Original Article

Fas Ligand mRNA Levels of Circulating Leukocytes Reflect Endothelial Dysfunction in Hyperlipidemic but Not in Non-Hyperlipidemic Patients

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To find a novel marker for identifying patients at high-risk for endothelial dysfunction among patients with atherosclerosis, we examined the correlation between mRNA levels of Fas ligand (FasL), an apoptosis-inducing factor, in circulating leukocytes and clinical parameters in these patients. FasL mRNA levels of circulating leukocytes were measured with the TaqMan-PCR method. A negative correlation was observed between brachial artery flow-mediated dilatation (%FMD) and FasL mRNA levels of leukocytes in hyperlipidemic but not in non-hyperlipidemic patients. %FMD was more impaired in patients with a high level of FasL mRNA than in those with a low level of FasL mRNA. Interestingly, the improvement of %FMD by treatment with a 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitor (simvastatin) was greater in the group showing a decrease in FasL mRNA than in the group with no such decrease. Additionally, simvastatin suppressed the FasL mRNA expression in leukocytes and decreased plasma oxidized low-density lipoprotein (OxLDL) levels. Furthermore, the supernatant of cultured leukocytes from hyperlipidemic patients induced cell death in Jurkat T cells, which was neutralized by an antibody against FasL. These findings suggest that high FasL mRNA expression in circulating leukocytes may be a marker of high-risk for endothelial dysfunction in hyperlipidemic but not in non-hyperlipidemic patients. This information may provide a novel basis for targeting of statin therapy in patients with vulnerable plaques. (*Hypertens Res* 2006; 29: 217–225)

***Key Words*: apoptosis, atherosclerosis, endothelium, hypercholesterolemia, inflammation**

Introduction

A growing body of evidence shows that rupture or erosion of vulnerable plaques plays a pivotal role in the development of atherosclerotic coronary events in hypertensive patients (*1*, *2*). Recent evidence shows that apoptosis occurs in human atherosclerotic plaques, potentially leading to plaque rupture or erosion, which are the triggers for atherothrombotic events (*3*, *4*). *In vivo* induction of endothelial cell (EC) apoptosis may also result in the development of endothelial dysfunction. Thus, EC apoptosis may be a critical step in the transition from a stable plaque to a vulnerable plaque in patients with atherosclerosis (*5*). Fas is a type I membrane protein belonging to a member of the tumor necrosis factor-α (TNF-α) receptor superfamily. Activation of Fas with Fas ligand (FasL) induces apoptosis of Fas-bearing cells, including ECs, through activation of the caspase cascade (*6*). Although ECs

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Table 1. Patient Characteristics and Laboratory Parameters of Patients with or without Hyperlipidemia

	Non-hyperlipidemia (n=39)	Hyperlipidemia (n=38)
Age (years)	65.5±2.2	66.3±1.4
Sex (men/women)	16/23	10/28
Hypertension	10/39	11/38
Smoking	5/39	5/38
Diabetes mellitus	6/39	3/38
Total cholesterol (mg/dl)	186±4.0	247±4.4*
LDL cholesterol (mg/dl)	99±4.0	137±5.7*
HDL cholesterol (mg/dl)	55±2.3	60±2.8
Triglycerides (mg/dl)	96±6.1	162±17*
Oxidized LDL (U/ml)	7.9±0.9	12.6±1.9*
IMT (mm)	0.76±0.04	0.79±0.06

Data are presented as mean±SEM. *$p<0.05$ *vs*. non-hyperlipidemia. LDL, low-density lipoprotein; HDL, high-density lipoprotein; IMT, intima-media thickness.

are highly resistant to Fas-mediated apoptosis, oxidized low-density lipoproteins (OxLDL) sensitize ECs to Fas-mediated apoptosis through down-regulation of the FLICE-inhibitory protein (FLIP), an intracellular caspase inhibitor (*7*). Importantly, FasL mRNA expression of circulating mononuclear cells is upregulated in patients with acute myocardial infarction and unstable angina, and 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors (statins) decrease FasL expression and cytotoxicity in activated T lymphocytes (*8*, *9*). Taken together, these findings suggest that FasL expressed on circulating leukocytes may be involved in the genesis of endothelial dysfunction and plaque vulnerability in patients with atherosclerosis.

