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Strategies to Antagonise the Cyclosporine A-Induced Proliferation of Human Pulmonary Artery Smooth Muscle Cells: Anti-endothelin-1 Antibodies, Verapamil, and Octreotide

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ABSTRACT. The present study investigated the mechanisms mediating the actions of the immunosuppressive drug cyclosporine A (CsA) on human pulmonary artery smooth muscle cell (PASMC) proliferation. The new hydroxyethyl derivative of D-serine^{8}-cyclosporine, SDZ IMM 125, was used for comparison. CsA-induced proliferation was determined by incorporation of [^{3}H]thymidine ([^{3}H]Thy). CsA in the concentration range between 0.1 nM and 0.1 μM induced a concentration-dependent increase in proliferation after 24, 48, and 72 hr of incubation. Higher CsA concentrations were cytotoxic. When proliferation experiments were performed in the presence of a monoclonal antibody against endothelin-1 (ET-1), CsA-induced proliferation was totally inhibited. No inhibition occurred in the presence of the same antibody when heat-inactivated or a non-specific monoclonal antibody. In parallel, CsA increased the production of ET-1, as determined by radioimmunoassay. Incubation of PASMCs with ET-1 at the concentration range at which the latter was released by CsA induced cell proliferation. The somatostatin derivative Sandostatin (SDT; octreotide), which is an inhibitor of the growth of smooth muscle cells as well as a potent inhibitor of ET-1 secretion, inhibited both the CsA-induced ET-1 release and the increase in [3H]Thy incorporation by PASMCs. A similar effect was observed for the calcium channel blocker verapamil (VP). SDZ IMM 125 induced weaker effects than CsA in terms of PASMC proliferation and ET-1 secretion. In conclusion, CsA increased the rate of proliferation of PASMCs, while SDZ IMM 125 induced a weaker effect. Anti-ET-1 antibody, VP, and SDT significantly inhibited CsA-induced PASMC proliferation. BIOCHEM PHARMACOL **59**;11:1459–1466, 2000. © 2000 Elsevier Science Inc.

KEY WORDS. cyclosporine A; SDZ IMM 125; smooth muscle cells; proliferation; endothelin-1; verapamil; octreotide

The immunosuppressive drug CsA† can profoundly influence lymphocyte activation. Lymphocytes play a central role in asthma, triggering the immunological reaction and controlling the mobilisation and recruitment of eosinophils and mast cells into lung tissue. Hence, it is appropriate to consider CsA as a novel anti-asthma therapy [1, 2]. Some clinical trials with CsA have already been carried out, with promising results for the treatment of chronic severe asthma [3, 4]. Likewise, CsA has shown efficacy in animal models of allergic rhinitis [5] and of sensitised airways [6]. Finally, aerosolised cyclosporine has been reported to be a safe and efficacious therapy for acute lung rejection [7], as well as for refractory chronic lung rejection [8]. In contrast to the oral or parenteral administration of CsA, the choice of the inhalative route presents the dual advantage of reducing side-effects related to systemic distribution of the drug (nephrotoxicity, hypertension) [9, 10], while attaining the effective concentration of the drug in the airways at lower doses. However, the high concentration of CsA attained in the lungs after administration by inhalation requires monitoring of potential local adverse effects, in particular in the vascular system. Chronic treatment with CsA has been occasionally associated with structural changes in the vasculature, characterised histologically as arterial myointimal hyperplasia occurring in the presence of an intact endothelium [9–11]. However, it is not clear whether these effects may be attributed to chronic graft rejection taking place in the transplanted animals or humans [12] or to an effect of the drug. The available data regarding the effects of CsA on the growth of smooth muscle cells are conflicting, with both stimulatory and inhibitory activities having been described [13–16]. The variations seem to be related to differences among species and among tissular origin of the smooth muscle cells.

The present experiments were designed to investigate the effects of CsA on human PASMC proliferation *in vitro*.

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† *Abbreviations:* CsA, cyclosporine A; ET-1, endothelin-1; [^{3}H]Thy, [^{3}H]thymidine; PASMCs, pulmonary artery smooth muscle cells; SDT, Sandostatin; and SmGM, smooth muscle growth medium.

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Special attention was devoted to the investigation both of intra- and extracellular mechanisms mediating CsA actions (calcium and ET-1, respectively) and of the different possibilities of interfering with these mechanisms, with the aim of antagonising CsA adverse effects. Finally, SDZ IMM 125, the hydroxyethyl derivative of D-serine[8]-cyclosporine ($C_{63}N_{11}O_{14}H_{115}$) which has been shown to be a promising alternative to current immunosuppressants [17, 18], was compared with CsA in the above-mentioned *in vitro* model.

MATERIALS AND METHODS

Materials

CsA was used as an orthorhombic microcrystalline form prepared as a micronised powder (Novartis Pharma AG). Normal human PASMC, culture medium SmGM (modified MCDB 131), supplements for the medium (human epidermal growth factor [hEGF], human hepatocyte growth factor [hHGF], fetal bovine serum [FBS], gentamicin, amphotericin B, and dexamethasone), and passage reagents (HEPES-buffered saline solution, trypsin–EDTA, and trypsin-neutralising solution) were purchased from Clonetics. [^{3}H]Thy, ET-1 radioimmunoassay kits, and C-2 columns were obtained from Amersham. Specific monoclonal antibody against ET-1 (no cross-reactivity with other members of the endothelin family) was purchased from American Qualex. Specific monoclonal antibody against human desmin was obtained from Sigma. All other chemicals were of the highest commercially available quality.

PASMC Culture

Commercially available PASMCs (third passage) were cultured in the medium recommended by the supplier (SmGM supplemented with 0.1 mg/mL of hEGF, 0.01 mg/mL of hHGF, 5% FBS, 3.9 mg/mL of dexamethasone, 0.5 mg/mL of gentamicin sulphate, and 0.5 mg/mL of amphotericin B) and maintained at 37° in an atmosphere of 5% CO_2 and 95% O_2. Media were changed every two days. For all the experiments, cells in passages three to six were used. The passages were always performed as described elsewhere [19] using the reagents recommended by Clonetics.

