Immunology 1990 **70** 398–404

The influence of FK-506 on the thymus: an immunophenotypic and structural analysis

R. G. P. PUGH-HUMPHREYS,* C. S. K. ROSS & A. W. THOMSON *Immunopathology Laboratory, Departments of Pathology and *Zoology, University of Aberdeen, Aberdeen*

Accepted for publication 16 March 1990

SUMMARY

The influence of the powerful new immunosuppressant FK-506 on the thymus was investigated in Sprague–Dawley rats that were immunized with sheep erythrocytes and treated with FK-506 (1 mg/kg/day i.m.) for 7 days. Suppression of humoral immunity in drug-treated animals was accompanied by reductions in circulating lymphocytes bearing activation markers (interleukin-2 receptor β-chain and OX40, activated CD4$^+$ cells) and by striking thymic medullary atrophy. There were, however, no significant differences in thymic weights or in thymocyte numbers between experimental and control groups during the period of FK-506 administration. Reduction of the medullary compartment was visualized immunohistochemically, by decreases in major histocompatibility complex (MHC) class I- and MHC class II-positive cells and in CD37$^+$ (mature medullary) thymocytes. Flow cytometric analysis of thymocytes showed that FK-506 induced increases in bright, Thy-1.1$^+$ cells and in numbers of CD4$^+$ and CD8$^+$ thymocytes, whilst CD37$^+$ cells were less numerous than in controls. Percentages of MHC class I- and MHC class II-positive cells varied little throughout the course of FK-506 administration. Evidence of selective damage to medullary epithelial cells, attributable to FK-506, was found at both the light and electron microscopic levels, whilst thymic macrophages in drug-treated rats displayed features of enhanced phagocytic activity, including ingestion of damaged epithelial cells. These FK-506-induced abnormalities were reversed within 14 days of drug withdrawal. These findings suggest that, like cyclosporin A, FK-506 reversibly disrupts the thymic microenvironment and may interfere with the function/maturation of T lymphocytes.

INTRODUCTION

FK-506 is a potent new anti-lymphocytic and immunosuppressive agent extracted from the fermentation broth of the soil fungus *Streptomyces tsukubaensis* (Kino *et al.*, 1987a). It is a macrolide antibiotic (822 MW), totally distinct in structure from the cyclic peptide cyclosporin A (CsA), but with the latter it shares a selective, inhibitory action on CD4$^+$ T-lymphocyte activation (Thomson, 1989). Notably, however, FK-506 inhibits the expression of a variety of murine and human cytokine genes, including those coding for interleukin-2 (IL-2), IL-3, IL-4, granulocyte–macrophage colony-stimulating factor (GM-CSF) and interferon -gamma (IFN-γ) (Kino *et al.*, 1987b; Yoshimura *et al.*, 1989; Tocci *et al.*, 1989), at at least 100-fold lower concentrations than CsA. Furthermore, FK-506 suppresses both humoral (Kino *et al.*, 1987a; Woo, Stephen & Thomson, 1988; Woo *et al.*, 1990a; Propper *et al.*, 1990) and cell-mediated immunity (Kino *et al.*, 1987a; Inamura *et al.*, 1988) in experimental animals at considerably lower doses than CsA. FK-506 is also highly effective in prolonging organ allograft survival in a variety of species, including rodents, dogs, non-human primates and man (Ochiai *et al.*, 1987a, b; Todo *et al.*, 1988; Starzl *et al.*, 1989; Thomson, 1990b). Although differential toxicity has been reported in various non-human species, preliminary clinical experience of FK-506 in organ transplantation indicates that the drug is well tolerated and may be free of major short-term side-effects, including nephrotoxicity (Starzl *et al.*, 1989; Thomson, 1990a).

In previous studies, it has been shown that the inhibitory action of FK-506 on immune reactivity is accompanied by structural changes within the thymus, in particular thymic medullary atrophy (Stephen *et al.*, 1989). Since effects of FK-506 on T-cell maturation/functional differentiation may contribute to its immunomodulatory activity, a detailed, sequential immunophenotypic and structural analysis of rat thymuses has been conducted during the course of systemic FK-506 administration to immunized rats and following its withdrawal.

Abbreviations: FCS, fetal calf serum; MNL, mononuclear leucocytes; PBS, phosphate-buffered saline; SRBC, sheep red blood cells.

Correspondence: Dr A. W. Thomson, Immunopathology Laboratory, Dept. of Pathology, University of Aberdeen, Foresterhill, Aberdeen AB9 2ZD, U.K.

MATERIALS AND METHODS

Animals

Male Sprague–Dawley rats (mean initial body weight 235 g) were purchased from Charles River, Manston Road, Margate, Kent.

Drugs

FK-506 (Fujisawa Pharmaceutical Co. Ltd, Osaka, Japan; Lot 011504) was provided in powder form and dissolved in absolute methanol. A 10% w/v solution of the FK-506-containing methanol in olive oil (Boots Company Ltd, Nottingham, Notts) was prepared, the methanol evaporated off at 60° for 5 min and the solution injected (0·1 ml; 1 mg/kg/day) intramuscularly (i.m.) into one of the four limbs, with daily rotation.