A number of clinical trials have shown that statins improve endothelial function and reduce atherosclerotic events independent of the degree of low-density lipoprotein (LDL)–lowering in hyperlipidemic patients (*10*). Accumulating evidence shows that these cholesterol-independent or "pleiotropic" effects of statins are mediated through an increase in the bioavailability of nitric oxide (NO) (*11*, *12*). Since NO inhibits EC apoptosis (*13*), it is expected that statin suppresses EC apoptosis. Previous studies, however, have shown that statin induces both pro-apoptotic and anti-apoptotic effects in vascular cells. For example, statins induce apoptosis in cultured vascular smooth muscle cells, suggesting a beneficial role of statins in the prevention of neointima thickening (*14*). On the other hand, low dose statin protects ECs from hypoxia-induced apoptosis, whereas high dose statin enhances EC apoptosis (*15*). Thus, the *in vivo* role of statin therapy in the prevention of EC apoptosis in hyperlipidemic patients remains to be elucidated.

In the present study we examined whether FasL mRNA levels of circulating leukocytes would be a clinically useful marker to identify patients at high-risk for endothelial dysfunction in patients with atherosclerosis. We found that there was a negative correlation between brachial artery flow-mediated dilatation (%FMD) and FasL mRNA levels of leukocytes in hyperlipidemic but not in non-hyperlipidemic patients. In addition, the improvement of endothelial function by statin therapy was greater in the group showing a decrease in FasL mRNA.

Methods

Patients

Thirty-eight patients with hyperlipidemia and 39 patients without hyperlipidemia were enrolled in this study. Patients with any serious diseases, including inflammatory disease or cancer, and patients treated with steroid hormone or other immunosuppressive drugs were excluded. Hypertension was defined as a systolic blood pressure (BP)≥140 mmHg and diastolic BP≥90 mmHg. Drug regimens were unchanged during the study. Patient characteristics are shown in Table 1. Written informed consent was acquired from all patients. The study was conducted according to the Declaration of Helsinki, and the Joint Commission on Ethics of the Osaka University Graduate School of Medicine approved the study protocol.

Ultrasound Imaging

Carotid ultrasound studies (*16–18*) were performed with a Toshiba Power Vision 8000 ultrasound system and 7.5-mHz liner-array transducer. The image was focused on the posterior wall of the left carotid artery. A minimum of 4 measurements of the common carotid far wall was taken 10 mm proximal to the bifurcation to derive mean carotid intima-media thickness (IMT).

Brachial artery ultrasound studies (*19*) were performed to assess endothelial function by measuring flow-mediated dilatation (FMD) induced by reactive hyperemia as previously described (*20*). The baseline diameter was calculated as the average diameter from all baseline images measured. The 60-s diameter was calculated as the average of all images measured between 55 and 65 s after cuff deflation. FMD induced by reactive hyperemia was expressed as actual FMD ([55-s diameter] – [baseline diameter] = FMD mm) and as the relative change from baseline (FMD mm/baseline diameter = %FMD) (*21*, *22*).

Clinical Study

After the determination of basal FMD levels, hyperlipidemic patients were treated for 12 weeks with simvastatin (10 mg/day), and blood samples were collected at 0, 2, 4 and 12 weeks during the treatment. Serum levels of high sensitive C-reactive protein (hs-CRP) were measured with a commercial

