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Cyclosporine A aggravates vascular endothelial injury in hyperlipidemic rats by down-regulating decay-accelerating factor

Wei Wang [a,1], Peng Zhang [a,1], Jinjing Wang [a], Hong Bu [b,*]

[a] Key Laboratory of Transplant Engineering and Immunology, Ministry of Health, West China Hospital, Sichuan University, Chengdu, PR China
[b] Department of Pathology, West China Hospital, Sichuan University, Chengdu 610041, Sichuan, PR China

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ABSTRACT

It has been reported that cyclosporine A (CsA) aggravates vascular injury in hyperlipidemic patients, but the specific mechanisms are unclear. We explored the hypothesis that CsA may result in complement-mediated endothelial cell lysis induced by down-regulation of decay-accelerating factor (DAF) in hyperlipidemic patients. Human umbilical vein endothelial cells (HUVECs) were treated with CsA or/and oxidized low-density lipoprotein (ox-LDL) before allowing DAF expression. Complement factor C3 cell binding was measured by flow cytometry. CsA exposure led to decreased DAF expression and aggravated cell lysis of the HUVECs pre-incubated with ox-LDL, in a dose-dependent fashion. In in vivo experiments using thoracic aortic endothelium from hyperlipidemic rats, CsA resulted in dose-dependent down-regulation of DAF, and accompanying endothelial damage. These observations provide new evidence that hyperlipidemic patients treated with CsA may have an increased vascular risk, at least in part through complement-mediated EC lysis following down-regulated DAF expression.

Although cyclosporine A (CsA) is an effective immunosuppressant in tissue/organ transplantation, long-term use is limited by side effects such as endothelial dysfunction and transplant vasculopathy [1,2]. It is known that CsA increases the risk of vascular disease in hyperlipidemic patients, but the specific mechanisms are unclear [3]. Some studies suggest the complement system may play an important role: for example, CsA can activate the complement system and induce C3 and C4d deposition in the renal tubule and interstitium [4,5]. A recent study suggests CsA inhibits up-regulation of complement inhibitor decay-accelerating factor (DAF) in vascular endothelium by decreasing the expression of vascular endothelial growth factor (VEGF) [6]. However, there is evidence that binding of oxidized low-density lipoprotein (ox-LDL) to its receptor (LOX-1) induces VEGF expression in hyperlipidemic patients [7]. This led us to consider the roles of CsA and ox-LDL/LOX-1 in regulating VEGF/DAF production in hyperlipidemic transplant patients.

It is known that DAF is inducible on the surface of vascular endothelial cells (ECs), that it protects ECs against complement-mediated cell lysis triggered by a variety of physiological agents, and that other membrane-bound complement regulatory proteins have no such biological effects [8]. DAF regulation is known to play a role in the pathogenesis of atherosclerosis, as well as in the accelerated atherosclerosis of transplantation and other inflammatory cardiovascular diseases [9,10]. However, it is unclear how DAF is expressed, what regulatory functions it performs, and its exact role in the pathogenesis of cardiovascular disease, particularly in special situations such as transplant-related hyperlipidemia.

In this study, in vivo and in vitro experiments demonstrate that CsA down-regulates DAF expression on vascular ECs in hyperlipidemia in a dose-dependent fashion through the VEGF pathway, and that this down-regulation of DAF enhances complement-mediated vascular EC lysis. We provide new evidence (i) that transplant patients with hyperlipidemia treated with CsA have a higher vascular risk than either transplant patients taking CsA who do not have hyperlipidemia or hyperlipidemic patients who are not taking CsA, and (ii) that this vascular injury occurs, at least in part, through complement-mediated mechanisms.

Materials and methods

Preparation and oxidation of LDL. Human LDL was isolated from fresh plasma by gradient ultracentrifugation as previously described [11]. The purity of the LDL was assessed by agarose gel electrophoresis, and the protein content was determined by the modified Lowry method. LDL was oxidized at a concentration of 3 mg protein/mL by exposure to 7.5 μm $CuSO_4$ for 24 h at 37 °C. Oxidation was monitored by measuring production of thiobarbituric acid-reactive substance (20.12 ± 3.25 nmol/mg protein), and the greater mobility of LDL on agarose gel electrophoresis—due to its

* Corresponding author. Fax: +86 28 85164034.
E-mail address: hongbu@scu.edu.cn (H. Bu).
[1] These authors contributed equally to this work.

