Transfusion Medicine

research paper

haematologica 2002; 87:679-687
http://www.haematologica.ws/2002_07/679.htm

FK506 in the maturation of dendritic cells

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Background and Objectives. FK506 (tacrolimus) is a potent immunosuppressive agent that inhibits interleukin-2 (IL-2) and interferon-γ production by CD4+ cells. The effect of this agent on dendritic cells (DCs), the highly professional antigen-presenting cells for T-cells, has not been completely defined. We investigated the effect of FK506 on DC differentiation from monocytes, and on the shift from immature to mature immunophenotypes.

Design and Methods. DCs were generated *in vitro* from monocytes of healthy donors. Cells were exposed to lipopolysaccharide (LPS) and two doses of FK506, with variations in time of exposure and sequence of FK506 and LPS addition. Immunophenotype analysis in immature and mature DCs under FK506 treatment was performed by flow cytometry at the end of cell culture. The Student's t-test was used for statistical analyses.

Results. FK506 did not affect dendritic cell generation or viability. There were no changes in cell surface markers with addition of FK506 at physiologic concentrations (10 ng/mL). We found a decrease in CD1a median fluorescence intensity (MFI) and an increase in percentage of CD86-positive cells with lengthy exposure (6 days) to FK506 at 5000 ng/mL. In the sequential study, 5000 ng/mL FK506 before LPS addition resulted in a significant decrease in CD1a MFI and in the percentage of cells co-expressing CD83 and CD86.

Interpretation and Conclusions. Our results indicate that lengthy exposure to 5000 ng/mL FK506 modified the expression of some DC-cell surface markers, maintaining DCs in a low maturity stage.

Key words: FK506, dendritic cells, cell-surface markers.

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The survival of patients with solid-organ transplants has increased significantly since the introduction of cyclosporine and tacrolimus (FK506) as immunosuppressive agents. FK506 (Prograf, Fujisawa Pharmaceutical Co., Osaka, Japan) is a macrolide lactone antibiotic isolated from a Japanese soil fungus (*Streptomyces tsukubaensis*). This agent, which has the same mechanism of action as cyclosporine, but is 10 to 100 times more potent, was introduced in 1989 by Starzl *et al.*[1] as an alternative to cyclosporine in liver transplant patients and, subsequently, in other solid-organ recipients. These drugs bind to various immunophilins and inhibit the activity of calcineurin, inducing quantitative limitations in interleukin (IL)-2 production by T-helper cells and in downstream lymphocyte proliferation.[2,3] Despite improvements in the prevention of rejection provided by immunosuppressive agents, chronic rejection remains among the main causes of long-term graft loss.

Experimental studies in mice,[4] in several rat strain combinations,[5] and in pigs[6] have demonstrated that in successful liver transplants, donor-specific tolerance across major histocompatibility complex barriers can be induced without immunosuppression. Tolerance to the allograft has also been observed in some patients after withdrawal of immunosuppression due to major toxicity, such as renal dysfunction, neurotoxicity or hypertension. This tolerogenicity is attributed to establishment of a stable mixed hematopoietic chimerism of recipient and donor bone marrow cells.[7-12]

Apart from microchimerism, professional antigen-presenting cells (APCs), such as dendritic cells (DCs), can also play an important role in the inherent tolerogenicity of liver allografts.[8,13] DCs, first described by Steinman and Cohn in 1973,[14] are the most potent APCs and are considered potential modulators of the peripheral immune response

because of their ability to generate primary and secondary immune responses against specific antigens.[15,16] DCs take up and internalize exogenous antigens by phagocytosis, macropinocytosis or adsorptive pinocytosis pathways. Peptides in the context of major histocompatibility complex (MHC) class I or class II molecules are then presented on the DC surface. With antigen exposure, DC immature phenotypes change to mature phenotypes and stimulate responses from naive and memory T-cells in the paracortical area of secondary lymphoid organs. Although B-cells and macrophages can also trigger T-cells, only DCs are able to induce primary responses in naive T-cells.[17,18] They efficiently internalize soluble antigens and constitutively express high levels of co-stimulatory and adhesion molecules required for antigen presentation to T-cells.[19,20] Subsequently, immunoregulatory and immunomodulatory cytokine production by DCs plays an important role in the cascade of events during T-cell priming.[21] Several groups have designed experimental models to determine the effects of immunosuppressive agents on APCs, based on the production of IL-12 p70, tumor necrosis factor-α and IL-6.[22-24]

Until now, FK506 exposure has been investigated mainly in T-lymphocytes, with special focus on its inhibition of the activation of transcription factors involved in IL-2 gene expression, such as nuclear factor (NF)-κB transcription factor.[25,26] More recently, Shimizu *et al.*[27] studied the ability of FK506 to modify the function of DCs derived from CD34$^+$ hematopoietic progenitor cells. These authors demonstrated that DCs cultured in the presence of FK506 display a reduced capacity to stimulate an allogeneic T-cell response and significantly lower interleukin-12 production. However, the effect of FK506 on the shift of monocyte-generated DCs from immature to mature phenotypes has been little studied.

Given that: a) APCs are responsible for the initiation of T-cell-mediated immunity, b) DCs are involved in the inherent tolerogenicity of liver allografts, c) deficiency or blockade of co-stimulatory molecules such as CD40, CD80 or CD86 is consistent with DC tolerogenicity, both in allograft models and experimental autoimmune disease,[13,28] and d) DCs can be generated *in vitro* from monocytes,[29] our group investigated the effect of FK506 on the immunophenotype and maturative capacity of DCs generated from monocytes of healthy donors and exposed to lipopolysaccharide (LPS).