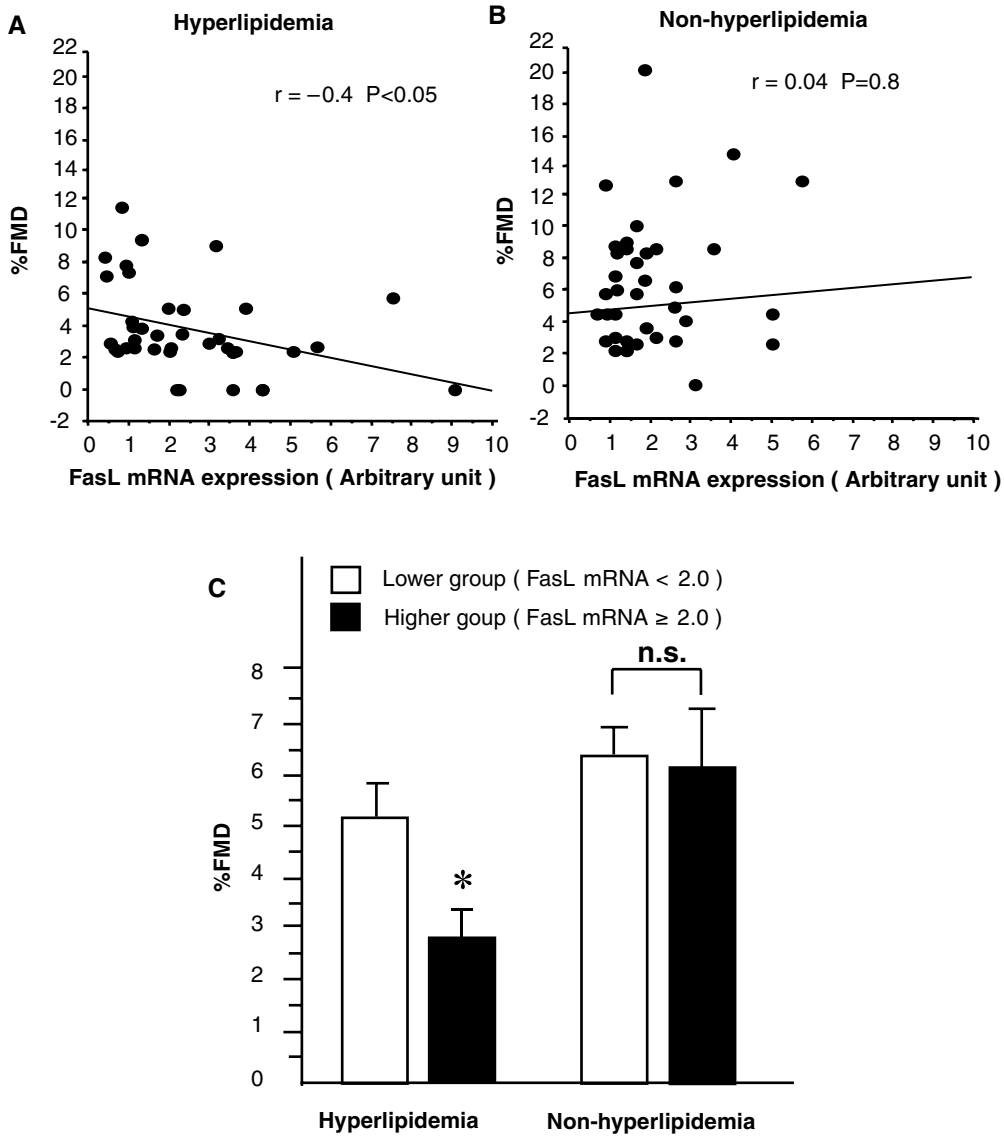

Fig. 1. *FasL mRNA levels of circulating leukocytes negatively correlated with %FMD in hyperlipidemic (A) but not in non-hyperlipidemic patients (B). Endothelial function was more impaired in the higher-level FasL mRNA group than in the lower-level FasL mRNA group in hyperlipidemic but not in non-hyperlipidemic patients (C). FasL mRNA levels and %FMD were measured as described in the text. Patients were classified into two groups according to the levels of FasL mRNA: a higher-level group (FasL mRNA≥2.0) and a lower-level group (FasL mRNA<2.0).* *$p < 0.05$, *significantly different from the lower-level group.*

kit (Dade Behring, Marburg, Germany). Plasma concentrations of OxLDL were determined by using a sandwich-type enzyme immunoassay kit (Kyowa-Medix, Tokyo, Japan). To isolate the mRNA, blood samples were immediately treated with the lysis solution after blood sampling, and total RNA was extracted with the ABI 6100 Nucleic Acid Prep Station system (Applied Biosystems, Tokyo, Japan).

Cell Culture

Peripheral blood mononuclear cells (PBMCs) were prepared by centrifugation at 1,600 rpm for 30 min with a leukocyte isolation solution (Nacalai Tesque Inc., Kyoto, Japan). 1A12 cells, a FasL-expressing T cell line, were provided by Shigekazu Nagata (Osaka University). These cells were cultured with RPMI 1640 medium (Nichiken Biomedical, Tokyo, Japan) supplemented with 10% fetal calf serum (FCS). The supernatant of leukocytes or 1A12 cells was

Table 2. Patient Characteristics of FasL mRNA Higher Group and Its Lower Group in Hyperlipidemic Patients

	FasL mRNA lower group (n=17)	FasL mRNA higher group (n=21)
Age (years)	63.6±1.9	68.4±1.8
Sex (men/women)	3/14	7/14
Hypertension	4/17	7/21
Smoking	2/17	3/21
Diabetes mellitus	1/17	2/21
Total cholesterol (mg/dl)	252±6.4	243±6.1
LDL cholesterol (mg/dl)	137±8.9	137±7.6
HDL cholesterol (mg/dl)	60±4.7	62±3.5
Triglycerides (mg/dl)	169±24	156±26
IMT (mm)	0.74±0.04	0.83±0.11
FMD (%)	5.0±0.7	2.7±0.5*