PASMC Proliferation Assay

The PASMC proliferation rate was determined by measuring the amount of [^{3}H]Thy incorporated by the cells, as follows: PASMCs were seeded in 24-well plates (2×10^3 cells/well) with one mL of SmGM and maintained in culture until 60–70% confluence was reached (approximately 7 days). At the time of the experiment, cells were washed three times with serum-free SmGM and incubated with the corresponding drugs for 24, 48, and/or 72 hr, using 4 replicates for each treatment. All drugs were initially dissolved in dimethylsulphoxide and further diluted with serum-free SmGM. The final dimethylsulphoxide concentration was 0.01% in all cases. Four hours before the end of each experimental period, 1 mCi/mL of [^{3}H]Thy was added to each well, and cells were maintained under normal culture conditions. Afterwards, media were siphoned off, cells were washed three times with cold phosphate-buffered saline solution, 0.5 mL of 10% trichloroacetic acid (TCA) was added to each well, and the plates were kept on ice for 5 min. This step was repeated three times. The TCA-precipitable material was then dissolved with 0.5 mL of 1 N NaOH at 37° for 1 hour. The supernatants were then neutralised with 1 N HCl, transferred into scintillation vials, mixed with scintillation liquid, and counted in a β-counter.

ET-1 Determination

Levels of ET-1 released by PASMCs in response to the corresponding drugs were assayed using a commercially available radioimmunoassay kit. For all determinations, cells were seeded in 6-well plates and at the time of the experiments were approximately 80% confluent. Four wells were set up for each treatment. Incubations were carried out in serum-free SmGM. Cell supernatants were purified using C-2 columns, which provided a recovery of 85 ± 6%, before being assayed. Results were corrected by the amount of protein in each well. A microassay procedure (Bio-Rad Laboratory) similar to the Lowry method [20] was used to determine the protein content.

Statistical Analysis

For the calculation of PASMC proliferation results, the percentage of this proliferation was determined because the variations in [^{3}H]Thy incorporation in different experiments invalidated their direct comparison. In every experiment (each of which was performed at least in triplicate), the mean value of the four control wells (serum-free SmGM) was assumed to be the 100% PASMC proliferation level. All proliferation values in each replicate of control and experimental samples were converted into percentage values by using the value described above as 100% proliferation. Means and SEM values were calculated from the obtained percentages.

The SAS software (SAS Institute Inc.) was used for the computations. The following tests were used as appropriate (see legends to the figures): two- or three-way analysis of variance, Dunnett test, two-sample Student's *t*-test, and Tukey's test.

RESULTS

Figure 1 (a–c) shows the concentration- and time- dependent effect of CsA and SDZ IMM 125 on PASMC [^{3}H]Thy incorporation. The maximum effect was observed after 72 hr of incubation with 0.1 μM CsA, while at higher concentrations a decrease in this parameter was observed. This decrease was related to a direct toxic effect on cells, as confirmed by an increase in the leakage of lactate dehydro-

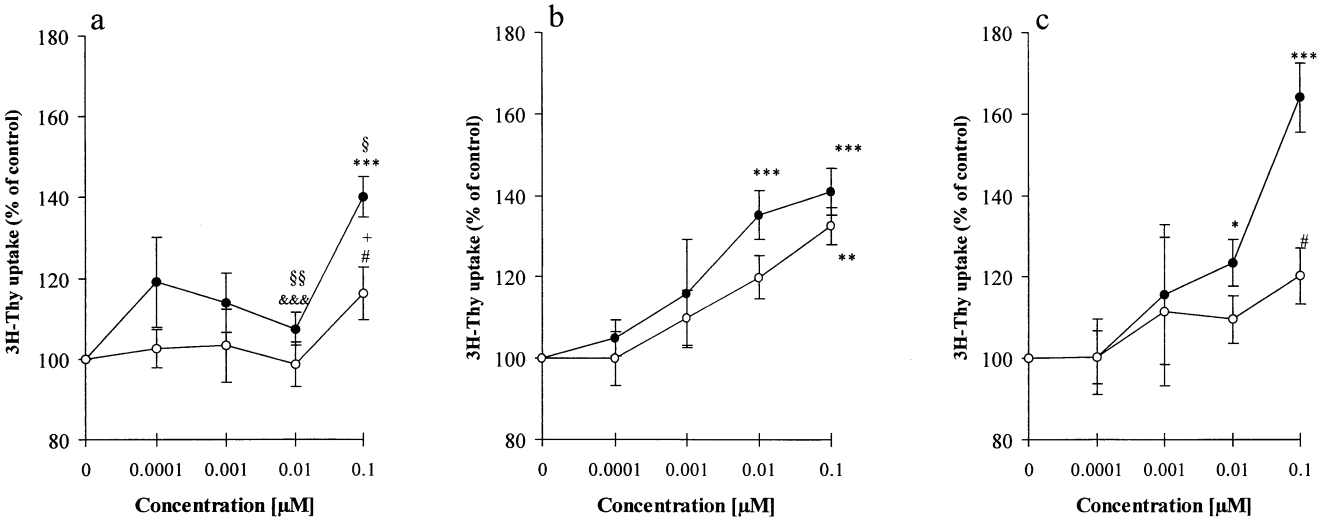

FIG. 1. Effect of cyclosporine A (closed circles) and SDZ IMM 125 (open circles) on [^{3}H]Thy incorporation by cultured human pulmonary artery smooth muscle cells after (a) 24 hr, (b) 48 hr, and (c) 72 hr of incubation. Results are expressed as a percentage of [^{3}H]Thy uptake as compared to control untreated cell uptake (100%). Values are means ± SEM of 3 experiments with N = 6–8. Concentration dependency of the effects: *$P < 0.05$ vs control; **$P < 0.01$ vs control; ***$P < 0.001$ vs control (Dunnett test). Comparison of cyclosporine A vs SDZ IMM 125: $^{+}P < 0.001$ between drugs (two-way ANOVA); $^{\#}P < 0.05$ vs corresponding CsA concentration (*t*-test). Time dependency of the effects: $^{\&\&\&}P < 0.001$ vs 48 hr; $^{\S}P < 0.05$ vs 72 hr; $^{\S\S}P < 0.01$ vs 72 hr (Tukey's test).

genase (data not shown). In all cases, SDZ IMM 125 induced a weaker proliferative effect than CsA, reaching statistical significance at certain points, as shown in the figures. Vehicle alone (0.01% DMSO) did not cause any effect at any time on this parameter (data not shown).