Design and Methods

Monocyte separation

Peripheral blood monocytes were isolated by Lymphoprep, density=1.077 g/mL (Nycomed) gradient centrifugation of buffy coats from 10 healthy blood donors at our hospital Transfusion Center and Tissue Bank. Briefly, mononuclear cells were isolated by centrifugation at 300 × g for 30 min at room temperature, and then washed at 450 × g for 15 min in 35 mL of phosphate-buffered saline (PBS) without Ca^{++} or Mg^{++}. The pellet obtained was washed twice at 250 × g for 15 min in the same conditions described above to eliminate platelets. The monocyte population was further enriched using MACS technology (MACS, Miltenyi). Between 3×10^8 and 4.5×10^8 mononuclear cells were labeled with CD14$^+$ microbeads during 15 min at 4°C and then separated using a high gradient magnetic separation column placed in a strong magnetic field. The monocyte population was analyzed by flow cytometry (FACS analysis), as described below. Monocyte yield was greater than 85% and cell viability was over 90%. Monocytes were frozen at –80°C until culture.

Generation of immature DCs

Monocytes were dispensed in 6-well culture plates (Costar) at a final density of 3×10^6 cells per well in 2.9 mL of culture medium (RPMI 1640, Gibco-BRL, Life Technologies), supplemented with 1% L-glutamine, 1% sodium pyruvate, 1% penicillin-streptomycin, 1‰ 2-β mercaptoethanol and 10% heat-inactivated fetal bovine serum (FBS) (Gibco-BR, Life Technologies). In all experiments, incubation was carried out in a humidified 5% CO_2 atmosphere at 37°C. To induce immature DCs from monocytes, the culture medium was supplemented with 800 U/mL of granulocyte/macrophage colony-stimulating factor (GM-CSF; Leucomax 300) and 1000 U/mL IL-4 (R & D Systems) on the first day of culture (day 0) and on day 3. Immature DCs were collected after 7 days of culture by pipetting and were subsequently washed with PBS without Ca^{++} or Mg^{++} at 450 × g for 5 min (*see Diagram 1*). An amount of 2×10^5 immature DCs was used for cell morphology and FACS analysis (see below). All experiments were performed with the same batch of FBS to avoid variations in the pattern of CD14 and CD1a expression.

Generation of FK506-DCs

After 24 h (d+1) of culture (3×10^6 cells per well in 2.9 mL), 100 μL of medium containing FK506

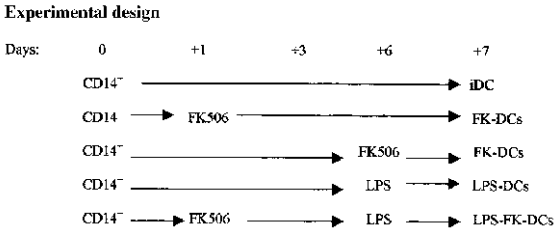

Diagram 1. Experimental design. The culture medium was supplemented with 800 U/mL GM-CSF (Leucomax 300) and 1000 U/mL IL-4 on the first day of culture and on day 3. FK506 was added at concentrations of 10 ng/mL and 5000 ng/mL at the two time points indicated. The concentration of LPS was 1000 ng/mL. *CD14⁺: monocytes. iDC: immature dendritic cells. FK-DCs: dendritic cells exposed to FK506 on day 1 or day 6 of 7 days of culture. LPS-DCs: LPS-stimulated dendritic cells on day 6 of culture. LPS-FK-DCs: LPS-stimulated FK-DCs.*

(Fujisawa Pharmaceutical Co., Osaka, Japan) at a final concentration of 10 ng/mL (physiologic concentration) or 5000 ng/mL were added to each experimental well and plates were incubated for the remaining 6 days of the culture protocol. Under these conditions, we obtained a population of DCs that had been exposed to immunosuppressant agent (FK-DCs) for a lengthy period. To study the effect of a brief exposure to the agent, 10 ng/mL and 5000 ng/mL of FK506 were added at day 6 (d+6) and plates were incubated for the last day of the culture protocol. In all experiments, cytokines (GM-CSF and IL-4) were added on days 0 and 3 (*Diagram 1*). Control wells received 100 μL of culture medium and all wells contained a final volume of 3 mL of culture medium.

Maturation of DCs and FK-DCs

DC and FK-DC maturation was performed on 3×10^6 cells per well in 6-well culture plates (Costar) using LPS (*Escherichia coli*, serotype 0128:B12) purchased from Sigma. LPS was reconstituted in RPMI 1640 medium and stored at –20ºC before use. For maturation, 1000 ng/mL LPS were added to the cells on day 6. Cell morphology and cell-surface markers were analyzed on day 7 (*Diagram*).

Analysis of cell morphology

DC and FK-DC morphology with and without LPS-induced cell maturation was studied in 2×10^5 cells. Cells on microscope slides were centrifuged at 600 rpm for 5 min in a Cytospin centrifuge (Shandon, Cytospin II). Cytospins were stained using May Grünwald-Giemsa stain, then examined by light microscopy (Olympus).

Analysis of cell-surface markers and cell viability by flow cytometry

Analysis of cell surface markers in immature DCs, LPS-stimulated DCs, FK-DCs and LPS-stimulated FK-DCs was performed on day 7 of cell culture. Cells were washed with Ca^{++} and Mg^{++} free PBS before flow cytometry analysis. An amount of 2×10^5 cells was double-stained with fluorescein isothiocyanate and phycoerythrin monoclonal antibodies in Ca^{++} and Mg^{++} free PBS for 15 minutes and washed twice before FACS analysis. The pairs of mouse anti-human monoclonal antibodies used for immunofluorescence staining of leukocyte population, monocyte yield and DCs were: CD45-FITC (J.33)/CD3-PE (UCHT1), CD14-FITC (RMO52)/CD1a-PE (BL6), CD64-FITC (22)/CD54-PE (84H10), CD83-FITC (HB159)/CD86-PE (HA5.2B7), IgG1-FITC (679.1Mc7)/IgG1-PE (679.1Mc7) and IgG2b-FITC (MOPC-195) (Coulter-Immunotech).