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increased negative charge—was compared with native LDL (ox-LDL was 2.0 ± 0.6 times more mobile). ox-LDL was then sterilized by passing it through a 0.22 μm filter.

Cell culture. Human umbilical vein endothelial cells (HUVECs) were obtained from the American Type Culture Collection (ATCC) and cultured in Medium199 (M199) supplemented with 10% foetal calf serum, 100 IU/mL penicillin, 0.1 mg/mL streptomycin, 2 mmol/L L-glutamine, 10 units/mL heparin, and 30 μg/mL EC growth factor at 37 °C in a 5% CO_2 humidified atmosphere. Cells used for experiments were passaged between three and seven times. The cells were incubated with ox-LDL (80 μg/mL) or/and CsA (0.1–5 μm) for 24 h before determining the amount of binding of complement factor C3 on the cell surface and the amount of expression of DAF and VEGF.

Flow cytometry. The effects of ox-LDL or/and CsA on DAF expression and C3 cell surface binding were determined using flow cytometry. A monolayer of HUVECs was pre-incubated for 24 h with ox-LDL (80 μg/mL) and then treated with a specific, blocking, anti-VEGF monoclonal antibody (mAb) (1 μg/mL, Sigma, USA) for 1 h or left untreated. Finally, cells were treated with CsA or left untreated and then harvested by exposure to trypsin/EDTA for 1 min at 37 °C. After repeated pipetting to ensure single-cell suspensions, the cells were stained with the appropriate primary MoAb for anti-DAF (Santa Cruz Biotechnology, CA, USA) and anti-C3d (DakoCytomation, USA), respectively, for 30 min at room temperature. After being washed twice in phosphate-buffered saline/2.5% FBS, the HUVECs were re-suspended in fluorescein isothiocyanate (FITC)-labeled goat anti-rat IgG and FITC-labeled rabbit anti-goat IgG (DakoCytomation, Carpentaria, CA), respectively, for 30 min at room temperature. The wash procedure was repeated, and the cells fixed in 4% paraformaldehyde. Cells were analyzed using an EPICS XL flow cytometer (Beckman Coulter, USA). The fluorescence intensity of 10,000 cells for each sample was quantified. Unstained cells were used as controls. The mean fluorescence intensity from each group was calculated and presented as a percentage of the control value.

Cell lysis assays. The complement-mediated cell lysis induced by CsA under hyperlipidemic conditions was estimated according to Elaine's methods [12]. Briefly, HUVECs were pre-incubated with ox-LDL (80 μg/mL) overnight in a 24-well plate (1×10^5/well) at 37 °C prior to the addition of CsA (0.5, 1, or 5 μm) for 24 h, with a control consisting of the medium alone. The cells were then incubated for 30 min at 37 °C in a medium containing 7 μmol/mL calcein-acetoxymeth1 ester (calcein AM, molecular probes). After being washed in M199/10% FBS, HUVECs monolayers were opsonized by incubation with anti-Endoglin MAb (DAKO) for 30 min at 37 °C. After being washed with HBSS/1% BSA, the cells were incubated with 250 μL of 5–20% fresh human serum in M199 for 30 min at 37 °C.

The supernatant from each well was transferred to a 96-well plate. After the cells were washed in M199/1% BSA, the calcein AM remaining in the cells was released by incubation with 250 μL of M199/1% BSA/0.1% Triton X-100. The lysate was then transferred to a new 96-well plate, and the calcein AM released by complement and detergent was estimated using a FL-600 microplate fluorescence reader (Biotek Instruments, Inc). The percentage of specific lysis in triplicate wells was calculated as follows: (complement-mediated release − spontaneous release)/(maximal release − spontaneous release) × 100%, where maximal release is complement-mediated release + detergent-mediated release. Spontaneous release was less than 20% in all experiments.