The two concentrations of CsA which showed the clearest effects were selected for the following experiments: 0.01 and 0.1 μM. Incubation of PASMCs with CsA in the presence of a specific monoclonal antibody against ET-1 (dilution 1:10,000) partially blocked the proliferative effect of the drug (Fig. 2, a–c), an effect which was not achieved with the use of the same antibody when heat-inactivated (80°, 10 min) or with a similar immunoglobulin G at the same dilution (anti-desmin antibody). In the absence of CsA, the antibodies had no effect on PASMC [^{3}H]Thy incorporation.

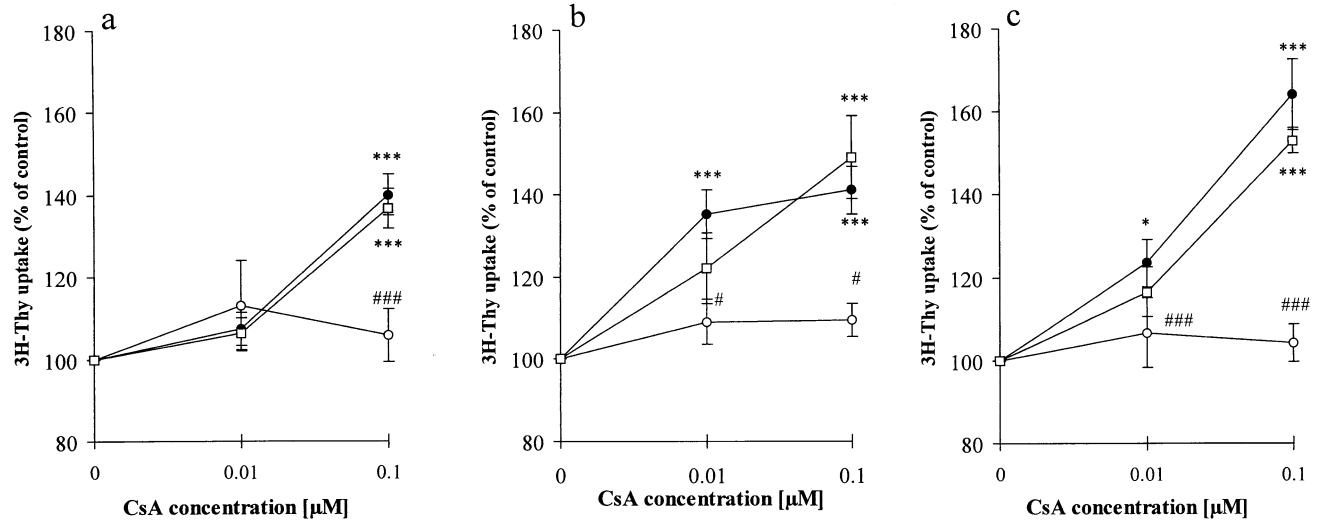

FIG. 2. Effect of an active (open circles) and a heat-inactivated (open squares) monoclonal antibody against endothelin-1 (dilution 1:10,000) on the cyclosporine A-induced increase in [^{3}H]Thy incorporation (closed circles; historical control data) by cultured human pulmonary artery smooth muscle cells after (a) 24 hr, (b) 48 hr, and (c) 72 hr of incubation. Results are expressed as a percentage of [^{3}H]Thy uptake as compared to control untreated cell uptake (100%). Values are means ± SEM of 3 experiments with N = 6–8. Concentration dependency of the effects: *$P < 0.05$ vs control; ***$P < 0.001$ vs control (Dunnett test). Comparison of cyclosporine A + antibody vs cyclosporine A alone: $^{\#}P < 0.05$ vs corresponding concentration of cyclosporine A alone; $^{\#\#\#}P < 0.001$ vs corresponding concentration of cyclosporine A alone (*t*-test).

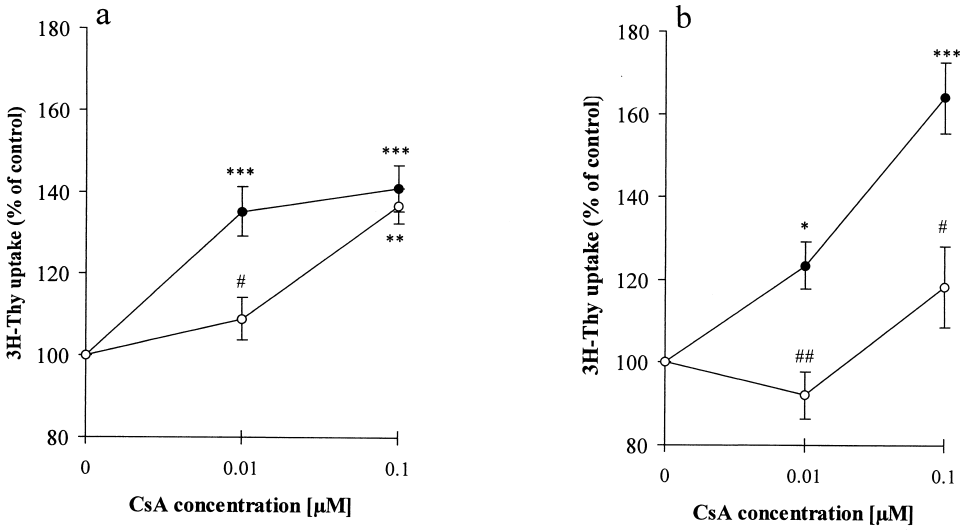

FIG. 3. Effect of verapamil (1 μM) (open circles) on the cyclosporine A-induced increase in [^{3}H]Thy incorporation (closed circles) by cultured human pulmonary artery smooth muscle cells after (a) 48 hr and (b) 72 hr of incubation. Results are expressed as a percentage of [^{3}H]Thy uptake as compared to control untreated cell uptake (100%). Values are means ± SEM of 3 experiments with N = 6–8. Concentration dependency of the effects: *$P < 0.05$ vs control; **$P < 0.01$ vs control; ***$P < 0.001$ vs control (Dunnett test). Comparison of cyclosporine A + verapamil vs cyclosporine A alone: $^{\#}P < 0.05$ vs corresponding concentration of cyclosporine A alone; $^{\#\#}P < 0.01$ vs corresponding concentration of cyclosporine A alone (*t*-test).