Cell viability was determined by a method using carboxyfluorescein diacetate (CFDA, Sigma). CFDA is internalized into living cells and subsequently hydrolyzed into a polar compound whose fluorescence is determined by flow cytometry. Briefly, 2×10^5 cells were incubated at 37°C for 15 min with CFDA at a final concentration of 0.5 mg/mL. Cells were washed twice with Ca^{++} and Mg^{++} free PBS (450 × g, 5 min) before flow cytometry analysis using a FACSCalibur flow cytometer (Becton Dickinson). Generally, 10,000 events were analyzed. Cell-surface markers were determined by means of a forward-versus-side scatter gate. All isotype controls were set to be within 2% positive for statistical analysis.

Statistical analysis

Descriptive statistics are used to report the mean values and standard deviation (SD) of the experimental data. Statistical analysis was performed using the Student's t-test with the SPSS computer software program. Significance for a two-tailed test was set at a p value of less than 0.05.

Results

Analysis of cell morphology

Figure 1 shows the morphologic appearance of immature DCs (A), LPS-stimulated DCs (B), FK-DCs (C) and LPS-stimulated FK-DCs (D). A homogeneous population of large cells displaying characteristic DC morphology (abundant cytoplasm, irregular membrane ruffling and cytoplasm protrusion) was observed in each experiment. Cell viability studies yielded results of around 90% viability for each experiment, indicating that FK506, even at the high

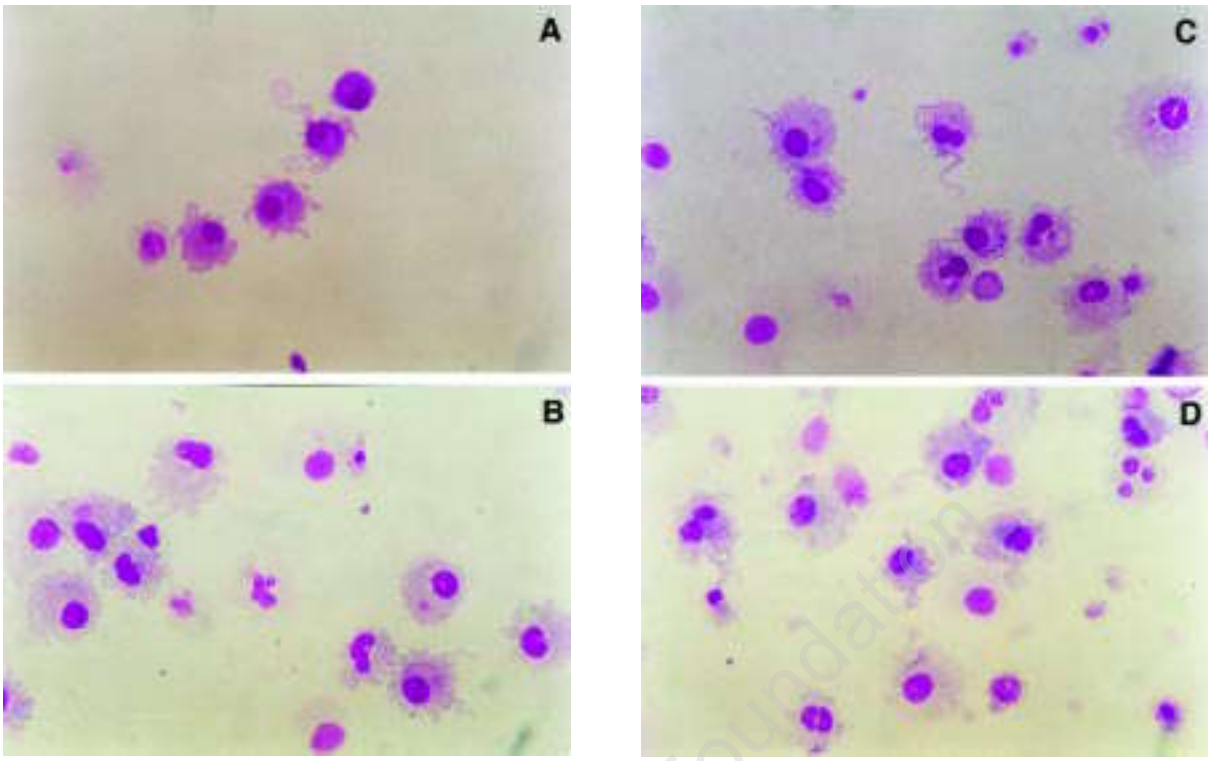

Figure 1. Morphology of monocyte-generated DCs with GM-CSF + IL-4 (May Grünwald-Giemsa staining). Immature DCs (a); LPS-stimulated DCs on day 6 of culture (b); FK-DCs on day 1 of culture (c); FK-DCs on day 1 of culture + LPS on day 6 of culture (d). The final concentrations used were: 1000 ng/mL LPS; 5000 ng/mL FK506. No drastic morphologic changes were seen under these culture conditions. Magnification × 400. The figure shows representative data from 5 independent experiments.

dose studied, was not cytotoxic in our experimental conditions.

Flow cytometry analysis of monocytes, immature and mature DCs

Figure 2 shows the surface phenotypes of monocytes, immature DCs and DCs stimulated by 1,000 ng/mL LPS at day 6 of culture. The process of differentiation and maturation of DCs from monocyte precursors was achieved with loss of CD14 and acquisition of CD1a. A new marker, CD83, as well as accessory molecules, such as CD54 and CD86, were expressed in DCs when they were stimulated by LPS.