Animal experiments. To induce hyperlipidemia, 50 male wistar rats each weighing 180–200 g were fed a high-fat diet (40% basic feeds, 40% lard, 5% eggs, 5% whole milk powder, 5% peanut powder, 5% sesame powder) for 20 days before hyperlipidemia was confirmed by evaluating blood-fat levels. Next, the rats (n = 10 for each experimental group) were treated with two doses of CsA (1 or 5 mg/kg. per day) for 7 days. At the end of this period, the rats were anesthetized using ether, a median sternotomy was performed, and the thoracic aorta was excised for tissue sampling. In addition, before tissue excision, 5 mL of blood from the right ventricle was collected for analysis of blood-fat and serum ox-LDL levels.

mRNA analysis for DAF and VEGF. Total cell RNA was isolated from the stripped rat thoracic aorta using an RNeasy mini kit (Qiagen). Reverse transcription-polymerase chain reaction (RT-PCR) was performed using the Qiagen Onestep RT-PCR Kit. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA was used as an internal control.

The following primers were synthesized: rat DAF: 5′-GCC TTG AGG AAT TAG TAT GG-3′ (sense), 5′-TGC ACT TGG GTG GTG CAC TA-3′ (antisense); rat VEGF: 5′-GCT CTC TTG GGT GCA CTG GAC-3′ (sense), 5′-CAC CGC CTT GGC TTG TCA C -3′ (antisense); rat GAPDH: 5′-GGT CGG TGT GAA CGG ATT TG-3′ (sense), 5′-GCC TTC TCC ATG GTG GTG AA-3′ (antisense).

Western blot analysis of DAF and VEGF. Western blot analysis was performed as described previously [13]. Anti-rat DAF polyclonal antibody was obtained from Santa Cruz Biotechnology, and the VEGF monoclonal antibody and the horseradish peroxidase (HRP)-conjugated rabbit anti-goat IgG from DakoCytomation. Finally, the signals of the bands were quantified with Image-Pro Plus 4.5 software. All results were normalized to β-actin.

Immunohistochemistry and Sudan III staining. For immunohistochemistry and Sudan III staining, segments of the rat thoracic aortas were either fixed in 10% neutral-buffered formalin and embedded in paraffin according to standard procedures or snap-frozen in liquid nitrogen. Frozen sections for Sudan III staining (7 μm) or paraffin sections (4 μm) for immunohistochemistry were cut. Immunohistochemistry analysis for DAF expression was performed according to a standard protocol. The results were quantified with Image Pro-Plus software.

Statistical analysis. Data are presented as means ± SD. Differences between groups means were determined by Student's t-test or one-way ANOVA followed by a Newman–Keuls test; $P < 0.05$ was considered statistically significant.

Results

CsA down-regulated vascular endothelial DAF expression

To examine how CsA regulates the expression of DAF in a hyperlipidemic situation, HUVECs were pre-incubated with ox-LDL (80 μg/mL) for 24 h and then exposed to CsA of various dosages, from 0.1 to 5 μm, for 24 h to determine the maximal response. Compared with no treatment, a dose-dependent decrease of DAF expression was observed after exposure to CsA at concentrations of up to 5 μm. The maximum reduction of DAF expression was 84.5% ± 1.04 and this was seen at the highest dose of CsA (Fig. 1A). However, there was no change in DAF expression when the VEGF pathway was blocked (Fig. 1B).

In the animal experiments, the rats were confirmed to be hyperlipidemia by detection of high blood-fat levels. We found the plasma glucose, total cholesterol, triglyceride; ox-LDL levels and body weight of rats fed the high-fat diet to be clearly higher than those of rats fed a standard diet (Table 1). DAF mRNA and protein expression in the thoracic aorta of CsA-treated hyperlipidemic rats was markedly down-regulated compared with levels in either hyperlipidemic control rats who were not exposed to CsA or normal control rats (Supplementary Material). This indicates that CsA inhibits DAF expression in hyperlipidemia in a dose-dependent fashion.