Figure 3 (a and b) shows the partial inhibitory effect of the calcium channel blocker verapamil (VP) (1 μM) on CsA-induced PASMC [^{3}H]Thy incorporation after 48 and 72 hr of incubation. Incubation of PASMCs with CsA in the presence of the long-acting somatostatin derivative octreotide (SDT) resulted in a lower increase in [^{3}H]Thy incorporation as compared with that induced by CsA alone (Fig. 4, a and b). The partial blocking effect of SDT was observed to be concentration-dependent in the range from 0.01 to 1 μM (data not shown). Higher concentrations induced a direct toxic effect on PASMCs (lactate dehydrogenase test).

Next, the ability of PASMCs to release ET-1 into the medium in response to the addition of the drugs was tested through radioimmunoassay. Determinations were done after 8, 24, and 48 hr of incubation (Fig. 5, a–c). The graphics show that CsA induced an increase in the release of ET-1 by PASMCs as compared to the basal level, an

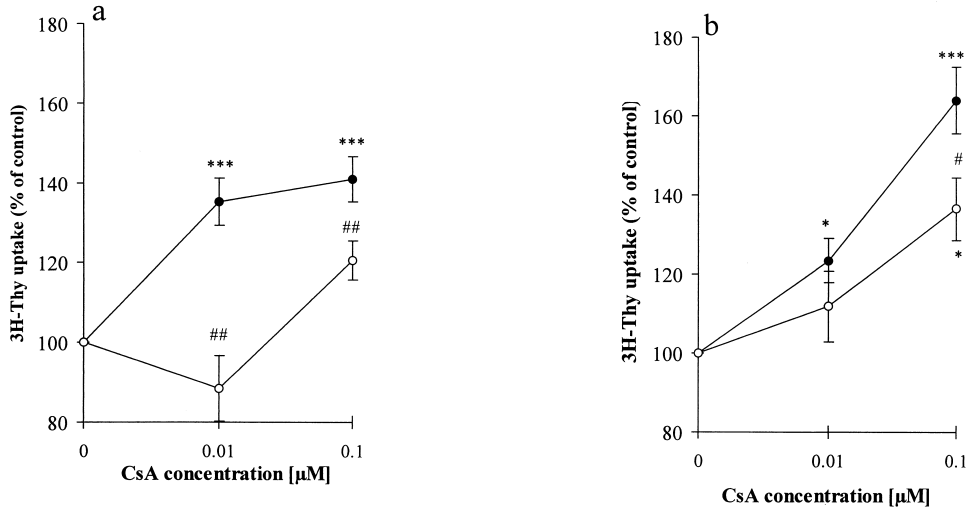

FIG. 4. Effect of Sandostatin (1 μM) (open circles) on the cyclosporine A-induced increase in [^{3}H]Thy incorporation (closed circles) by cultured human pulmonary artery smooth muscle cells after (a) 48 hr and (b) 72 hr of incubation. Results are expressed as a percentage of [^{3}H]Thy uptake as compared to control untreated cell uptake (100%). Values are means ± SEM of 3 experiments with N = 6–8. Concentration dependency of the effects: *$P < 0.05$ vs control; ***$P < 0.001$ vs control (Dunnett test). Comparison of cyclosporine A + Sandostatin vs cyclosporine A alone: $^{\#}P < 0.05$ vs corresponding concentration of cyclosporine A alone; $^{\#\#}P < 0.01$ vs corresponding concentration of cyclosporine A alone (*t*-test).

