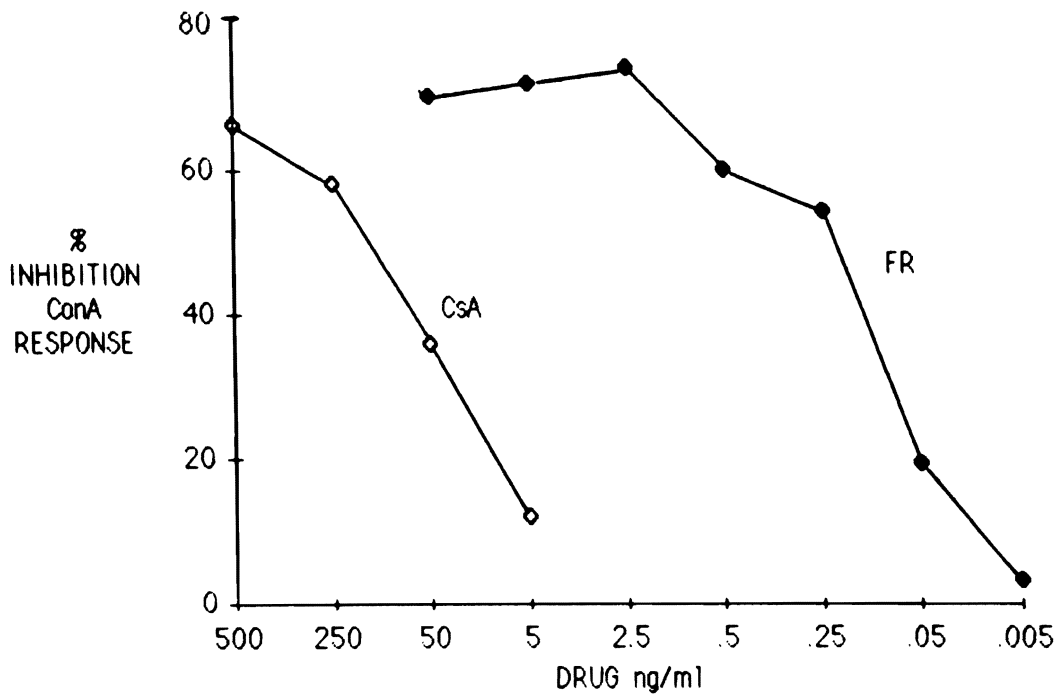


FIGURE 1 Inhibition of ConA induced proliferation of lymphocytes by FR and CsA.

Similar differences were observed in the dose effects of FR and CsA-induced inhibition of the primary MLR (Figure 2).

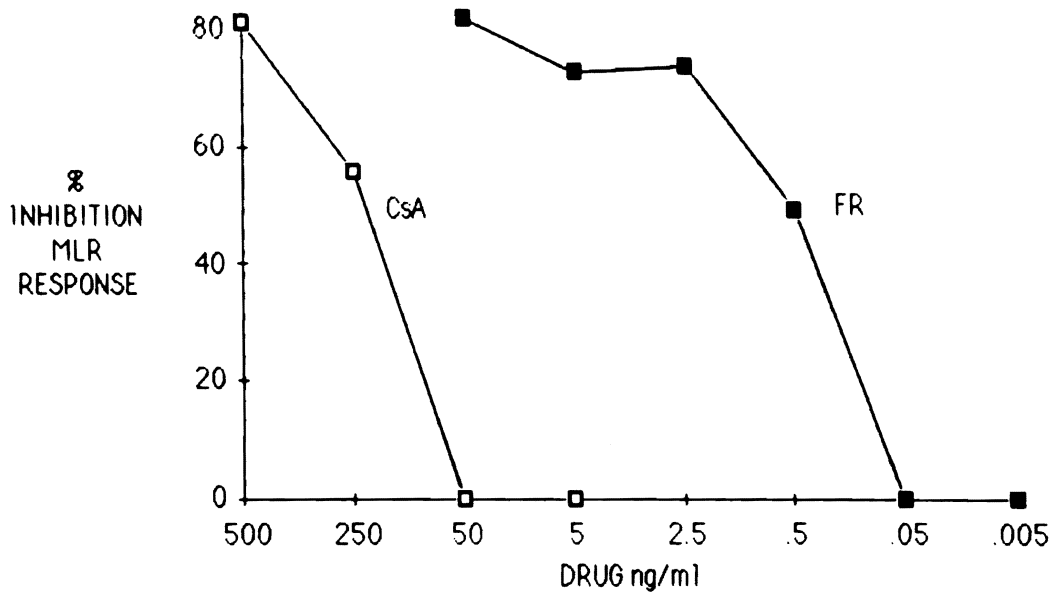


FIGURE 2 Dose effects of FR and CsA on MLR induced lymphocyte proliferation.

Kinetic studies of MLR inhibition by FR showed that the strongest effect was observed when FR was added during the first day of culture (Table 1).

**TABLE I Inhibition of MLR response by FR added at various times during culture**

| FR ug/ml | % Inhibition of MLR Response (FR added on) |       |       |       |
|----------|--|-------|-------|-------|
|          | Day 1                                      | Day 2 | Day 3 | Day 4 |
| .5       | 84   | 55    | 30    | 24    |
| .05      | 68   | 48    | 27    | 0     |
| .005     | 65   | 37    | 20    | 0     |

The inhibitory effects of FR and CsA were also determined in secondary proliferation assays with alloactivated T cells. Figure 3 depicts the findings with an MLR-generated alloreactive T cell clone DB29 which was specific for a DQw1-associated cellular determinant on class II molecules encoded by the HLA-DQ subregion (6).