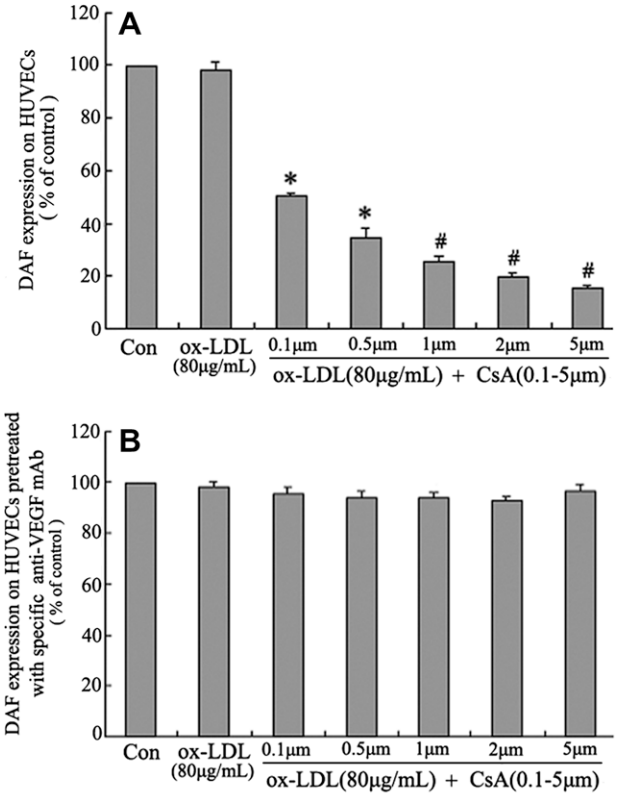

Fig. 1. Analysis of DAF expression in HUVECs following stimulation by ox-LDL or/and CsA. ECs were pre-incubated for 24 h with ox-LDL (80 μg/mL) and then treated with specific blocking anti-VEGF mAb for 1 h. Next, cells were exposed to CsA at concentrations of 0.1 or 5 μm, followed by analysis of DAF expression by flow cytometry. All data are expressed as means ± SD; $^{*}P < 0.05$, $^{\#}P < 0.01$ VS control; $n = 10$ per group. The results represent three independent experiments.

Table 1
The hyperliepma model's analysis of blood-fat value.

Parameter	Control (n:10)	Hyperlipemia model (n:10)
Glucose (mg/d1 serum)	79.63 ± 2.48	154.44 ± 3.74*
Total cholesterol (mg/d1 serum)	75.42 ± 1.78	269.40 ± 3.79#
Triglyceride (mg/d1 serum)	62.17 ± 1.91	197.86 ± 5.09#
Body weight (g)	282.33 ± 11.98	395.17 ± 10.91*
Serum ox-LDL (% of control)	100%	285.72% ± 8.93 *

The results are expressed as means ± SD.
After fed a high-fat diet for 20 days, the plasma glucose, total cholesterol, triglyceride and body weight are obviously higher than control.
* 0.05.
# 0.01 VS control.

CsA-induced down-regulation of DAF aggravates complement-mediated vascular injury

To address the functional significance of DAF down-regulation, the effect of CsA on the binding of complement factor C3 to the surface of HUVECs was measured. ox-LDL slightly increased C3 binding and CsA markedly increased binding to a maximum of 161.33 ± 4.03% (Fig. 2A). The relevance of this increase in C3 binding was confirmed by the concomitant significant increase in complement-mediated cell lysis observed in the HUVECs pre-incubated with ox-LDL and treated with CsA (Fig. 2B). Hyperlipidemia alone did not induce cell lysis, although C3 binding was slightly higher than that of normal controls. However, when CsA was added, there was marked, dose-dependent cell lysis. In the animal experiments, CsA increased the amount of damage seen in the thoracic aortic