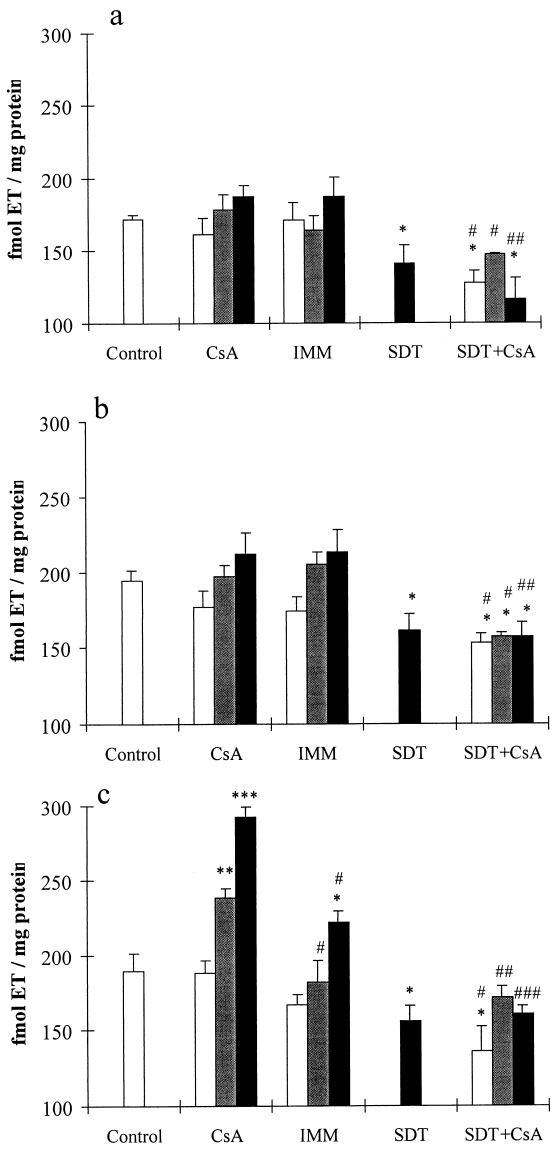

FIG. 5. Effect of cyclosporine A (CsA), SDZ IMM 125 (IMM), Sandostatin (SDT), and Sandostatin + cyclosporine A (SDT + CsA) on endothelin-1 (ET-1) release by cultured human pulmonary artery smooth muscle cells after (a) 8 hr, (b) 24 hr, and (c) 48 hr of incubation. Drug concentrations were 0.01 μM (white bars), 0.1 μM (grey bars), and 1 μM (black bars). The amount of ET-1 in the supernatants was measured through radioimmunoassay. Results are expressed as fmol ET-1/mg cellular protein. Values are means ± SEM of 3 experiments with N = 4–6. Concentration dependency of the effects: *P < 0.05 vs control; **P < 0.01 vs control; ***P < 0.001 vs control (Dunnett test). Comparison of cyclosporine A + Sandostatin vs cyclosporine A alone: #P < 0.05 vs corresponding concentration of cyclosporine A alone; ##P < 0.01 vs corresponding concentration of cyclosporine A alone; ###P < 0.001 vs corresponding concentration of cyclosporine A alone (*t*-test).

effect which was statistically significant after 48 hr with the concentrations of 0.1 and 1 mM (at shorter times, only a trend toward an increase was observed). On the other hand, SDZ IMM 125 induced only a slight increase in ET-1 production by the cells after 48 hr at the concentration of

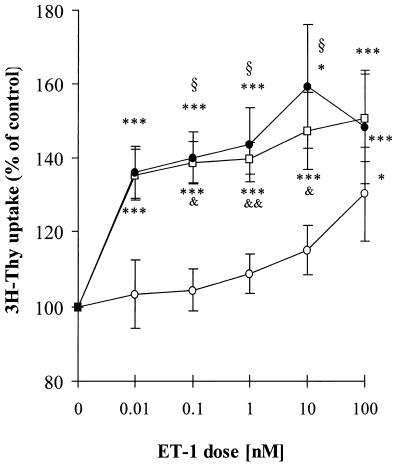

FIG. 6. Effect of endothelin-1 on [^{3}H]Thy incorporation by cultured human pulmonary artery smooth muscle cells after 24 hr (open circles), 48 hr (open squares), and 72 hr (closed circles) of incubation. Results are expressed as a percentage of [^{3}H]Thy uptake as compared to control untreated cells uptake (100%). Values are means ± SEM of 3 experiments with N = 6–8. Concentration dependency of the effects: *P < 0.05 vs control; ***P < 0.001 vs control (Dunnett test). Time dependency of the effects: &P < 0.05, 48 hr vs 24 hr; &&P < 0.01, 48 hr vs 24 hr; §P < 0.05, 72 hr vs 24 hr.

1 mM, and even in this case, the amount of ET-1 released was significantly lower than that produced by CsA. Furthermore, SDT (1 μM) was able to significantly reduce the production of ET-1 by PASMCs at all times tested, under control conditions, and in the presence of CsA (Fig. 5, A–C).

Finally, the ability of human recombinant ET-1 to modify the proliferative state of PASMCs was tested. Figure 6 shows that ET-1 induced a marked concentration- and time-dependent increase in the incorporation of [^{3}H]Thy by PASMCs which was statistically significant after 24 hr of incubation with the concentration of 100 nM and after 48 hr with 10 pM ET-1.

DISCUSSION

CsA is one of the most efficacious immunosuppressant drugs. However, since the vascular system represents a potential target for adverse effects, care has to be taken to monitor possible alterations. The renal dysfunction and hypertension that have been associated with CsA administration are probably related to its effects on the regulation mechanisms of vascular tone [9]. On the other hand, prolonged administration leads to anatomical, rather than functional, changes in the vascular structures. However, it is still a matter of discussion whether the vasculopathies related to CsA administration are the consequence of CsA action or the result of a variety of factors, including chronic graft rejection, vascular remodelling (in the case of asthma), etc. [11, 21, 22]. Chronic administration of CsA by inhalation as a novel therapy for asthma or lung transplantation would require a special attention to the vascular

effects, especially at the local site of administration. The present study was designed to investigate the *in vitro* effects of CsA on PASMCs as well as the mechanisms involved, in order to find alternatives to antagonise the potential CsA-induced effects.

CsA induced a direct proliferative effect on human PASMC. Although a proliferative effect of CsA is shown in the present study, the precise effects on the PASMC cell cycle are still not known. Further experiments aimed at studying how CsA affects cell cycle parameters and regulatory checkpoints would certainly contribute to the understanding of the specific transduction pathways triggered by CsA. SDZ IMM 125 induced PASMC proliferation to a lesser extent than CsA. This suggests that the application of this drug to humans might be better tolerated than CsA *in vivo* at the vascular level. Similar conclusions supporting this hypothesis have been previously reported [23].

There is controversy regarding the direct action of CsA on the growth rate of cultured smooth muscle cells from different origins: antiproliferative [14, 24], non-active [25], and proliferative [13, 16]. Differences among species and among tissular origin of the smooth muscle cells probably account for these discrepancies. Some authors also propose a different effect of CsA depending on the concentration used in the experiments [15]. The vasoconstrictor peptide ET-1 plays a central role in CsA-induced vascular effects. It is released by smooth muscle cells [26] and induces proliferation in some smooth muscle cell types [27]. Furthermore, ET-1 is increased in response to CsA both *in vivo* and in different *in vitro* systems [25, 26, 28–30]. The involvement of ET-1 in the pathophysiology of a number of lung pathologies, including asthma, has been demonstrated [31].

The partial inhibition of the CsA-induced effect by the pretreatment of PASMCs with an anti-ET-1 monoclonal antibody indicates that the secondary release of ET-1 may be one of the possible mediating mechanisms. This action was specific for this functional antibody, because neither the heat-inactivated antibody nor the use of a similar non-relevant immunoglobulin G had similar inhibitory ability. Anti-ET-1 antibodies, as well as ET-1 receptor antagonists, have previously been used to block CsA-induced effects *in vivo* [32, 33], *ex vivo* [34], and *in vitro* [35]. However, to our knowledge, this is the first report showing CsA-induced alterations in pulmonary vascular cells mediated by ET-1.