Immunization

Sheep red blood cells (SRBC), prepared as previously described (Woo *et al.*, 1988), were administered (10^9) by a single intraperitoneal injection.

Experimental protocol

Groups of six rats were immunized on Day 0 and treated with FK-506 from Days 0 to 6, inclusive. An olive oil vehicle-treated control group was investigated in parallel. Measurements of antibody titres were performed on samples of tail blood and were undertaken immediately before immunization (Day 0) and on Days 4, 7, 14 and 21. At the same time-points, groups of animals were killed by terminal ether anaesthesia. On Day 4, spleen cell suspensions were prepared for estimation of antibody plaque-forming cells (see below). Blood (removed by cardiac puncture) and thymuses were taken at all time-points for flow cytometric analysis. In addition, blocks of thymic tissue were taken for histological (including immunohistological) or electron microscopic examination (see below).

Preparation of cell suspensions

Mononuclear leucocytes (MNL) were isolated from blood anticoagulated with acid citrate dextrose by centrifugation over Ficoll Isopaque (density 1·077; Nyegaard, Oslo, Norway). Suspensions of blood MNL or thymocytes were prepared as described previously (McIntosh *et al.*, 1986). Contaminating red cells were lysed in 0·83% w/v NH_4Cl, pH 7·2. The washed cells were resuspended at 10^7/ml in RPMI-1640 with 10% v/v fetal calf serum (FCS; Gibco, Paisley, Renfrewshire).

Immunofluorescence staining

An indirect immunofluorescence method (Stephen *et al.*, 1989) was used to demonstrate cell phenotypes, using appropriate primary mouse anti-rat monoclonal antibodies (Serotec Ltd, Bicester, Oxfordshire) at the following dilutions: OX19 ($CD5^+$ T cells), 1:500; W3/25 ($CD4^+$ helper/inducer T cells, macrophages), 1:500; OX8 ($CD8^+$ cytotoxic/suppressor T cells, natural killer cells), 1:400; OX7 (Thy-1.1^+ T cells), 1:250; OX44 ($CD37^+$ mature medullary thymocytes), 1:500; OX40 (activated $CD4^+$ cells, a gift from Dr A. F. Williams; University of Oxford) 1:400; OX12 (κ-light chains, pan B cells), 1:500; OX18 (MHC class I monomorphic), 1:500; OX6 (MHC class II monomorphic), 1:300; OX39 (CD25; IL-2 receptor; IL-2R β-chain gp55), 1:300. Lymphocyte analysis was performed using an 'EPICS C' microcytofluorograph (Coulter Electronics, Luton, Beds).

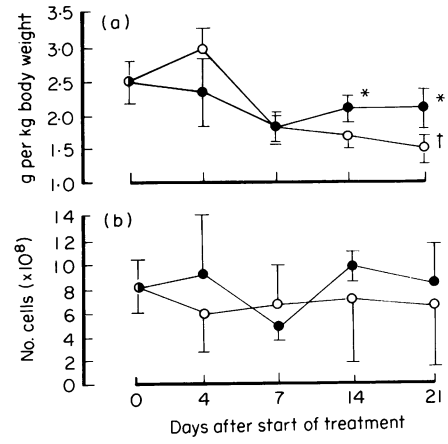

Figure 1. (a) Thymic weights and (b) total numbers of thymocytes in rats given FK-506 (1 mg/kg) (●) or drug vehicle (○) from Days 0 to 6, inclusive. Symbols indicate significance of differences from vehicle-treated controls ($*P<0·05$) or pretreatment values ($\dagger P<0·05$). Six animals per group.

Immunohistochemistry

Immunohistochemical staining of cryostat sections was by an indirect procedure, using the appropriate primary mouse monoclonal antibody and a secondary, peroxidase-conjugated rabbit anti-mouse Ig (Dakopatts, Copenhagen, Denmark).

Electron microscopy

Samples of thymuses were fixed in 0·5% v/v glutaraldehyde, then rinsed 0·1 M phosphate buffer prior to further fixation in 1% w/v osmium tetroxide. The samples were then dehydrated in graded ethanols and immersed in propylene oxide before embedding in Epon (Taab Laboratories, Reading, Berks). Thin sections were doubly stained with uranyl acetate and lead citrate and then examined within a Philips 301 transmission electron microscope.

Antibody-producing cells and circulating antibody

Single, antibody (IgM)-producing plasma cells in spleen cell suspensions were estimated by the plaque assay of Cunningham & Szenberg (1968). Total haemagglutinin titres to SRBC in heat-inactivated (56°, 30 min) serum samples were determined by standard procedures, using a Titertek multiple diluter and U-shaped, 96-well plates.

Statistics

The significances of differences between means were determined using the Student's *t*-test for dependent or independent means, as appropriate.