Data are presented as mean±SEM. *$p<0.05$ *vs*. FasL mRNA lower group. FasL, Fas ligand; LDL, low-density lipoprotein; HDL, high-density lipoprotein; IMT, intima-media thickness; FMD, flow-mediated dilatation.

obtained by culturing these cells for 24 h with a serum-free medium (leukocytes: 6×10^6 cells/ml; 1A12 cells: 2.5×10^5 cells/ml). For the determination of FasL mRNA expression, leukocytes were pretreated for the indicated times (from 1 to 24 h) with simvastatin at the indicated concentrations (from 1 to 30 μmol/l) and then treated for 1 h with OxLDL (200 μg/ml) in the presence or absence of 5 μmol/l geranylgeranylpyrophosphate (GGPP). After the treatment, total RNA was extracted with a commercial kit (Promega, Madison, USA).

Quantification of Cell Viability in Jurkat T Cells

Jurkat T cells were cultured in 96-well plates with 200 μl of RPMI 1640 medium supplemented with 10% FCS and 100 μl of the supernatant of 1A12 cells or leukocytes isolated from hyperlipidemic patients in the presence or absence of a neutralizing antibody against FasL (4H9), and then cell viability was quantified by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay (*23*). The medium was removed, and 0.2 ml of RPMI 1640 containing 20 μl of 5 mg/ml MTT was added to each well. Following incubation for 2 h, 0.2 ml of acid-isopropyl alcohol (0.04 Eq/l HCl in isopropyl alcohol) was added, and the cells were stored in darkness. The absorbance was read at 570 nm with background subtraction at 630 nm.

Real-Time PCR

To quantify the levels of FasL mRNA expression, the TaqMan-PCR method was used with an ABI Prism 7700 sequence detector system (Applied Biosystems, Tokyo, Japan), which can detect mRNA amounts in real time using a fluorescent probe complementary to the RNA sequence. FasL mRNA expression was normalized to that of the control (18S rRNA). Primers and probes were purchased from Applied Biosystems.

Statistical Analysis

Results were expressed as the mean±SEM, unless stated otherwise. Baseline comparisons between clinical parameters were performed using an F-test. A paired Student's t-test or non-parametric Mann-Whitney U-test, as appropriate, was used for comparisons of the two treatment groups. For correlation analysis, Spearman's correlation coefficients for skewed variables were calculated. A p value of <0.05 was considered statistically significant.

Results

FasL mRNA Levels Negatively Correlated with Endothelial Function in Hyperlipidemic but Not in Non-Hyperlipidemic Patients

We first examined whether FasL mRNA levels of leukocytes reflect endothelial function. A negative correlation was observed between FasL mRNA levels and %FMD in patients with hyperlipidemia (Fig. 1A), whereas no correlation was observed in patients without hyperlipidemia (Fig. 1B). We next classified these patients into two groups according to the levels of FasL mRNA (higher-level group: FasL mRNA≥2.0; lower-level group: FasL mRNA<2.0). Clinical backgrounds and laboratory findings were not significantly different between the groups except for FMD (Table 2). Interestingly, endothelial function was more impaired in the higher-level FasL mRNA group than in the lower-level group in hyperlipidemic but not in non-hyperlipidemic patients (Fig. 1C).

Beneficial Effects of Statin Therapy Were Greater in the Higher-Level FasL mRNA Group than in the Lower-Level FasL mRNA Group

Next, we examined whether the effects of statin therapy were different between the groups in hyperlipidemic patients. Twenty-nine hyperlipidemic patients were treated with simvastatin (10 mg/day) for 12 weeks. As reported previously (*10*), statin therapy restored endothelial function and decreased the levels of hs-CRP in total hyperlipidemic patients (data not shown). However, the degree of restoration of endothelial function and decrease in the levels of hs-CRP by statin therapy was statistically significant in the higher-level FasL mRNA group but not in the lower-level FasL mRNA group (Fig. 2), suggesting that the beneficial effects of statin therapy are greater in the former group.