Flow cytometry analysis of cell surface markers in immature DCs, LPS-stimulated DCs, FK-DCs and LPS-stimulated FK-DCs

The median fluorescence intensity (MFI) values of CD45, CD1a, CD54, CD83 and CD86 on DCs produced after exposure to FK506 are listed in Table 1. There were no significant differences in MFI values between control DCs and FK-DCs exposed to physiologic concentrations (10 ng/mL) of immunosuppressant. However, in cells exposed to the high concentration of FK506, changes were found in CD1a, CD86 and CD83/CD86 expression according to the duration of exposure. Brief exposure caused a decrease in CD86 MFI values (Table 1), with no changes in the percentage of CD86-positive cells (Figure 3B), whereas long exposure reduced CD1a expression. Additionally, the percentage of cells co-expressing CD83 and CD86 was slightly decreased at both experimental times (Figure 3A). In LPS-pulsed DCs, the CD54 and CD86 FMI values and percentage of CD83/CD86-positive cells were statistically increased as compared to those of the control group (Table 1). However, significant decreases in CD1a values (Table 1) and percentage of CD83/CD86-positive cells were found in LPS-stimulated DCs previously incubated with 5,000 ng/mL of FK506 (Figure 3A). In the same set of experiments, an increase in the percentage of CD86-positive cells as compared to control DCs was observed with 5000 ng/mL FK506 (d+1) (Figure 3B), suggesting that the surface-marker was up-regulated by FK506 at this high concentration.

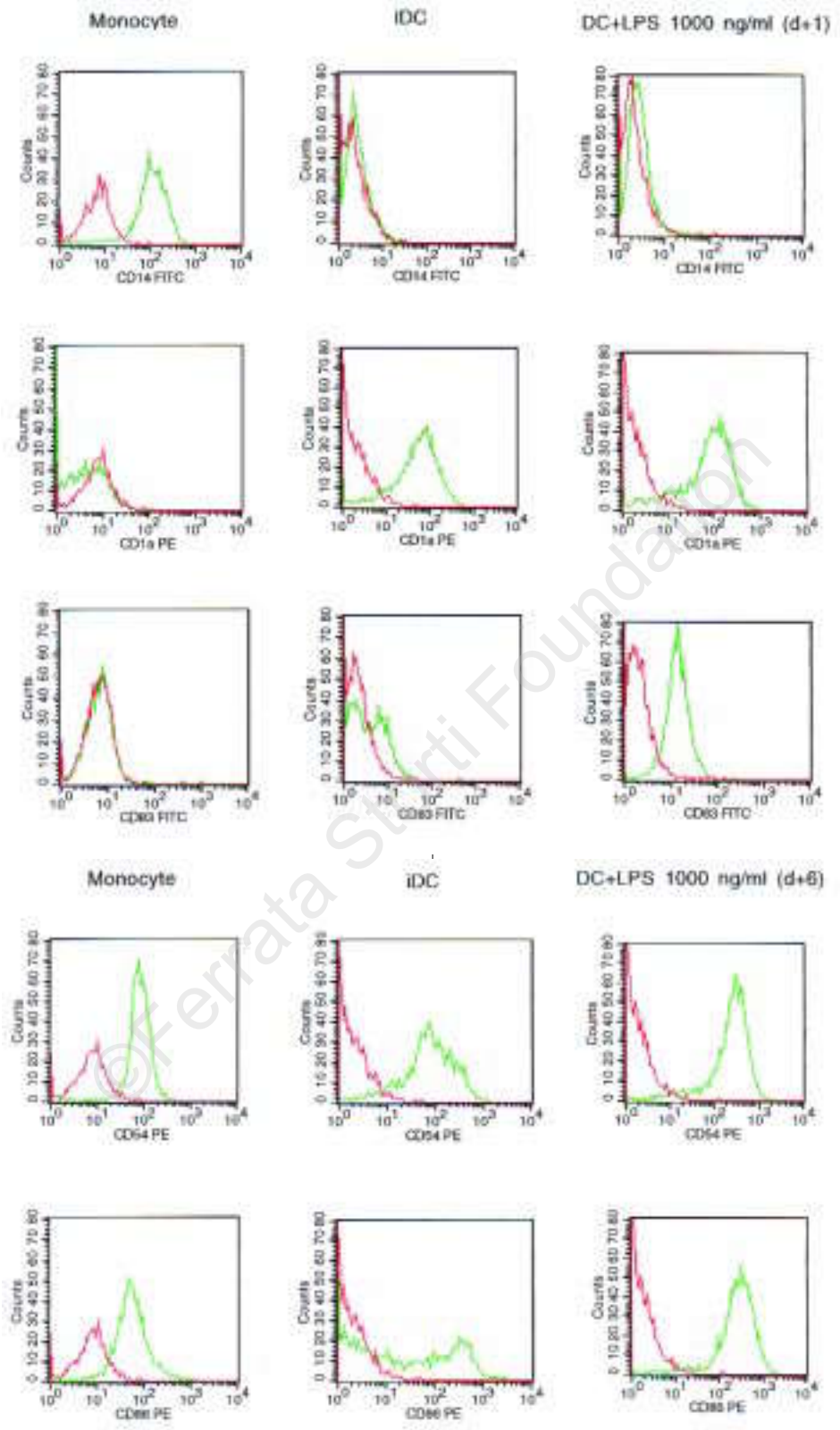

Figure 2. Surface phenotypes of monocytes, immature DCs and mature DCs. Monocytes were cultured for 7 days with complete culture medium without cytokines. Immature DCs were cultured with complete medium and GM-CSF plus IL-4. For DC maturation, 1,000 ng/mL LPS were added to cultures on day 6. Flow cytometry analysis was performed on day 7. The red line represents the isotypic control. The figure shows representative data from 4 independent experiments.