RESULTS

Effects of FK-506 on thymic weights and thymocyte numbers

During the course of FK-506 administration (Days 0–6), there were no significant differences in thymic weights (expressed on a body weight basis) between experimental and control groups (Fig. 1a). By Day 21, a reduction in thymic weights, compared to pretreatment values, was observed in the control but not in the FK-506-treated group. Indeed, organ weights were significantly

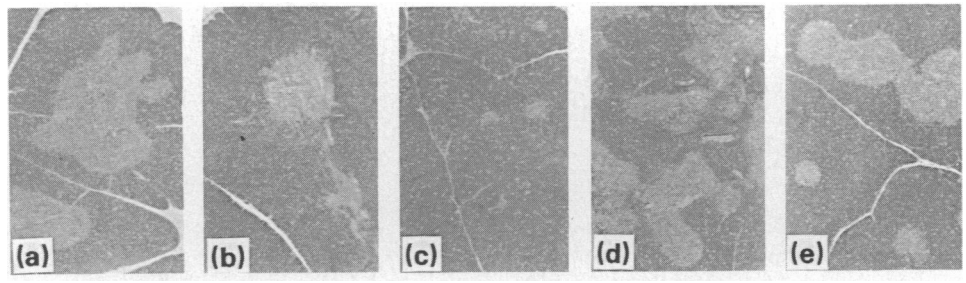

Figure 2. Reversible thymic medullary atrophy in FK-506-treated rats. (a) Day 0, (b) Day 4, (c) Day 7, (d) Day 14, (e) Day 21 after start of treatment. FK-506 administered from Days 0 to 6, inclusive.

higher ($P < 0.05$) in the latter compared with the former group on Days 14 and 21. The total number of cells within the thymus however, was similar in both FK-506 and control groups throughout the experimental period (Fig. 1b).

Appearance and immunohistochemical analysis of the thymus

FK-506 administration resulted in a progressive and marked reduction in the thymic medulla; by Day 7, the medulla was almost totally depleted, whereas the cortex was comparatively preserved (Fig. 2). Following FK-506 withdrawal, the medullary atrophy was reversed and reconstitution of the medulla appeared complete by Day 21.

Virtually all thymocytes in normal and drug-vehicle treated rats stained with OX7 (Thy-1.1); W3/25$^+$ (CD4$^+$) and OX8$^+$ (CD8$^+$) cells were also uniformly distributed throughout the thymus. Strongly stained OX6$^+$ dendritic cells were observed in the medulla, which also contained the majority of strongly stained OX18$^+$ (MHC class I$^+$) thymocytes and also a proportion of OX44$^+$ (CD37$^+$) cells of varying staining intensity. FK-506 treatment did not significantly affect the staining pattern observed for Thy-1.1, CD4 or CD8, but the reduction of the medulla could be visualized by decreases in MHC class I-, MHC class II- and CD37-positive cell numbers.

Flow cytometric analysis of thymocytes

The incidences of Thy-1.1$^+$, CD4$^+$, CD8$^+$ and CD37$^+$ thymocytes in FK-506- and vehicle-treated rats determined by flow cytofluorometry are shown in Fig. 3. Although FK-506 had no significant effect on the percentage of Thy 1.1$^+$ cells, the peak of cell membrane fluorescence intensity shifted to the right (increased) during drug administration, but reverted to normal by Day 21. Increases in the incidences of CD4$^+$ and CD8$^+$ cells above normal values were observed during FK-506 administration, although the change was statistically significant only with respect to CD8 (Day 7). No difference in fluorescence intensity for either marker was found between the groups at any time-point. The minor incidence of CD37$^+$ cells did not change with FK-506 treatment, but was significantly lower than that of the control group, which showed minor increases between Days 0 and 14. Percentages of MHC class I- and MHC class II-positive thymocytes varied very little throughout the course of FK-506 treatment, and no significant differences in fluorescence intensity were observed between the groups (data not shown).

Histological and ultrastructural features

Epithelial cells. Light microscopic examination of thymuses from FK-506-treated rats at the time of maximal medullary atrophy (Day 7) revealed that approximately 10% of the medullary epithelial cells had marked cytoplasmic compaction and eosinophilia; moreover, the nuclei were hyperchromatic, shrunken and flattened. The other epithelial cells had a normal appearance (approximately 30%) or were swollen (approximately 60%), with very vacuolated cytoplasm and reduced cytoplasmic eosinophilia. Epithelial cells of vehicle-treated rats did not show these features (Fig. 4a, b). Swollen epithelial cells, containing remains of necrosed thymocytes were also observed in the thymic cortex of drug-treated but not vehicle control rats (Fig. 4c, d).

At the ultrastructural level, the cortical and medullary epithelial cell injury attributable to FK-506 was reflected in a