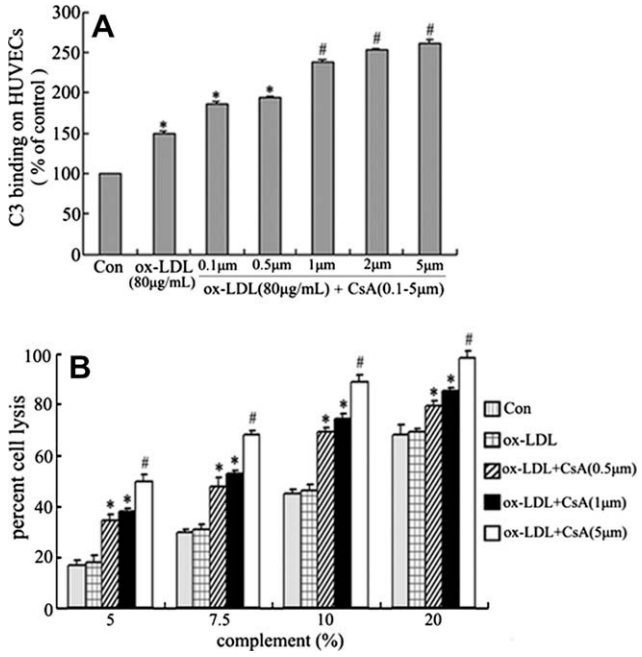

Fig. 2. ox-LDL or/and CsA-induced HUVEC damage by complement-mediated lysis. (A) The effect of CsA on surface C3 binding was determined by flow cytometry using FITC-conjugated anti-C3. (B) CsA-induced damage in HUVECs pre-incubated with ox-LDL. HUVECs were cultured with ox-LDL (80 μg/mL) overnight in 24-well plate (1×10^5/well) at 37 °C prior to the addition of CsA (0.5 or 5 μm) for 24 h before conducting the cell lysis assay, using the medium alone as a control. The percentage cell lysis was calculated. All data are expressed as means ± SD; $^{*}P < 0.05$, $^{\#}P < 0.01$ VS control; $n = 10$ per group. The resulted represent 3 independent experiments.

endothelium of hyperlipidemic rats in a dose-dependent fashion (Fig. 3A).

In addition, Sudan III staining showed that there was little lipid deposition within the thoracic aortic subendothelium of hyperlipidemic rats, some deposition in the subendothelium of hyperlipidemic rats treated with low-dose CsA (1 mg/kg), and a large amount of deposition in the subendothelium of hyperlipidemic rats treated with high-dose CsA (5 mg/kg) (Fig. 3B). This suggests that CsA increases the risk of atherosclerosis in hyperlipidemic rats above that seen with hyperlipidemia alone.

CsA induces down-regulation of DAF by inhibiting VEGF expression

When the VEGF pathway was blocked, DAF expression in HUVECs treated with CsA was not significantly different to that of normal controls (Fig. 1B). To further observe the effect of CsA on VEGF expression in a hyperlipidemic situation, we investigated both mRNA transcription and protein levels. Results of both the in vivo and in vitro experiments show that VEGF expression is up-regulated when there is hyperlipidemia. This finding accords with the previous studies. However, in the present study, we found that CsA dose-dependently inhibited this effect of hyperlipidemia on VEGF induction (Fig. 4).

Discussion

Many studies have confirmed that hyperlipidemia, as an independent risk factor, may influence the short- and long-term survival of transplant patients [14]. Furthermore, it is now recognized that hyperlipidemic patients treated with CsA have a higher vascular risk than other hyperlipidemic patients, although the mechanism is not fully understood [15]. Our in vitro data show that treatment of ox-LDL-pre-incubated HUVECs with CsA resulted