Table 1. Effect of FK506, LPS, and FK506 plus LPS on DC surface markers. DCs were exposed to 10 ng/mL (FK10) and 5000 ng/mL (FK5000) FK506 for a short period (d+6: FK506 was added on day 6 and incubation was continued for the one remaining day) and a long period (d+1: FK506 was added on day 1 and incubation was continued for the 6 remaining days). Immature DCs were activated to mature DCs with addition of 1000 ng/mL LPS on day 6. Results are given as mean ± SD of median fluorescence intensity (MFI) in several independent experiments (n) from 10 healthy blood donors. Immature DCs (control cells) (n=10), FK10 (d+1) (n=6), FK10 (d+6) (n=4), FK5000 (d+1) (n=7), FK5000 (d+6) (n=5), LPS 1000 (d+6) (n=4), FK10 (d+1) + LPS 1000 (d+6) (n=5) and FK5000 (d+1) + LPS 1000 (d+6) (n=5). *p values of < 0.05 were considered statistically significant.

Treatments	*Cell-surface markers*						
	CD45-FITC	*CD83-FITC*	*Control antibody (FITC)*	*CD1a-PE*	*CD54-PE*	*CD86-PE*	*Control abntibody (PE)*
Control cells	92.65±1.64	5.57±1.24	3.10±0.73	105.03±30.97	162.76±38.93	128.97±67.79	2.80±0.91
FK10(d+1)	93.97±1.25	5.10±0.96	3.23±0.81	111.05±27.55	159.16±45.19	113.16±89.08	2.99±1.04
FK10 (d+6)	93±2.60	4.33±0.84	2.79±0.22	111.31±24.17	173.31±20.43	87.56±31.10	2.41±0.22
FK5000 (d+1)	96.08±1.13	4.61±1.20	3.46±1.37	31.99±20.90*	155.18±56.18	143.26±103.19	3.80±1.60
FK5000 (d+6)	92.99±1.90	4.10±0.64	3.22±0.35	86.11±22.13	142.91±19.09	77.27±9.89*	2.82±0.51
LPS1000 (d+6)	96.69±1.89	13.65±6.60	3.24±1	113.93±23.92	280.91±48.22*	272.35±119.67*	2.85±0.52
FK10 (d+1) + LPS1000 (d+6)	95.58±1.61	11.17±5.57	3.02±0.44	107.02±18.52	261.15±45.95*	218.77±138.84	3.07±0.70
FK5000 (d+1) + LPS1000 (d+6)	95.22±1.25	8.57±1.96	3.31±1.19	25.60±5*	234.55±44.48*	294.48±139.27	3.69±1.40

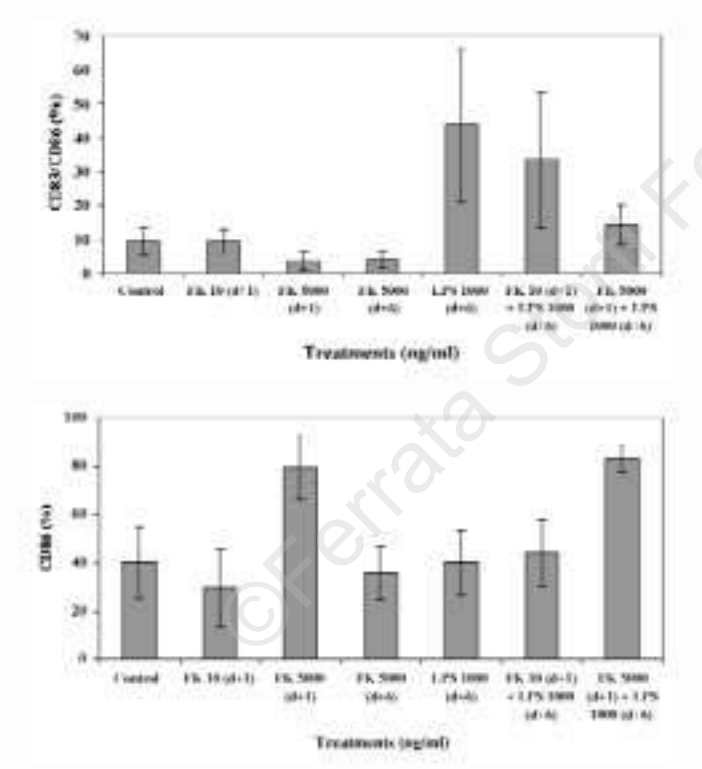

Figure 3. Effect of FK506, LPS, and FK506 plus LPS on percentage of cells positive for CD83/CD86 (A) and CD86 (B). DCs were treated with 10 ng/mL (FK10) and 5000 ng/mL (FK5000) of FK506, 1000 ng/mL LPS (LPS 1000) and with sequential treatment consisting in administration of 10 ng/mL (FK10) or 5000 ng/mL (FK5000) with 1000 ng/mL LPS (LPS1000). d+1 indicates the longer exposure time (FK506 concentrations were added on day 1 of culture). d+6 indicates the shorter exposure time (FK506 concentrations were added on day 6 of culture). Data are given as mean ± SD of MFI in several independent experiments (n) from 10 healthy blood donors. Immature DCs (control cells) (n=10), FK10 (d+1) (n=6), FK5000 (d+1) (n=7), FK5000 (d+6) (n=5), LPS 1000 (d+6) (n=4), FK10 (d+1) + LPS 1000 (d+6) (n=5), FK5000 (d+1) + LPS 1000 (d+6) (n=5). *p values < 0.05 were considered statistically significant.

Discussion

Immunosuppressive drugs such as cyclosporin A and tacrolimus are essential for prolongation of allograft survival. In this context, the persistence of donor-derived leukocyte populations such as DCs in recipient tissues seems to play an important role in the induction of organ tolerance.[30] The DCs used in the present study were generated from monocytes of healthy donors cultured in the presence of GM-CSF and IL-4.[29] FK-506 concentrations were determined on the basis of the interaction of this immunosuppressant agent in monocytes, B-cells, T-cells, and $CD34^+$-derived DCs, and concentrations in plasma and whole blood during clinical use.[27,31,32,33] The effect of FK506 on DCs was tested in a set of experiments evaluating the following: the concentration of FK506 (10 ng/mL vs. 5,000 ng/mL), the period of exposure to FK506 (6 days vs. 1 day) and the sequence of FK506 treatment and LPS addition.