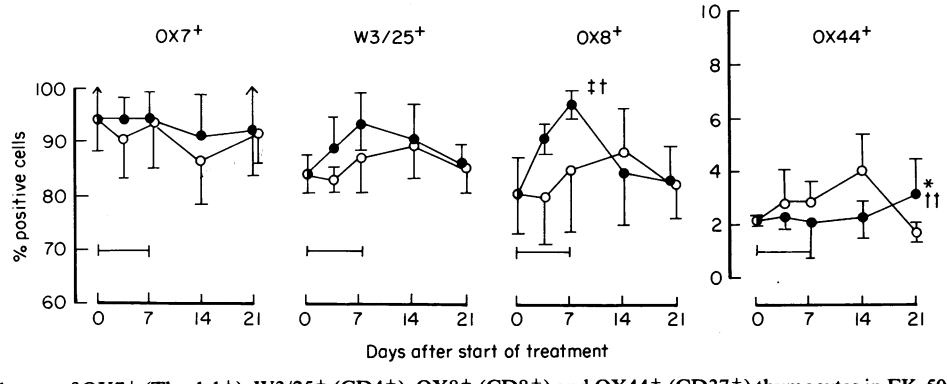

Figure 3. Incidences of OX7$^+$ (Thy-1.1$^+$), W3/25$^+$ (CD4$^+$), OX8$^+$ (CD8$^+$) and OX44$^+$ (CD37$^+$) thymocytes in FK-506 (●), or drug vehicle-treated (○) rats. FK-506 was administered from Days 0 to 6 inclusive (bar). Symbols indicate significance of differences from vehicle-treated controls: †$P < 0.05$; *$P < 0.01$) or pretreatment values (††$P < 0.05$; ‡‡$P < 0.01$).

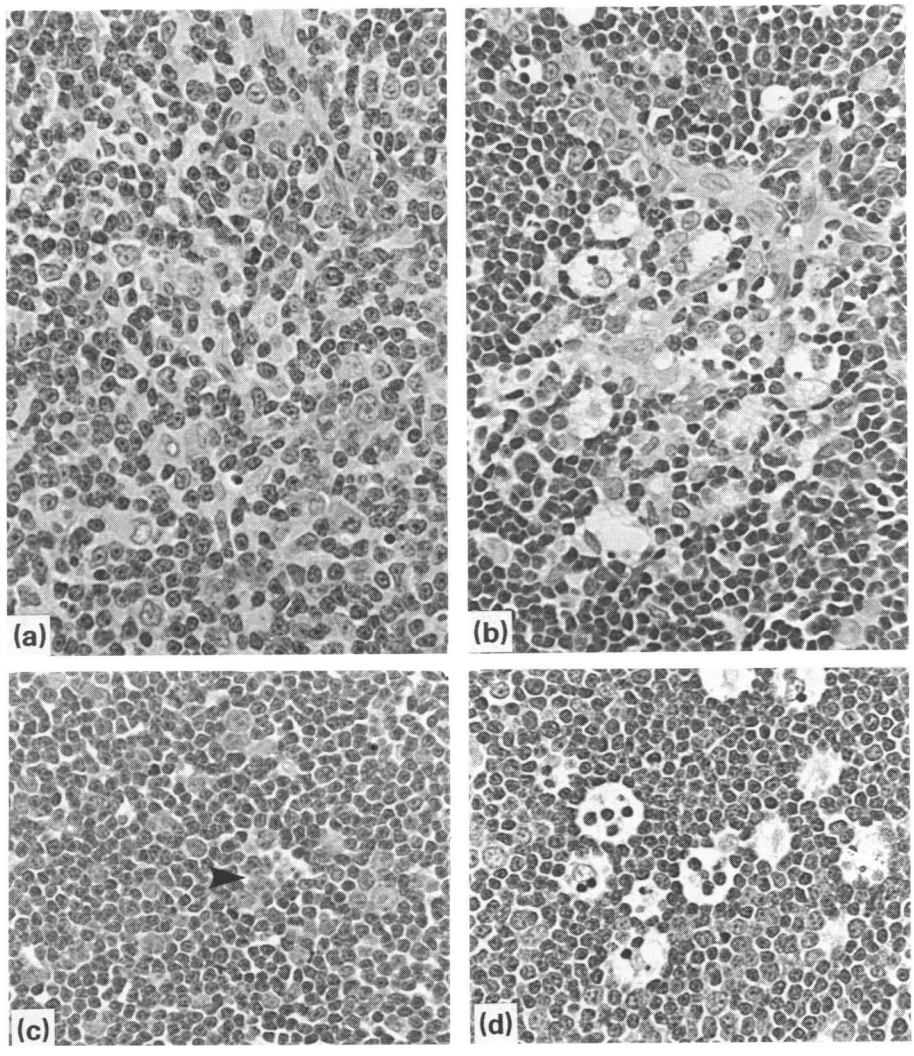

Figure 4. (a) Medulla of vehicle-treated rat, showing a reticulum of predominantly epithelial cells with interspersed thymocytes; (b) atrophied medulla of FK-506-treated rat, showing sparse and swollen epithelial cells. (c) Thymic cortex of drug vehicle-treated rat, showing predominance of normal thymocytes, fewer epithelial cells and a probable thymic 'nurse' cell (arrow), with perinuclear clustering of lymphocytes; (d) cortex of FK-506-treated rat, showing numerous swollen cells containing remains of, presumably, necrosed thymocytes. (a–d) × 384.

marked rarifaction of cytoplasmic electron density, coarse cytoplasmic vacuolation, elevation in lysosome numbers and clumping of cytokeratin filaments (Fig. 5). Membrane-bound inclusions, presumed to be phagosomes and containing a variety of inclusions, were frequently observed within the affected cortico-medullary epithelial cells of the FK-506-treated rats (Fig. 5c, d). Although phagosome-like structures were also observed within the epithelial cells of vehicle-treated animals (Fig. 5a), these inclusions were smaller, more uniform in size and less heterogeneous in ultrastructural appearance than those observed within the epithelial cells of FK-506-treated animals. In some instances, thymocytes within dug-treated rats were observed enveloped by damaged cortico-medullary epithelial cells (Fig. 5d). Of the reticular epithelial cells within the cortex of FK-506-treated rats, the majority (approximately 70%) exhibited the swollen appearance described for medullary epithelial cells, but no other features of damage. Within 14 days of drug withdrawal, the epithelial cells within both the medulla and cortex appeared normal.