Smooth muscle cells express and release ET-1 in response to CsA [35]. As shown in Fig. 5, the increased release caused by the drug was statistically significant after 48 hr at 0.1 and 1 mM. With such stimulus, PASMCs released between 250 and 300 fmol ET-1/mg rotein (equivalent to 8–9.6 pM in the supernatant). These ET-1 concentrations were considered to be sufficient to induce PASMC proliferation, because in parallel experiments, synthetic human ET-1 induced a marked increase in the proliferation of PASMCs which was significant after 48 hr at the dose of 10 pM. Taken together, these results indicate that ET-1 may be one of the possible mediators of the CsA-evoked proliferative effect on PASMCs.

Next, the involvement of calcium in the observed effects of CsA was investigated. Calcium is needed for the binding of CsA, cyclophilin, and calcineurin that takes place during CsA immunosuppression [36]. CsA may also alter calcium homeostasis at the cellular level, resulting in an impaired cell function (this may be, at least in part, the mechanism responsible for the marked vasoconstriction causing hypertension and nephrotoxicity) [37, 38]. The use of calcium channel blockers inhibits some of the *in vitro* effects of CsA [39, 40], prevents some of the CsA adverse effects in treated animals [41, 42], and might even improve initial graft function, rejection frequency, and long-term graft survival of treated patients [43–45].

Verapamil induced a partial inhibition of the CsA-induced effects, thereby suggesting that the mechanism of action of CsA involves an intracellular increase in cytosolic calcium concentration. These findings are consistent with our hypothesis that ET-1 mediates CsA effects, because ET-1 induces many of its actions (contraction, mitogenesis, etc.) through an increase in the concentration of intracytosolic free calcium, which acts as a second messenger [46]. Although ET-1 is not a ligand of L-type calcium channels, verapamil was able to block CsA action (which, as shown above, is mediated by ET-1). The reason for this may be an indirect opening of voltage-operated channels through an unknown mechanism in response to ET-1 [47] or the involvement of mediators other than ET-1 in the cell response (platelet-activating factor, thromboxane A_2, etc.) The analysis of these possibilities requires further investigation.

SDT is a long-acting derivative of somatostatin [48]. It potently inhibits secretions, mainly in pancreas and gastrointestinal tract, but also in kidney, thyroid, parathyroid, cerebral cortex, and peripheral nervous system [48, 49]. Furthermore, it inhibits the growth of different cell types, including smooth muscle cells [50]. Interestingly, octreotide and other somatostatin analogues protect arteries from myointimal hyperplasia in several experimental models [51–53]. Takahashi *et al.* [54] demonstrated that administration of SDT to rats was able to inhibit the development of monocrotaline-induced medial proliferation of pulmonary arteries in rats. In our experiments, SDT partially inhibited the effect of CsA (Fig. 4) and decreased significantly, although not completely, the release of ET-1 by PASMCs under basal conditions and upon stimulation with CsA (Fig. 5). This indicates that SDT might inhibit CsA effects on PASMC through a mechanism involving, at least in part, inhibition of the release of ET-1 by the cells (although other mechanisms should not be discarded), and therefore, preventing its secondary action.

The observations reported here provide evidence for two of the possible mechanisms involved in the CsA-induced proliferation of cultured normal human PASMCs: ET-1 and calcium. However, additional mediators might be involved in the *in vivo* situation, where other cell types and

regulatory mechanisms are present: for instance, adjacent vascular endothelial cells, which can release antiproliferative mediators such as transforming growth factor beta, nitric oxide, heparin, heparan sulfate, atrial natriuretic peptide, etc.; and epithelial cells and fibroblasts, able to release prostaglandins and cytokines [55]. Finally, PASMCs themselves could release other active mediators (platelet-derived growth factor, basic fibroblast growth factor, insulin-like growth factor, heparin-binding epidermal growth factor-like growth factor, transforming growth factor, interferon-γ, etc.) [56] which have not been evaluated in this study and might be produced in response to CsA, and which might indeed potentiate or counterbalance the effects triggered by ET-1.

In conclusion, CsA increased the rate of proliferation of PASMCs, while SDZ IMM 125 induced a weaker effect. The CsA-induced effect was blocked by an anti-ET-1 antibody, as well as by verapamil, thus indicating the involvement of ET-1 and calcium in CsA-induced effects. Preincubation of PASMCs with SDT partially blocked the effect of CsA. The findings reported here point to the possibility of using CsA in conjunction with other pharmacological modulators, such as anti-ET-1 antibodies, ET-1 receptor antagonists, calcium channel blockers, or SDT, to increase the safety for the treatment of asthma or for lung transplantation.