Under our culture conditions we found the features of mature DCs[34,35] and typical DC morphology without cytotoxic effects in LPS-stimulated DCs, FK-DCs and LPS-stimulated FK-DCs. There were no differences in MFI values between control DCs and FK-DCs exposed to physiologic concentrations (10 ng/mL) of the immunosuppressant. Thus, contact with FK506 did not influence the generation of morphologically normal DCs (Figure 1), i.e., high MFI levels for CD45, CD1a, CD54, CD86, low levels for CD83 (Table 1), presence of CD83/CD86 (Figure 3A), absence of CD14 and CD64, as well as percentage of double staining ≤1.6% for CD45/CD3, ≤3% for CD14/CD1a and ≤5% for CD64/CD54 (data not shown). Similar results were obtained by Woltman *et al.*[36] when freshly isolated monocytes were cultured with GM-CSF, IL-4 and 10^{-6} M (804 ng/mL) FK506 for 6 days, and by Szabo *et al.*[37] when myeloid DCs were exposed to 10^{-8} M FK506. However, when FK506 was added at the high concentration, changes in CD1a, CD86 and CD83/CD86 expression were found according to the duration of exposure. Although low levels of CD1a were detected, long exposure to FK506 blocked normal generation of DCs during their differentiation from monocytes, as evidenced by the significant decrease in MFI values for this marker. Recent studies by Shimizu's group[27] showed high levels of CD1a on DCs generated from $CD34^+$ hematopoietic progenitor cells cultured for 14 days in the presence of FK506. Moreover, they found that FK-DCs co-expressed CD1a and CD14, as if they had the characteristics of an intermediate precursor cell with the ability to differentiate into either DCs ($CD1a^+$ $CD14^-$) or monocytes ($CD1a^-$ $CD14^+$).[38] In our experiments, using the monocyte precursor, these intermediate precursor cells were not detected. Though the half-life of FK506 in cell culture conditions is unknown, the different exposure periods to the immunosuppressive agent and DC-progenitor used in each study could explain the changes in CD1a and CD14 patterns.

In the present study results for mature DC generation with or without long exposure to FK506 indicate a loss of maturative capacity in the presence of the agent. The maturation process, characterized by an up-regulation of co-stimulatory and adhesion molecules, confers the DCs a greater capacity for T-cell recruitment and activation.[39,40] According to the CD54 and CD86 FMI values and percentage of CD83/CD86 positive cells (Table 1), LPS-DCs displayed the characteristic changes to the mature state[41] with 24 hours of exposure to LPS (Figure 3A). However, significant decreases in CD1a MFI values (Table 1) and percentage of cells co-expressing CD83 and CD86 were found when LPS-stimulated DCs had been previously incubated with 5,000 ng/mL of FK506 (Figure 3A). Some features that predispose DCs to tolerogenicity are: exposure to IL-10, ultraviolet B radiation, inhibition of IL-12 production by prostaglandin E_2, co-stimulatory molecule deficiency, blockade of co-stimulatory molecule expression by CTLA4-Ig and transduction with genes encoding immunosuppressive molecules.[30] Other immunosuppressive agents, such as corticosteroids, transforming growth factor-β and IL-10 can render monocyte-derived DCs tolerogenetic.[42,43] However, little is known about the biochemical pathways that regulate the maturation process in these cells.

Mature DCs express high levels of nuclear factor (NF)-κB transcription factor. In DC culture systems, LPS induces nuclear translocation of NF-κB, which plays an important role in the expression of genes involved in DC maturation.[44-47] In our experiments the loss of the maturation process could be due to inhibition of NF-κB activation by FK506. Several groups have demonstrated that DC antigen presentation is NF-κB-dependent when this transcription factor is inhibited by serine protease inhibitor, proteasome inhibitor, SN50 synthetic peptide and adenoviral transfer of the endogenous inhibitor of NF-κB. These observations suggest that NF-κB is an important target for blocking DC antigen presentation and inhibiting T-cell-dependent immune responses.[45,48-51]

Though 5,000 ng/mL FK506 is not used in clinical trials, we conclude that this concentration is not toxic to DCs and can operate, at least in our experimental conditions, as a drug maintaining DCs in a low maturity stage to confer immunologic privilege. Further studies are needed to elucidate the importance of changes in DC immunophenotype in liver transplant patients receiving FK506 and the possible significance of these alterations in the immune response to the allograft. Along this line, knowledge of the capacity of DCs for antigen uptake and the molecular basis underlying FK506's capacity to block the shift from the immature to mature stage could contribute to explaining the therapeutic potential of tacrolimus. The application of these findings might be useful in future trials aimed toward reducing the dose of this immunosuppressant agent and, therefore, its toxic effects.

Contributions and Acknowledgments

JC designed the study, performed all the in vitro assays and wrote the paper. TV contributed to the

positive selection of monocytes and the generation of dendritic cells. RP contributed to the flow cytometry analysis and reviewed the paper. DG contributed to the experimental design and analysis of results. IB and CM performed the literature review and gave advice on manuscript preparation. LM supplied the buffy-coats from healthy donors, offered technical support, and contributed to the analysis of results and the review of the paper. The authors thank Dr. Acebedo and Dr. Irriguible (Servei d'Hematologia, Vall d'Hebron Hospitals) for technical support in the cell morphology analysis and Ms. Celine Cavallo for English language editing.

Funding

This study was supported by Fundació La Marató de TV3 (nº 002110), Spain.

Disclosures

Conflict of interest: none.

Redundant publications: no substantial overlapping with previous papers.