Thymocytes. In marked contrast to the abnormalities observed within the epithelial cells of FK-506-treated rats, neither cortical nor medullary thymocytes within these animals displayed any overt ultrastructural abnormalities not seen in vehicle-treated animals.

Macrophages. 'Tingible bodies' containing remnants of phagocytosed thymocytes were a conspicuous feature of approximately 10% of cortical macrophages within thymuses of vehicle-treated rats (Fig. 5b). In FK-506-treated rats (Days 4 and 7), however, cortical macrophages were more frequently vacuolated, their cytoplasm had a reduced electron opacity and the phagosomes contained a wider variety of inclusions than those seen within phagosomes of cortical macrophages in vehicle-treated rats (Fig. 5e, f). The phagocytosed material was presumed, because of the presence of cytokeratin filaments, to

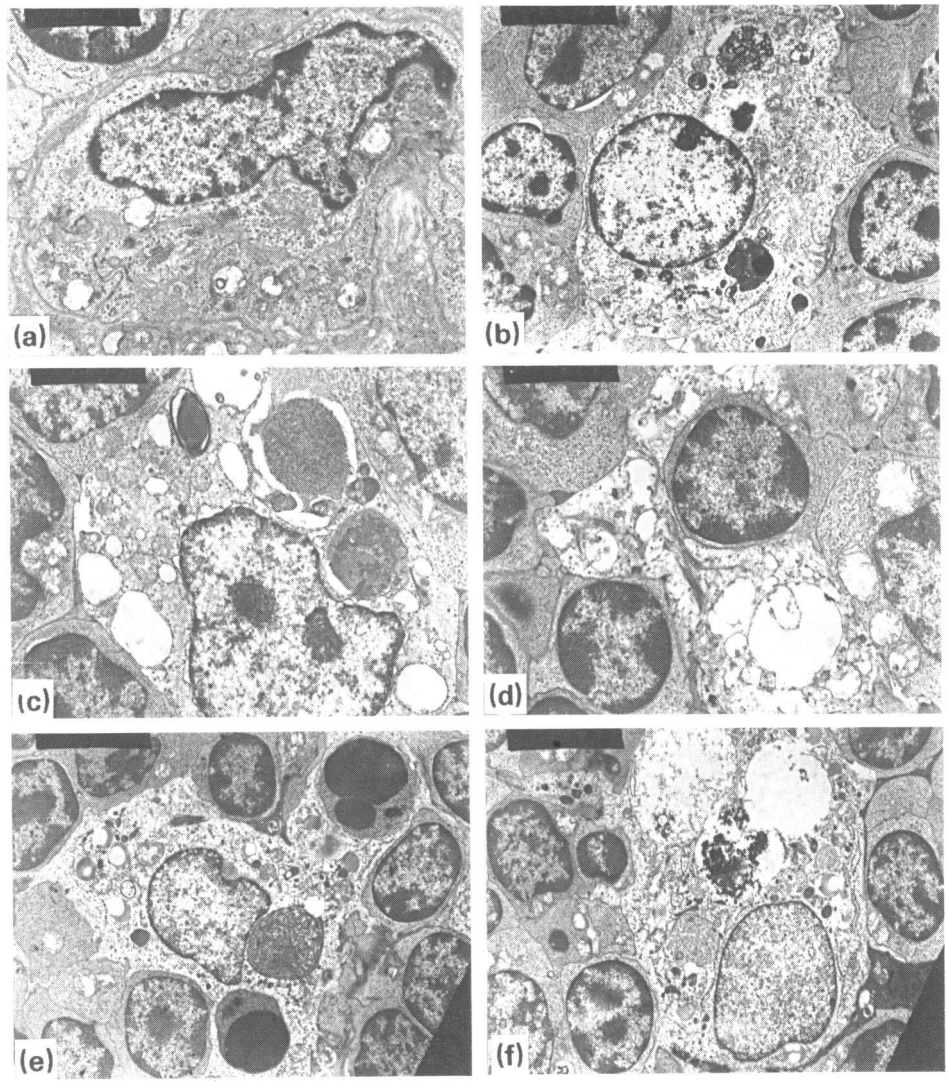

Figure 5. Electronmicrographs of thymus cells, showing damage attributable to FK-506 within the medulla and cortex. (a) Typical reticular epithelial cell and (b) characteristic 'tingible body' macrophage within the thymic cortico-medullary zone of drug vehicle-treated controls. (c)–(f) FK-506-treated rats (Day 4). (c) Highly vacuolated, damaged cortico-medullary epithelial cell with phagosomes containing material of variable morphology. (d) Thymocytes surrounded by a very vacuolated cortico-medullary epithelial cell. (e)–(f) Cortical macrophages showing numerous inclusions, including necrosing thymocytes. (a) × 5850; (b), (e), (f) × 2925; (c), (d) × 4375.

be the remnants of damaged thymic epithelial cells. No similar inclusions were observed within medullary macrophages of vehicle-treated rats.