References

1. Morley J, Cyclosporin A in asthma therapy: A pharmacological rationale. *J Autoimmun* **5** (Suppl A): 265–269, 1992.
2. Deykin A and Israel E, Newer therapeutic agents for asthma. *Dis Mon* **45:** 117–144, 1999.
3. Alexander AG, Barnes NC and Kay AB, Trial of cyclosporin in corticosteroid-dependent chronic severe asthma. *Lancet* **339:** 324–328, 1992.
4. Lock SH, Kay AB and Barnes NC, Double-blind, placebo-controlled study of cyclosporin A as a corticosteroid-sparing agent in corticosteroid-dependent asthma. *Am J Respir Crit Care Med* **153:** 509–514, 1996.
5. Narita SI, Asakura K, Shiraski H, Isobe M, Ogasawara H, Saito H and Kataura A, Effects of cyclosporin A and glucocorticosteroids on antigen-induced hypersensitivity to histamine in a guinea pig model of allergic rhinitis. *Inflamm Res* **47:** 62–66, 1998.
6. Ceyhan BB, Sungur M, Celikel CA and Celikel T, Effect of inhaled cyclosporin on the rat airway: Histologic and bronchoalveolar lavage assessment. *Respiration* **65:** 71–78, 1998.
7. Iacono AT, Smaldone GC, Keenan RJ, Diot P, Dauber JH, Zeevi A, Burckart GJ and Griffith BP, Dose-related reversal of acute lung rejection by aerosolized cyclosporine. *Am J Respir Crit Care Med* **155:** 1690–1698, 1997.
8. Iacono AT, Keenan RJ, Duncan SR, Smaldone GC, Dauber JH, Paradis IL, Ohori NP, Grgurich WF, Burckart GJ, Zeevi A, Delgado E, O'Riordan TG, Zendarsky MM, Yousem SA and Griffith BP, Aerosolized cyclosporine in lung recipients with refractory chronic rejection. *Am J Respir Crit Care Med* **153:** 1451–1455, 1996.
9. Mason J, Pharmacology of cyclosporine (sandimmune). VII. Pathophysiology and toxicology of cyclosporine in humans and animals. *Pharmacol Rev* **41:** 423–434, 1990.
10. Noble S and Markham A, Cyclosporin. A review of the pharmacokinetic properties, clinical efficacy and tolerability of a microemulsion-based formulation (Neoral). *Drugs* **50:** 924–941, 1995.
11. Bujan J, Bellon JM, Jurado F, Hernando A Contreras L, Assessment of cyclosporine A-induced ultrastructural changes in vascular wall using an experimental arterial autograft model. *Histol Histopathol* **10:** 567–576, 1995.
12. Kuwahara M, Jacobsson J, Kuwahara M, Kagan E, Ramwell PW and Foegh ML, Coronary artery ultrastructural changes in cardiac transplant atherosclerosis in the rabbit. *Transplantation* **52:** 759–765, 1991.
13. Leszczynski D, Zhao Y, Yeagley TJ and Foegh ML, Direct and endothelial cell-mediated effect of cyclosporin A on the proliferation of rat smooth muscle cells *in vitro*. *Am J Pathol* **142:** 149–155, 1993.
14. Mohacsi PJ, Tuller D, Hulliger B and Wijngaard PL, Different inhibitory effects of immunosuppressive drugs on human and rat aortic smooth muscle and endothelial cell proliferation stimulated by platelet-derived growth factor or endothelial cell growth factor. *J Heart Lung Transplant* **16:** 484–492, 1997.
15. Tavares P, Martinez-Salgado C, Eleno N, Teixeira F and Lopez Novoa JM, Effect of cyclosporin A on rat smooth-muscle cell proliferation. *J Cardiovasc Pharmacol* **31:** 46–49, 1998.
16. Hu SJ, Fernandez R and Jones JW Jr, Cyclosporine A stimulates proliferation of vascular smooth muscle cells and enhances monocyte adhesion to vascular smooth muscle cells. *Transplant Proc* **31:** 663–665, 1999.
17. Hiestand PC, Gräber M, Hurtenbach U, Herrmann P, Cammisuli S, Richardson BP, Eberle MK and Borel JF, The new cyclosporine derivative, SDZ IMM 125: *In vitro* and *in vivo* pharmacologic effects. *Transplant Proc* **24** (Suppl 2): 31–38, 1992.
18. Donatsch P, Mason J, Richardson BP and Ryffel B, Toxicologic evaluation of the new cyclosporin derivative, SDZ IMM 125, in a comparative, subchronic toxicity study in rats. *Transplant Proc* **24** (Suppl 2): 39–42, 1992.
19. Hatakeyama H, Miyamori I, Fujita T, Takeda Y, Takeda R and Yamamoto H, Vascular aldosterone. Biosynthesis and a link to angiotensin II-induced hypertrophy of vascular smooth muscle cells. *J Biol Chem* **269:** 24316–24320, 1994.
20. Lowry OH, Rosebrough NG, Farr AL and Randal RJ, Protein measurement with the folin phenol reagent. *J Biol Chem* **193:** 265–275, 1951.
21. Myers BD, Newton L, Boshkos C, Macoviak JA, Frist WH, Derby GC, Perlroth MG and Sibley RK, Chronic injury of human renal microvessels with low-dose cyclosporine therapy. *Transplantation* **46:** 694–703, 1988.
22. Selby DM, Rudzki JR, Bayever ES and Chandra RS, Vasculopathy of small muscular arteries in pediatric patients after bone marrow transplantation. *Hum Pathol* **30:** 734–740, 1999.
23. Medina J, Robinson S, Kammermann R, Cordier A, Soler M and de Fraissinette AB, Cytokine profile of human bronchoalveolar macrophages and bronchial epithelial cells in response to inhalation particles of the cyclosporine derivative IMM 125. *Inhal Toxicol* **11:** 675–691, 1999.
24. Thyberg J and Hansson GK, Cyclosporine A inhibits induction of DNA synthesis by PDGF and other peptide mitogens in cultured rat aortic smooth muscle cells and dermal fibroblasts. *Growth Factors* **4:** 209–219, 1991.
25. Bunchman TE and Brookshire CA, Cyclosporine-induced synthesis of endothelin by cultured human endothelial cells. *J Clin Invest* **8:** 310–314, 1991.
26. Resink TJ, Hahn AW, Scott-Burden T, Powell J, Weber E and Buhler FR, Inducible endothelin mRNA expression and