References

1. Starzl TE, Todo S, Fung J, Demetris AJ, Venkataramman R, Jain A. FK 506 for liver, kidney, and pancreas transplantation. Lancet 1989; 2:1000-4.
2. Pazderka F, Enns J, Batiuk TD, Halloran PF. The functional consequences of partial calcineurin inhibition in human peripheral blood mononuclear leucocytes. Transpl Immunol 1996; 4:23-31.
3. Halloran PF. Immunosuppressive agents in clinical trials in transplantation. Am J Med Sci 1997; 313:283-8.
4. Qian S, Demetris AJ, Murase N, Rao AS, Fung JJ, Starzl TE. Murine liver allograft transplantation: tolerance and donor cell chimerism. Hepatology 1994; 19:916-24.
5. Kamada N, Davies HS, Roser B. Reversal of transplantation immunity by liver grafting. Nature 1981; 292:840-2.
6. Calne RY, Sells RA, Pena JR, Davis DR, Millard PR, Herbertson BM, et al. Induction of immunological tolerance by porcine liver allografts. Nature 1969; 223:472-6.
7. Reyes J, Zeevi A, Ramos H, Tzakis A, Todo S, Demetris AJ, et al. Frequent achievement of a drug-free state after orthotopic liver transplantation. Transplant Proc 1993; 25:3315-9.
8. Starzl TE, Demetris AJ, Murase N, Ildstad S, Ricordi C, Trucco M. Cell migration, chimerism, and graft acceptance. Lancet 1992; 339:1579-82.
9. Starzl TE. Cell migration and chimerism –a unifying concept in transplantation- with particular reference to HLA matching and tolerance induction. Transplant Proc 1993; 25:8-12.
10. Starzl TE, Demitris AJ, Murase N, Thomson AW, Trucco M, Ricordi C. Donor cell chimerism permitted by immunosuppressive drugs: a new view of organ transplantation. Immunol Today 1993; 14:326-32.
11. Suberbielle C, Caillat-Zucman S, Legendre C, Bodemer C, Noel LH, Kreis H, et al. Peripheral microchimerism in long-term cadaveric-kidney allograft recipients. Lancet 1994; 343:1468-9.
12. Schlitt HJ, Hundrieser J, Hisanaga M, Uthoff K, Karck M, Wahlers T, et al. Patterns of donor-type microchimerism after heart transplantation. Lancet 1994; 343:1469-71.
13 Thomson AW, Lu L, Murase N, Demetris AJ, Rao AS, Starzl TE. Microchimerism, dendritic cell progenitors and transplantation tolerance. Stem Cells 1995; 13:622-39.
14. Steinman RM, Cohn ZA. Identification of a novel cell type in peripheral lymphoid organs of mice I. Morphology, quantitation, tissue distribution. J Exp Med 1973; 137: 1142-62.
15. Banchereau J, Steinman RM. Dendritic cells and the control of immunity. Nature 1998; 392:245-52.
16. Watts C, Powis S. Pathways of antigen processing and presentation. Rev Immunogenet 1999; 1:60-74.
17. Steinman RM. The dendritic cell system and its role in immunogenicity. Annu Rev Immunol 1991; 9:271-96.
18. Peters JH, Gieseler R, Thiele B, Steinbach F. Dendritic cells: from ontogenetic orphans to myelomonocytic descendants. Immunol Today 1996; 17:273-8.
19. Inaba K, Metlay JP, Crowley MT, Witmer-Pack M, Steinman RM. Dendritic cells as antigen presenting cells in vivo. Int Rev Immunol 1990; 6:197-206.
20. Guinan EC, Gribben JG, Boussiotis VA, Freeman GJ, Nadler LM. Pivotal role of the B7:CD28 pathway in transplantation tolerance and tumor immunity. Blood 1994; 84:3261-82.
21. Lanzavecchia A, Sallusto F. Dynamics of T lymphocyte responses: intermediates, effectors, and memory cells. Science 2000; 290:92-7.
22. Blotta MH, DeKruyff RH, Umetsu DT. Corticosteroids inhibit IL-12 production in human monocytes and enhance their capacity to induce IL-4 synthesis in CD4+ lymphocytes. J Immunol 1997; 158:5589-95.
23. Vieira PL, Kalinski P, Wierenga EA, Kapsenberg ML, de Jong EC. Glucocorticoids inhibit bioactive IL-12p70 production by in vitro-generated human dendritic cells without affecting their T cell stimulatory potential. J Immunol 1998; 161:5245-51.
24. de Jong EC, Vieira PL, Kalinski P, Kapsenberg ML. Corticosteroids inhibit the production of inflammatory mediators in immature monocyte-derived DC and induce the development of tolerogenic DC3. J Leukoc Biol 1999; 66: 201-4.
25. Frantz B, Nordby EC, Bren G, Steffan N, Paya CV, Kincaid RL, et al. Calcineurin acts in synergy with PMA to inactivate IkB/MAD3, an inhibitor of NF-kB. EMBO J 1994; 13: 861-70.
26. Mattila PS, Ullman KS, Fiering S, Emmel EA, McCutcheon M, Crabtree GR, et al. The actions of cyclosporine A and FK506 suggest a novel step in the activation of T lymphocytes. EMBO J 1990; 9:4425-33.
27. Shimizu K, Fujii S, Fujimoto K, Kawa K, Yamada A, Kawano F. Tacrolimus (FK506) treatment of CD34+ hematopoietic progenitor cells promote the development of dendritic cells that drive CD4+ T cells toward Th2 responses. J Leukoc Biol 2000; 68:633-40.
28. Khoury SJ, Gallon L, Verburg RR, Chandraker A, Peach R, Linsley PS, et al. Ex vivo treatment of antigen-presenting cells with CTLA4Ig and encephalitogenic peptide prevents experimental autoimmune encephalomyelitis in the Lewis rat. J Immunol 1996; 157:3700-5.
29. Sallusto F, Lanzavecchia A. Efficient presentation of soluble antigen by cultured human dendritic cells is maintained by granulocyte/macrophage colony-stimulating factor plus interleukin 4 and downregulated by tumor necrosis factor a. J Exp Med 1994; 179:1109-18.
30. Thomson AW, Lu L. Are dendritic cells the key to liver transplant tolerance? Immunol Today 1999; 20:27-32.
31. Kino T, Hatanaka H, Hashimoto M, Nishiyama M, Goto T, Okuhara M, et al. FK506, a novel immunosuppressant isolated from a Streptomyces. I. Fermentation, isolation, and physico-chemical and biological characteristics. J Antibiot [Tokyo] 1987; 40:1249-55.
32. Keicho N, Sawada S, Kitamura K, Yotsumoto H, Takaku F. Effects of an immunosuppressant, FK506, on interleukin 1 alpha production by human macrophages and a macrophage-like cell line, U937. Cell Immunol 1991; 132:285-94.