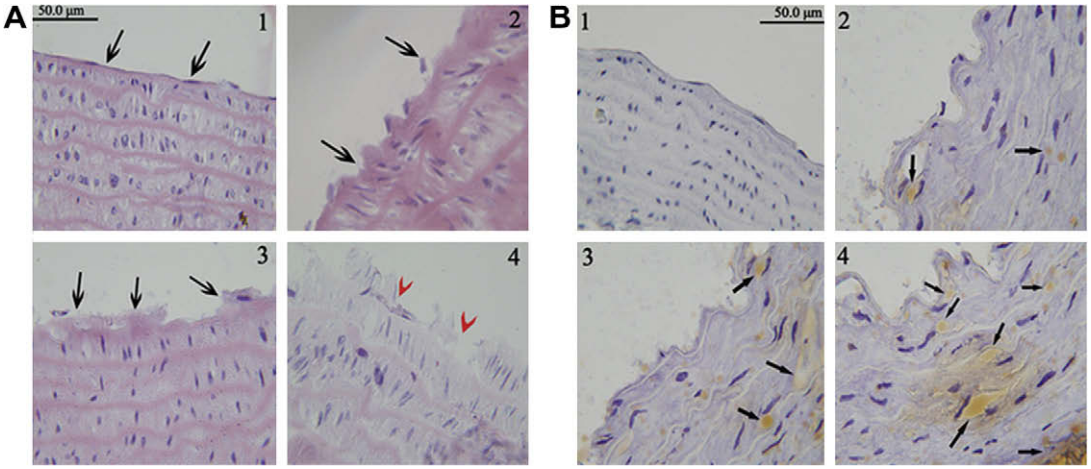

Fig. 3. CsA-induced vascular endothelium damage in hyperlipidemic rats via complement-mediated lysis. (A) Vascular endothelium was examined for damage using H&E staining. (1) The integrity of the endothelium can be observed in normal rat controls (arrows). (2) Slight endothelial damage was observed in hyperlipidemic rats (arrows). (3) The vascular endothelium suffered moderate injury, and little remaining endothelium can be observed, in hyperlipidemic rats treated with low-dose CsA (arrows). (4) The vascular wall has suffered serious damage, the endothelium is completely invisible, and the subendothelium has also suffered varying degrees of damage, in hyperlipidemic rats treated with high-dose CsA (arrowheads). (B) Observation of lipid deposition under the endothelium using Sudan III staining. (1) No lipid was observed within the endothelium of normal control rats. (2) A small amount of lipid was deposited within the subendothelium of hyperlipidemic rats (arrows). (3) Some lipid deposition was observed within the subendothelium of hyperlipidemic rats treated with low-dose CsA (arrows). (4) Large amounts of lipid were deposited within the subendothelium (arrows) of hyperlipidemic rats treated with high-dose CsA. All data are expressed as means ± SD; $^{*}P < 0.05$, $^{\#}P < 0.01$ VS control; $n = 10$ per group. The result represent three independent experiments.

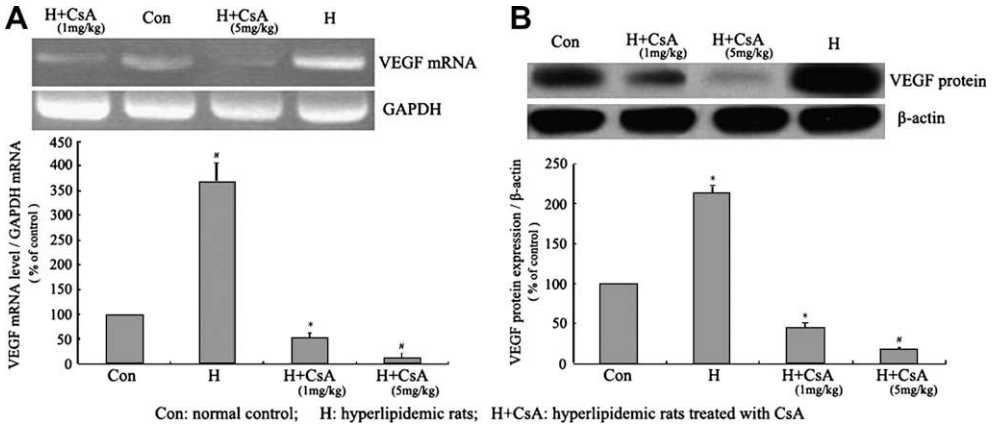

Fig. 4. Effect of CsA on VEGF expression in the thoracic aorta of hyperlipidemic rats. (A) Hyperlipidemic rats treated with CsA (1 or 5 mg/kg per day) for 7 days or left unexposed before analysis of VEGF mRNA transcription by RT-PCR. (B) Analysis of VEGF protein expression in the rat vascular endothelium using Western blot. All data are expressed as means ± SD; $^{*}P < 0.05$, $^{\#}P < 0.01$ VS control; $n = 10$ per group. The result represent three independent experiments.

in a dose-dependent down-regulation of DAF expression and an increase of C3 binding on the HUVEC surface, with subsequent complement-mediated cell lysis. In the animal experiments, we likewise found that CsA led to DAF down-regulation and vascular endothelial injury in a dose-dependent fashion. In addition, our finding that more lipid was deposited within the subendothelium of hyperlipidemic rats treated with high-dose CsA than those treated with low-dose CsA, or not exposed, suggests that CsA enhances the risk of atherosclerosis following hyperlipidemia.

To investigate whether this CsA-mediated DAF down-regulation occurs through VEGF induction, we blocked the VEGF pathway with specific anti-VEGF mAb and found that DAF expression in HUVECs did not change. In both the in vivo and in vitro experiments, mRNA and protein levels of VEGF progressively decreased with increasing doses of CsA, with a corresponding reduction of DAF expression. This suggests that ox-LDL/LOX-1 can induce VEGF expression but that CsA prevents this effect, leading to down-regulated DAF expression. Some studies have shown that CsA, a known anti-angiogenic medication, can interfere with VEGF production when blood-fat levels are normal. For example, Cho et al. found that treating synovial fibroblasts with CsA decreased VEGF expression at both the protein and mRNA level and that it did so by inhibiting activation of activator protein 1 (AP-1) [16]. Koji et al. also reported that CsA exerts its anti-angiogenic effects by inhibiting VEGF production by way of a c-jun N-terminal Kinase (JNK)-dependent pathway [17]. Moreover, CsA is able to directly inhibit many downstream signaling pathways involved in VEGF induction, including p38 MAPK, which is also an important signaling pathway for DAF production [6,18].