Immunosuppressive effect of FK-506. FK-506 exerted a powerful suppressive effect on antibody production to SRBC. Numbers of splenic direct IgM plaque-forming cells on Day 4 were reduced (88%) from $51{\cdot}4 \pm 12{\cdot}5 \times 10^3$/spleen in vehicle-treated controls, to $6{\cdot}3 \pm 3{\cdot}3 \times 10^3$/spleen ($P < 0{\cdot}01$) in the FK-506 group. Peripheral blood B cells on Day 7 were not so elevated in FK-506-treated rats ($33{\cdot}3 \pm 9{\cdot}7\%$) compared with controls ($57{\cdot}4 \pm 4{\cdot}8\%$; $P < 0{\cdot}01$), although both values were significantly higher than normal ($20{\cdot}3 \pm 7{\cdot}6\%$; $P < 0{\cdot}01$). The inhibitory effect of FK-506 on activation antigen expression on blood lymphocytes, determined by flow cytometry, is shown in Table 1. The increase in incidence of $OX40^+$, $OX39^+$ and $OX6^+$ lymphocytes observed following immunization (Day 7) was significantly inhibited by FK-506.

DISCUSSION

The only previous reports concerning the effects of FK-506 on the thymus have been restricted to histological observations of reversible medullary atrophy (Nalesnik *et al.*, 1987; Stephen *et al.*, 1989), similar in appearance to those described for CsA (Blair *et al.*, 1982; Beschorner *et al.*, 1987). The present findings show that at an immunotherapeutic dosage, FK-506 inflicted differential cytopathic effects on thymic cortical and medullary epithelial cells and on thymic macrophages, whilst no structural abnormalities attributable to the drug were observed in thymocytes. Nevertheless, immunocytochemical evidence was obtained of interference by FK-506 with thymocyte differentiation and maturation. All these changes were reversed within 2 weeks of drug withdrawal.

The differential injurious effect of FK-506 on medullary epithelial cells is consistent with the atrophy of the medullary

Table 1. Influence of FK-506 on lymphocyte activation antigen expression in blood

Treatment	Day	Activation antigen: % positive cells		
		OX40 (activated CD4$^+$ cells)	OX39 (IL-2 receptor)	OX6 (MHC class II)
Untreated	0	0·9±0·3	0·5±0·1	12·3±6·3
Control	7	1·5±0·6	1·2±0·2	43·2±5·6
FK-506	7	0·8±0·4†	0·5±0·3*	23·3±5·9**
Control	14	1·6±0·4	1·8±0·1	16·7±10·5
FK-506	14	2·2±1·3	1·6±0·5	27·7±9·5
Control	21	1·9±0·6	1·7±0·8	24·7±6·6
FK-506	21	2·0±0·8	1·3±0·5	23·4±9·1

FK-506 was administered from Days 0 to 6, inclusive.
Results are means ± 1 SD obtained from groups of six rats.
† $P<0·05$; * $P<0·02$; ** $P<0·001$ compared with vehicle-treated controls.

compartment. It appears that these cells are eliminated intrathymically, via phagocytosis by macrophages, within which remnants of epithelial cells are readily detected. The relative increase in cortical thymocytes, which are considered to be less mature than their medullary counterparts (Mason *et al.*, 1983) is consistent with the relative increase in bright OX7$^+$ (Thy-1.1$^+$) cells during FK-506 administration—a finding that has also recently been made in CsA-treated rats (Tanaka *et al.*, 1988). The observation of increased numbers of CD4$^+$ CD8$^+$ thymocytes is also consistent with interference by FK-506 in thymocyte differentiation and reduction in cell migration from cortex to medulla. Similar findings have been made in thymuses of CsA-treated rats (Kanariou *et al.*, 1989), whilst interference with rat peripheral T-cell activation at the CD4$^+$ CD8$^+$ stage has been described by Bevan & Chisholm (1986). Moreover, there is evidence that CsA interferes with thymocyte differentiation in foetal thymic organ cultures *in vitro* (Matsuhashi, Kuwase & Suzuki, 1989).

Interference with peripheral blood T-cell activation by FK-506 in immunized animals was confirmed in this study by the reduction in blood-borne activated CD4$^+$ T cells and IL-2R$^+$ cells. Similar effects of FK-506 on human blood T-cell activation *in vitro* have been reported (Woo, Sewell & Thomson, 1990b). Although the present studies also show that FK-506 inhibits the increase in MHC class II-positive blood lymphocytes following immunization (a finding which this laboratory has found to reflect increases in circulating B cells), no concomitant changes were observed in the intensity of intrathymic class II MHC antigen expression (thymocytes or epithelial cells). The numbers of OX6 (MHC class II-positive) epithelial cells in the medulla were reduced, however, a finding which could, as has been suggested for CsA (Cheney & Sprent, 1985), reduce the efficiency by which self-reactive T cells are eliminated (Fowlkes & Pardoll, 1989). A further consideration is that FK-506 may, like CsA (Thomson & Duncan, 1989), interfere with antigen presentation by dendritic cells. No data, however, are yet available concerning the influence, if any, of FK-506 on antigen presentation. The observed maintenance of MHC class II expression on thymic cortical and residual medullary epithelial cells (which do not constitutively express these antigens) under cover of FK-506 does suggest that sufficient IFN-γ is available within the thymic microenvironment in these immunized rats, despite evidence that FK-506 inhibits production of this and other cytokines by stimulated CD4$^+$ T cells (Kino *et al.*, 1987b; Yoshimura *et al.*, 1989; Tocci *et al.*, 1989). In a previous study, CsA has been shown not to decrease Ia molecule expression on thymic stromal cells in culture (Takeuchi *et al.*, 1989).