33. McMichael J, Irish W, McCauley J, Shapiro R, Gordon R, van Thiel DH, et al. Evaluation of a novel "intelligent" dosing system for optimizing FK506 therapy. Transplant Proc 1991; 23:2780-2.
34. Caux C, Liu Y, Banchereau J. Recent advances in the study of dendritic cells and follicular dendritic cells. Immunol Today 1995; 16:2-4.
35. Di Nicola M, Lemoli RM. Dendritic cells: specialized antigen presenting cells. Haematologica 2000; 85:202-7.
36. Woltman AM, de Fijter JW, Kamerling SW, Paul LC, Daha MR, van Kooten C. The effect of calcineurin inhibitors and corticosteroids on the differentiation of human dendritic cells. Eur J Immunol 2000; 30:1807-12.
37. Szabo G, Gavala C, Mandrekar P. Tacrolimus and cyclosporine A inhibit allostimulatory capacity and cytokine production of human myeloid dendritic cells. J Investig Med 2001; 49:442-9.
38. Ratta M, Rondelli D, Fortuna A, Curti A, Fogli M, Fagnoni F, et al. Generation and functional characterization of human dendritic cells derived from CD34 cells mobilized into peripheral blood: comparison with bone marrow $CD34^+$ cells. Br J Haematol 1998; 101:756-65.
39. Kapsenberg ML, Kalinski P. The concept of type 1 and type 2 antigen-presenting cells. Immunol Lett 1999; 69:5-6.
40. Ferlazzo G, Semino C, Melioli G. HLA class I molecule expression is up-regulated during maturation of dendritic cells, protecting them from natural killer cell-mediated lysis. Immunol Lett 2001; 76:37-41.
41. Banchereau J, Steinman RM. Dendritic cells and the control of immunity. Nature 1998; 392:245-52.
42. Lu L, Khoury SJ, Sayegh MH, Thomson AW. Dendritic cell tolerogenicity and prospects for dendritic cell based therapy of allograft rejection and autoimmunity. In: Lotze MT, Thomson AW, editors. Dendritic Cells. San Diego: Academic Press; 1998. p. 487-511.
43. Vanderheyde N, Verhasselt V, Goldman M, Willems F. Inhibition of human dendritic cells functions by methylprednisolone. Transplantation 1999; 67:1342-7.
44. Granelli-Piperno A, Pope M, Inaba K, Steinman RM. Coexpression of NF-κ B/Rel and Sp1 transcription factors in human immunodeficiency virus 1-inuced, dendritic cell-T-cell syncytia. Proc Natl Acad Sci USA 1995; 92:10944-8.
45. Rescigno M, Martino M, Sutherland CL, Gold MR, Ricciardi-Castagnoli P. Dendritic cells survival and maturation are regulated by different signaling pathways. J Exp Med 1998; 188:2175-80.
46. Baldwin AS Jr. The NF-κB and IκB proteins: new discoveries and insights. Annu Rev Immunol 1996; 14:649-83.
47. Koski GK, Lyakh LA, Cohen PA, Rice NR. $CD14^+$ monocytes as dendritic cell precursors: diverse maturation-inducing pathways lead to common activation of NK-κ/RelB. Crit Rev Immunol 2001; 21:179-89.
48. Lin YZ, Yao SY, Veach RA, Torgerson TR, Hawiger J. Inhibition of nuclear translocation of transcription factor NF-κ B by a synthetic peptide containing a cell membrane-permeable motif and nuclear localization sequence. J Biol Chem 1995; 270:14255-8.
49. Ardeshna KM, Pizzey AR, Devereux S, Khwaja A. The PI3 kinase, p38 SAP kinase, and NFκB signal transduction pathways are involved in the survival and maturation of lipopolysaccharide-stimulated human monocyte-derived dendritic cells. Blood 2000; 96:1039-46.
50. Yoshimura S, Bondeson J, Brennan FM, Foxwell BM, Feldmann M. Role of NFκB in antigen presentation and development of regulatory T cells elucidated by treatment of dendritic cells with the proteasome inhibitor PSI. Eur J Immunol 2001; 31:1883-93.
51. Yoshimura S, Bondeson J, Foxwell BM, Brennan FM, Feldmann M. Effective antigen presentation by dendritic cells is NF-kappaB dependent: coordinate regulation of MHC, co-stimulatory molecules and cytokines. Int Immunol 2001; 13:675-83.

Peer Review Outcomes

Manuscript processing

This manuscript was peer-reviewed by two external referees and by Professor Mario Cazzola, Editor-in-Chief. The final decision to accept this paper for publication was taken jointly by Prof. Cazzola and the Editors. Manuscript received January 23, 2002; accepted May 6, 2002.

What is already known on this topic

FK506 (tacrolimus) is a potent immunosuppressive agent that inhibits IL-2 and INF-γ production by CD4-positive cells. Its effects on dendritic cells are largely unknown.

What this study adds

This study shows that lengthy exposure to high concentrations of FK506 can modify the immunophenotype of dendritic cells, apparently preventing their maturation.

Potential implications for clinical practice

These findings may allow better immunosuppressive strategies after transplantation to be implemented.

Mario Cazzola, Editor-in-Chief