peptide secretion in cultured human vascular smooth muscle cells. *Biochem Biophys Res Commun* **168:** 1303–1310, 1990.
27. Bobik A, Grooms A, Millar JA, Mitchell A and Grinpukel S, Growth factor activity of endothelin on vascular smooth muscle. *Am J Physiol* **258:** C408–C415, 1990.
28. Haug C, Duell T, Voisard R, Lenich A, Kolb HJ, Mickley V, Hombach V and Grunert A, Cyclosporine A stimulates endothelin release. *J Cardiovasc Pharmacol* **26:** S239–S241, 1995.
29. Asberg A, Attramadal H, Midtvedt K, Sund S, Hartmann A and Berg KJ, Gene expression of the renal endothelin system in renal transplant recipients on cyclosporine A based immunosuppression. *Transplantation* **67:** 1056–1060, 1999.
30. L'Azou B, Medina J, Frieauff W, Cordier A, Cambar J and Wolf A, *In vitro* models to study mechanisms involved in cyclosporine A-mediated glomerular contraction. *Arch Toxicol* **73:** 337–345, 1999.
31. Hay DW, Putative mediator role of endothelin-1 in asthma and other lung diseases. *Clin Exp Pharmacol Physiol* **26:** 168–171, 1999.
32. Davis LS, Haleen SJ, Doherty AM, Cody WL and Keiser JA, Effects of selective endothelin antagonists on the hemodynamic response to cyclosporin A. *J Am Soc Nephrol* **47:** 1448–1454, 1994.
33. Bartholomeusz B, Hardy KJ, Nelson AS and Phillips PA, Bosentan ameliorates all cyclosporin A-induced hypertension in rats and primates. *Hypertension* **27:** 1341–1345, 1996.
34. Perico N, Dadan J and Remuzzi G, Endothelin mediates the renal vasoconstriction induced by cyclosporine in the rat. *J Am Soc Nephrol* **1:** 76–83, 1990.
35. Takeda Y, Itoh Y, Yoneda T, Miyamori I and Takeda R, Cyclosporine A induces endothelin-1 release from cultured rat vascular smooth muscle cells. *Eur J Pharmacol* **233:** 299–301, 1993.
36. Sigal NH and Dumont FJ, Cyclosporin A, FK-506, and rapamycin: Pharmacologic probes of lymphocyte signal transduction. *Annu Rev Immunol* **10:** 519–560, 1992.
37. Lo Russo A, Passaquin AC, André P, Skutella M and Rüegg UT, Effect of cyclosporin A and analogues on cytosolic calcium and vasoconstriction: Possible lack of relationship to immunosuppressive activity. *Br J Pharmacol* **118:** 885–892, 1996.
38. Avdonin PV, Cottet-Maire F, Afanasjeva GV, Loktionova SA, Lhote P and Ruegg UT, Cyclosporine A up-regulates angiotensin II receptors and calcium responses in human vascular smooth muscle cells. *Kidney Int* **55:** 2407–2414, 1999.
39. Gotze S, Auch-Schwelk W, Bossaller C, Thelen J and Fleck E, Preventive effects of dilitiazem on cyclosporin A-induced vascular smooth muscle dysfunction. *Transpl Int* **77:** 157–162, 1994.
40. Gallego MJ, Garcia Villalon AL, López Farre AJ, Garcia JL, Garron MP, Casado S, Hernando L and Caramelo CA, Mechanisms of the endothelial toxicity of cyclosporin A. Role of nitric oxide, cGMP, and Ca^{2+}. *Circ Res* **74:** 477–484, 1994.
41. Shaikh MG, Heys SD, Brown PA and Whiting PH. Chronic cyclosporin A (CsA) nephrotoxicity in the rat: The effect of calcium blockade with verapamil. *Int J Exp Pathol* **74:** 389–396, 1993.
42. Ar'Rajab A, Dawidson IJ, Harris RB, Mileski WJ and Sentementes JT, Deleterious effect of cyclosporins on the ischemic kidney in the rat and the protection by the calcium antagonist verapamil. *J Am Soc Nephrol* **5:** 93–101, 1994.
43. van der Dorpel MA, Zietse R, Ijzermans JN, Schalekamp MA and Weimar W, Effect of isradipine on cyclosporin A-related hypertension. *Blood Press Suppl* **1:** 50–53, 1994.
44. Harper SJ, Moorhouse J, Abrams K, Jurewicz A, Nicholson M, Horsburgh T, Harris K, Combe C, Bell PR, Walls J, Donnelly PK, Veitch PS and Feehally J. The beneficial effects of oral nifedipine on cyclosporin-treated renal transplant recipients—a randomised prospective study. *Transpl Int* **9:** 115–125, 1996.
45. Ahmed K, Michael B and Burke JF Jr, Effects of isradipine on renal hemodynamics in renal transplant patients treated with cyclosporine. *Clin Nephrol* **48:** 307–310, 1997.
46. Simonson MS and Dunn MJ, Endothelin. Pathways of transmembrane signaling. *Hypertension* **15:** 15–I12, 1990.
47. Rubanyi GM and Polokoff MA. Endothelins: Molecular biology, biochemistry, pharmacology, physiology and pathophysiology. *Pharmacol Rev* **46:** 325–415, 1994.
48. Battershill PE and Clissold SP, Octreotide. A review of its pharmacodynamic and pharmacokinetic properties and therapeutic potential in conditions associated with excessive peptide secretion. *Drugs* **38:** 658–702, 1989.
49. Mozell EJ, Woltering EA and O'Dorisio TM, Non-endocrine applications of somatostatin and octreotide acetate: Facts and flights of fancy. *Dis Mon* **37:** 749–848, 1991.
50. Grant MB, Wargovich TJ, Ellis EA, Caballero S, Mansour M and Pepine CJ, Localization of insulin-like growth factor I and inhibition of coronary smooth muscle cell growth by somatostatin analogues in human coronary smooth muscle cells. A potential treatment for restenosis? *Circulation* **89:** 1511–1517, 1994.
51. Santoian ED, Schneider JE, Gravanis MB, Foegh M, Tarazona N, Cipolla GD and King SB, Angiopeptin inhibits intimal hyperplasia after angioplasty in porcine coronary arteries. *Circulation* **88:** 11–14, 1993.
52. Ulus AT, Iscan Z, Saritas Z, Can C, Yamak B, Katircioglu SF and Bayazit M, Inhibition of myointimal proliferation by octreotide in canine vein interposition grafts. *Eur Surg Res* **30:** 318–325, 1998.
53. Sakamoto H, Sakamaki T, Kanda T, Ito Y, Sumino H, Masuda H, Ohyama Y, Ono Z, Kurabayashi M, Kobayashi I and Nagai R, The somatostatin analog, octreotide, inhibits *in vitro* outgrowth of smooth muscle cells from canine coronary and carotid atherosclerotic plaque tissues. *Res Commun Mol Pathol Pharmacol* **101:** 25–34, 1998.
54. Takahashi T, Kanda T, Imai S, Suzuki T and Murata K. Sandostatin inhibits development of medial proliferation of pulmonary arteries in a rat model of pulmonary hypertension. *Life Sci* **57:** PL91–PL95, 1995.
55. Scott-Burden T and Vanhoutte PM, Regulation of smooth muscle cell growth by endothelium-derived factors. *Tex Heart Inst J* **21:** 91–97, 1994.
56. Owens GK, Regulation of differentiation of vascular smooth muscle cells. *Physiol Rev* **75:** 487–517, 1995.