In addition to the expression of MHC class II antigens, thymic epithelial cells produce thymic hormones (thymulin, thymosin and thymopoietin) that stimulate T-cell differentiation (Savino & Dardenne, 1984) within the thymic microenvironment. Whilst the present studies clearly show that FK-506 injures epithelial cells, the influence of the drug on hormone secretion by these cells and on cellular interactions between thymocytes and thymic stromal cells (in particular epithelial cells) requires more intensive investigation.

Although FK-506 and CsA appear to share many properties, these two anti-T cell agents differ markedly in potency; moreover, they bind to distinct cytosolic proteins (Harding *et al.*, 1989) and the inhibitory action of FK-506 on T-cell activation is more difficult to reverse than that of CsA (Tocci *et al.*, 1989; Kay *et al.*, 1989). Further studies are already in progress to further elucidate the influence of FK-506 on the functional maturation of T cells and the maintenance of self-tolerance.

ACKNOWLEDGMENTS

We thank the Fujisawa Pharmaceutical Co. for providing FK-506, Mr J. I. Milton for operating the flow cytometer and the staff of the electron microscopy unit, Department of Pathology for technical assistance. The manuscript was typed by Mrs I. M. Watson. The work of the authors' laboratory is supported by grants from the Medical Research Council, the Cancer Research Campaign and the Wellcome Trust.

REFERENCES

Beschorner W.E., Namnoum J.D., Hess A.D., Shinn C.A. & Santos G.W. (1987) Cyclosporin A and the thymus: immunopathology. *Am. J. Pathol.* **126,** 487.

BEVAN D.J. & CHISHOLM P.M. (1986) Co-expression of CD4 and CD8 molecules and *de novo* expression of MHC class II antigens on activated rat T cells. *Immunology*, **59,** 621.

BLAIR J.T., THOMSON A.W., WHITING P.H. & SIMPSON J.G. (1982) Toxicity of the immune suppressant cyclosporin A in the rat. *J. Pathol.* **138,** 163.

CHENEY R.T. & SPRENT J. (1985) Capacity of cyclosporine to induce autograft-versus-host disease and impair intrathymic T cell differentiation. *Transplant. Proc.* **17,** 528.

CUNNINGHAM A. & SZENBERG A. (1968) Further improvement in the plaque assay for detecting single antibody-producing cells. *Immunology*, **14,** 599.

FOWLKES B.J. & PARDOLL D.M. (1989) Molecular and cellular events of T cell development. *Adv. Immunol.* **44,** 207.

HARDING M.W., GALAT A., UEHLING D.E. & SCHREIBER S.L. (1989) A receptor for the immunosuppressant FK-506 is a *cis-trans* peptidyl-prolyl isomerase. *Nature (Lond.)*, **341,** 758.

INAMURA N., NAKAHARA K., KINO T., GOTO T., AOKI H., YAMAGUCHI I., KOHSAKA M. & OCHIAI T. (1988) Prolongation of skin allograft survival in rats by a novel immunosuppressive agent FK-506. *Transplantation*, **45,** 206.

KANARIOU M., HUBY R., LADYMAN H., COLIC M., SIVOLAPENKO G., LAMPERT I. & RITTER M. (1989) Immunosuppression with cyclosporin A alters the thymic microenvironment. *Clin. exp. Immunol.* **78,** 263.

KAY J.E., BENZIE C.R., GOODIER M.R., WICK C.J. & DOE S.E.A. (1989) Inhibition of T-lymphocyte activation by the immunosuppressive drug FK-506. *Immunology*, **67,** 473.

KINO T., HATANAKA H., HASHIMOTO M., NISHIYAMA M., GOTO T., OKUHARA M., KOHSAKA M., AOKI H. & IMANAKA H. (1987a) FK-506, a novel immunosuppressant isolated from a *Streptomyces*. I. Fermentation, isolation and physicochemical and biological characteristics. *J. Antibiot.* **40,** 1249.

KINO, T., HATANAKA H., MIYATA S., INAMURA N., NISHIYAMA A., YAJIMA T. *et al.* (1987b) FK-506, a novel immunosuppresant isolated from a *Streptomyces*. II. Immunosuppressive effect of FK-506 *in vitro*. J. Antibiot. **40,** 1256.

MCINTOSH L.C., MORRICE L.M., UDAGAWA Y. & THOMSON A.W. (1986) The influence of tumour carriage on the production of lymphokines affecting macrophage behaviour. *Immunology*, **57,** 359.