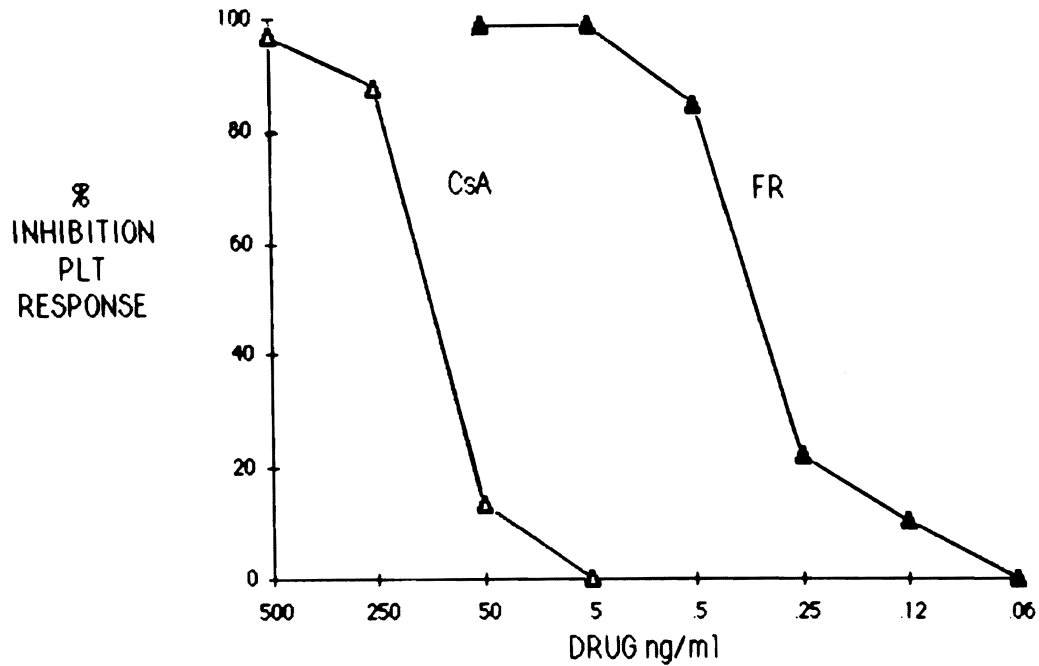


FIGURE 3 Immunosuppressive effect of FR and CsA on the PLT reactivity of alloreactive T cell clone DB29.

It may be seen that significant inhibition of the PLT response of DB29 occurred at about 300-fold lower concentrations of FR than of CsA. Similar differences between FR and CsA were observed with the donor-specific PLT response of alloactivated T cells propagated from a liver transplant biopsy (Figure 4).

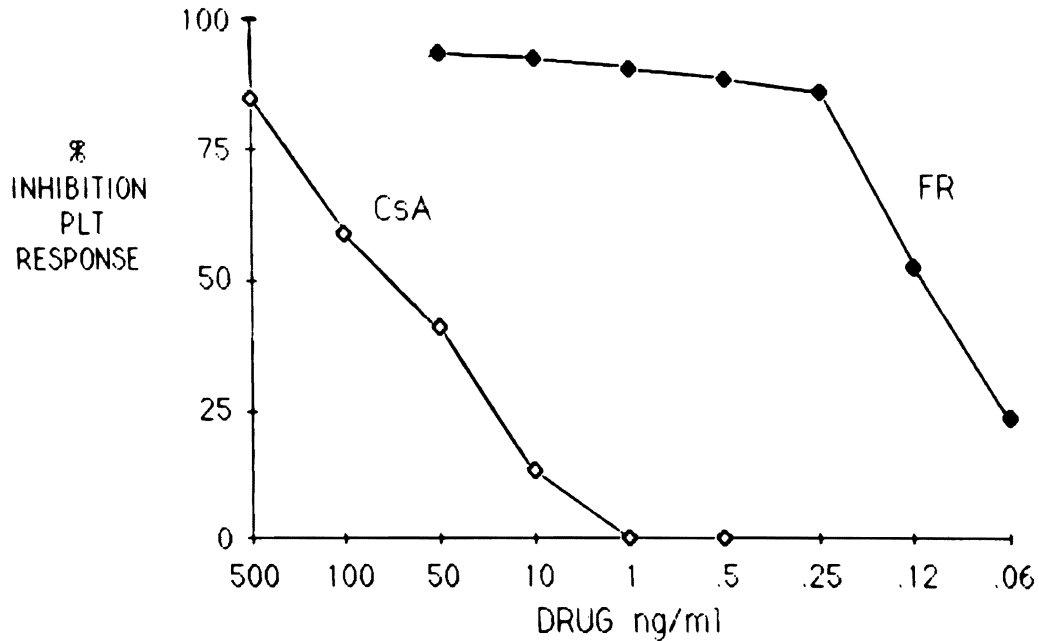


FIGURE 4 Inhibition of donor specific PLT response of alloreactive T cells propagated from a liver transplant biopsy.

## **DISCUSSION**

These results extend the original observations by Ochiai *et al.* (1), that FR has a potent *in vitro* suppressive effect on human lymphocyte activation. This was observed not only in primary activation of lymphocytes induced by Con-A or in MLR but also in secondary stimulation of alloactivated T cells generated in MLR or propagated from liver transplant biopsies. Thus, FR not only suppresses T lymphocyte alloactivation but also inhibits the alloantigen induced expansion of activated T cells including cells which may be found in cellular infiltrates of organ transplants. On the other hand, FR has no effect on the IL2-induced proliferation of alloactivated T cells



(5). This is compatible with the concept that the mechanism of the immunosuppressive effect of FR is mediated through an inhibition of IL2 release (1). Indeed, preliminary studies in our laboratory also suggest that FR blocks IL2 release (unpublished observations). The mechanism of FR seems similar to that of CyA.

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