Cumulatively, the data suggest that CsA-induced DAF regulation is dependent on VEGF expression. Additionally, we not only found that VEGF production increases when there is hyperlipidemia, but that hyperlipidemia increases the ability of CsA to inhibit VEGF expression and downstream signaling. Reduced VEGF expression then leads to a decrease in DAF expression that induces complement-mediated lysis of the vascular endothelium.

Interestingly, in the absence of exposure to CsA, DAF production does not increase with VEGF up-regulation when there is hyperlipidemia. This finding may be explained by the fact that binding of ox-LDL to LOX-1 activates the complement system. Previous researchers have reported that both the classic and alternative complement pathways can be activated by lipids isolated from atherosclerotic lesions and by LDL which has been modified enzymatically [19,20]. Recently, Adrienn et al. showed that modified LDL binds to and actives the C1 complement complex, and that this activation may be independent of C-reactive protein [21].

It is reasonable to conclude that CsA aggravates vascular endothelial injury in hyperlipidemic situations in vivo and in vitro, at least in part by complement-mediated EC lysis. This lysis follows CsA-induced down-regulation of DAF expression in the vascular endothelium, and the cytopathic effect increases with increasing exposure to CsA. Our data emphasize the importance of understanding complement-mediated vascular endothelial injury in patients with transplant-related hyperlipidemia who use CsA for immunosuppressive therapy. This may in turn identify novel therapeutic targets for preventing and treating transplant-related atherosclerosis.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbrc.2008.11.153.