MASON D.W., ARTHUR R.P., DALLMAN M.J., GREEN J.R., SPICKETT G.P. & THOMAS M.L. (1983) Functions of rat T-lymphocyte subsets isolated by means of monoclonal antibodies. *Immunol. Rev.* **74,** 426.

MATSUHASHI N., KUWASE Y. & SUZUKI G. (1989) Effects of cyclosporine A on thymocyte differentiation in fetal thymus organ culture. *Cell. Immunol.* **123,** 307.

NALESNIK M.A., TODO S., MURASE N., GRYZAN S., LEE P.H., MAKOWKA L. & STARZL T.E. (1987) Toxicology of FK-506 in the Lewis rat. *Transplant. Proc.* **19** (Suppl. 6), 89.

OCHIAI T., NAGATA M., NAKAJIMA K., SUZUKI T., SAKAMOTO K., ENOMOTO K. *et al.* (1987a) Studies of the effects of FK-506 on renal allografting in the beagle dog. *Transplantation*, **44,** 729.

OCHIAI T., NAKAJIMA K., NAGATA M., HORI S., ASANO T. & ISONO K. (1987b) Studies on the induction and maintenance of long-term graft acceptance by treatment with FK-506 in heterotopic cardiac allotransplantation in rats. *Transplantation*, **44,** 734.

PROPPER, D.J., WOO J., THOMSON A.W., CATTO G.R.D. & MACLEOD A.M. (1990) FK-506: influence on anti-class I MHC alloantibody responses to blood transfusions. *Transplantation* (in press).

SAVINO W. & DARDENNE M. (1984) Thymic hormone-containing cells VI. Immunohistologic evidence for the simultaneous presence of thymulin, thymopoietin and thymosin α_1 in normal and pathological human thymuses. *Eur. J. Immunol.* **14,** 987.

STARZL T.E., TODO S., FUNG J., DEMETRIS A.J., VENKATARAMMAN R. & JAIN A. (1989) FK-506 for liver, kidney and pancreas transplantation. *Lancet*, **ii,** 1000.

STEPHEN M., WOO J., HASAN N.U., WHITING P.H. & THOMSON A.W. (1989) Immunosuppressive activity, lymphocyte subset analysis and acute toxicity of FK-506 in the rat. *Transplantation*, **47,** 60.

TAKEUCHI Y., HABU S., OKUMURA K. & SUZUKI G. (1989) Cyclosporin A and anti-Ia antibody cause a maturation defect of $CD4^+8^-$ cells in organ-cultured fetal thymus *Immunology*, **66,** 362.

TANAKA M., SHINOHARA K., FUKUMOTO T., TANAKA H. & KANEKO T. (1988) Effect of cyclosporin A on rat thymus: time course analysis by immunoperoxidase technique and flow cytofluorometry. *Clin. exp. Immunol.* **72,** 216.

THOMSON A.W. (1989) FK-506. How much potential? *Immunol. Today*, **10,** 6.

THOMSON A.W. (1990a) FK-506 enters the clinic. *Immunol. Today*, **11,** 35.

THOMSON A.W. (1990b) Interspecies comparison of the immunosuppressive efficacy and safety of FK-506. *Transplant Proc.* **22,** Suppl. 1, 96.

THOMSON A.W. & DUNCAN J.I. (1989) The influence of cyclosporin A on T cell activation, cytokine gene expression and cell-mediated immunity. In: *Cyclosporin. Mode of Action and Clinical Applications* (ed. A. W. Thomson), pp. 50–81. Kluwer Academic Publishers, London.

TOCCI M.J., MATKOVICH D.A., COLLIER K.A., KWOK P., DUMONT F., LIN S., DEGUDICIBUS S., SIEKIERKA J.J., CHI J. & HUTCHINSON N.I. (1989) The immunosuppressant FK-506 selectively inhibits expression of early T cell activation genes *J. Immunol.* **143,** 718.

TODO S., UEDA Y., DEMETRIS J.A., IMVENTARZA O., NALESNIK M., VENKATARAMANAN R., MAKOWKA L. & STARZL T.E. (1988) Immunosuppression of canine, monkey and baboon allografts by FK-506: with special reference to synergism with other drugs and to tolerance induction. *Surgery*, **104,** 239.

WOO J., ROSS C.S.K., MILTON J.I. & THOMSON A.W. (1990a) Immunosuppressive activity of FK-506 in rats: flow cytometric analysis of lymphocyte populations in blood, spleen and thymus during treatment and following drug withdrawal. *Clin. exp. Immunol.* **79,** 109.

WOO J., SEWELL H.F. & THOMSON A.W. (1990b) The influence of FK-506 and low-concentration ciclosporin on human lymphocyte activation antigen expression and blastogenesis: a flow cytometric analysis. *Scand J. Immunol.* **31,** 297.

WOO J., STEPHEN M. & THOMSON A.W. (1988) Spleen lymphocyte populations and expression of activation markers in rats treated with the potent new immunosuppressive agent FK-506. *Immunology*, **65,** 153.

YOSHIMURA N., MATSUI S., HAMASHIMA T. & OKA T. (1989) Effect of a new immunosuppressive agent FK-506, on human lymphocyte responses in vitro. II. Inhibition of the production of IL-2 and γ-IFN, but not B-cell stimulating factor 2. *Transplantation*, **47,** 356.