References

[1] S. Dohgu, T. Nishioku, N. Sumi, F. Takata, S. Nakagawa, M. Naito, T. Tsuruo, A. Yamauchi, H. Shuto, Y. Kataoka, Adverse effect of cyclosporin A on barrier functions of cerebral microvascular endothelial cells after hypoxia-reoxygenation damage in vitro, Cell. Mol. Neurobiol. 27 (2007) 889–899.
[2] G. Wieczorek, M. Bigaud, K. Menninger, S. Riesen, V. Quesniaux, H.J. Schuurman, M. Audet, A. Blancher, M.J. Mihatsch, V. Nickeleit, Acute and chronic vascular rejection in nonhuman primate kidney transplantation, Am. J. Transplant. 6 (2006) 1285–1296.
[3] H. Valantine, Cardiac allograft vasculopathy after heart transplantation: risk factors and management, J. Heart Lung Transplant. 23 (2004) S187–193.
[4] E.A. Emmel, C.L. Verweij, D.B. Durand, K.M. Higgins, E. Lacy, G.R. Crabtree, Cyclosporin A specifically inhibits function of nuclear proteins involved in T cell activation, Science 246 (1989) 1617–1620.
[5] Y.O. Kim, S.W. Lim, C. Li, H.J. Kang, K.O. Ahn, H.J. Yang, J.Y. Ghee, S.H. Kim, J.Y. Kim, B.S. Choi, J. Kim, C.W. Yang, Activation of intrarenal complement system in mouse model for chronic cyclosporine nephrotoxicity, Yonsei Med. J. 48 (2007) 517–525.
[6] J.C. Mason, R. Steinberg, E.A. Lidington, A.R. Kinderlerer, M. Ohba, D.O. Haskard, Decay-accelerating factor induction on vascular endothelium by vascular endothelial growth factor (VEGF) is mediated via a VEGF receptor-2 (VEGF-R2)- and protein kinase C-alpha/epsilon (PKCalpha/epsilon)-dependent cytoprotective signaling pathway and is inhibited by cyclosporin A, J. Biol. Chem. 279 (2004) 41611–41618.
[7] M. Inoue, H. Itoh, T. Tanaka, T.H. Chun, K. Doi, Y. Fukunaga, N. Sawada, J. Yamshita, K. Masatsugu, T. Saito, S. Sakaguchi, M. Sone, K. Yamahara, T. Yurugi, K. Nakao, Oxidized LDL regulates vascular endothelial growth factor expression in human macrophages and endothelial cells through activation of peroxisome proliferator-activated receptor-gamma, Arterioscler. Thromb. Vasc. Biol. 21 (2001) 560–566.
[8] J.C. Mason, Z. Ahmed, R. Mankoff, E.A. Lidington, S. Ahmad, V. Bhatia, A. Kinderlerer, A.M. Randi, D.O. Haskard, Statin-induced expression of decay-accelerating factor protects vascular endothelium against complement-mediated injury, Circ. Res. 91 (2002) 696–703.
[9] R. Ross, Atherosclerosis—an inflammatory disease, N. Engl. J. Med. 340 (1999) 115–126.
[10] S. Huber, W.C. Song, D. Sartini, Decay-accelerating factor (CD55) promotes CD1d expression and Vgamma4+ T-cell activation in coxsackievirus B3-induced myocarditis, Viral Immunol. 19 (2006) 156–166.
[11] H. Kataoka, N. Kume, S. Miyamoto, M. Minami, H. Moriwaki, T. Murase, T. Sawamura, T. Masaki, N. Hashimoto, T. Kita, Expression of lectin like oxidized low-density lipoprotein receptor-1 in human atherosclerotic lesions, Circulation 99 (1999) 3110–3117.
[12] E.A. Lidington, D.O. Haskard, J.C. Mason, Induction of decay-accelerating factor by thrombin through a protease-activated receptor 1 and protein kinase C-dependent pathway protects vascular endothelial cells from complement-mediated injury, Blood 96 (2000) 2784–2792.
[13] W. Wang, P. Yu, P. Zhang, Y. Shi, H. Bu, L. Zhang, The infection of human primary cells and cell lines by human cytomegalovirus: new tropism and new reservoirs for HCMV, Virus Res. 131 (2008) 160–169.
[14] J.A. Kobashigawa, R.C. Starling, M.R. Mehra, R.L. Kormos, G. Bhat, M.L. Barr, C.S. Sigouin, J. Kolesar, W. Fitzsimmons, Multicenter retrospective analysis of cardiovascular risk factors affecting long-term outcome of de novo cardiac transplant recipients, J. Heart Lung Transplant. 25 (2006) 1063–1069.
[15] M. Boratynska, M. Banasik, E. Watorek, M. Klinger, A. Dorobisz, P. Szyber, Influence of hypercholesterolemia and acute graft rejection on chronic nephropathy development in renal transplant recipients, Transplant. Proc. 35 (2003) 2209–2212.
[16] M.L. Cho, C.S. Cho, S.Y. Min, S.H. Kim, S.S. Lee, W.U. Kim, D.J. Min, J.K. Min, J. Youn, S.Y. Hwang, S.H. Park, H.Y. Kim, Cyclosporine inhibition of vascular endothelial growth factor production in rheumatoid synovial fibroblasts, Arthritis Rheum. 46 (2002) 1202–1209.
[17] K. Naruishi, F. Nishimura, H. Yamada-Naruishi, K. Omori, M. Yamaguchi, S. Takashiba, C-jun N-terminal kinase (JNK) inhibitor, SP600125, blocks interleukin (IL)-6-induced vascular endothelial growth factor (VEGF) production: cyclosporine A partially mimics this inhibitory effect, Transplantation 76 (2003) 1380–1382.
[18] R. Oksjoki, P.T. Kovanen, M.I. Mayranpaa, P. Laine, A.M. Blom, S. Meri, M.O. Pentikainen, Complement regulation in human atherosclerotic coronary lesions. Immunohistochemical evidence that C4b-binding protein negatively regulates the classical complement pathway, and that C5b–9 is formed via the alternative complement pathway, Atherosclerosis 192 (2007) 40–48.
[19] E. Wieland, B. Dorweiler, U. Bonitz, S. Lieser, I. Walev, S. Bhakdi, Complement activation by oxidatively modified low-density lipoproteins, Eur. J. Clin. Invest. 29 (1999) 835–841.
[20] K. Yasojima, C. Schwab, E.G. McGeer, P.L. McGeer, Complement components, but not complement inhibitors, are upregulated in atherosclerotic plaques, Arterioscler. Thromb. Vasc. Biol. 21 (2001) 1214–1219.
[21] A. Biro, N.M. Thielens, L. Cervenak, Z. Prohaszka, G. Fust, G.J. Arlaud, Modified low density lipoproteins differentially bind and activate the C1 complex of complement, Mol. Immunol. 44 (2007) 1169–1177.