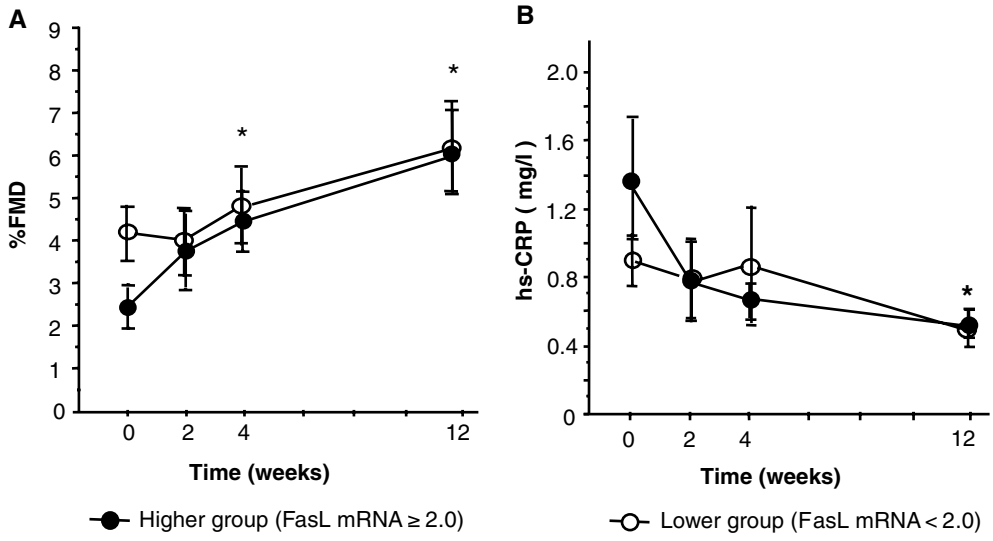

Fig. 2. *Statin therapy restored endothelial function (A) and serum hs-CRP levels (B) in the higher-level FasL mRNA group but not in the lower-level group. %FMD and hs-CRP levels were measured at 0, 2, 4, and 12 weeks after the start of statin therapy (simvastatin 10 mg per day) as described in the text.* *$p < 0.05$, *significantly different from the data before the treatment in the higher-level FasL mRNA group.*

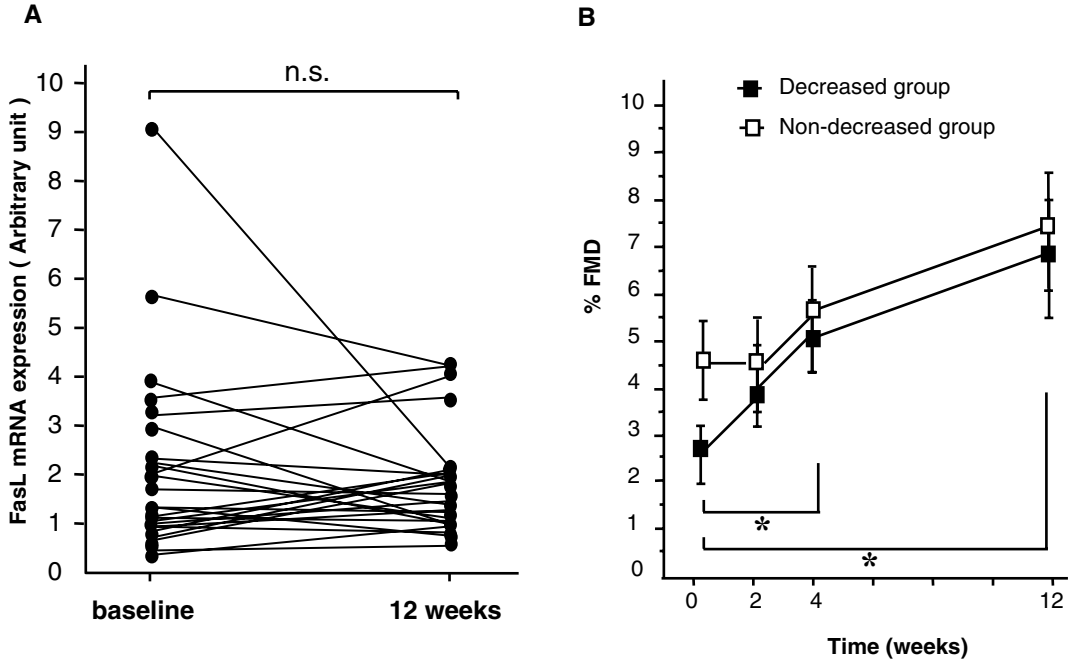

Fig. 3. *The improvement of endothelial function by statin therapy was greater in the group showing a decrease in FasL mRNA than in the group showing no change in FasL mRNA. We measured the changes of FasL mRNA levels of circulating leukocytes at 12 weeks after the start of statin therapy in total hyperlipidemic patients (A). We next classified these patients into two groups according to the degree of decrease in FasL mRNA levels after the start of statin therapy (decreased group [*$n = 16$*]: a more than 10% decrease from the baseline level; non-decreased group [*$n = 13$*]: a less than 10% decrease from the baseline level) and measured endothelial function in these groups (B). %FMD was measured as described in the text.* *$p < 0.05$, *significantly different from the data before the treatment in the decreased group.*

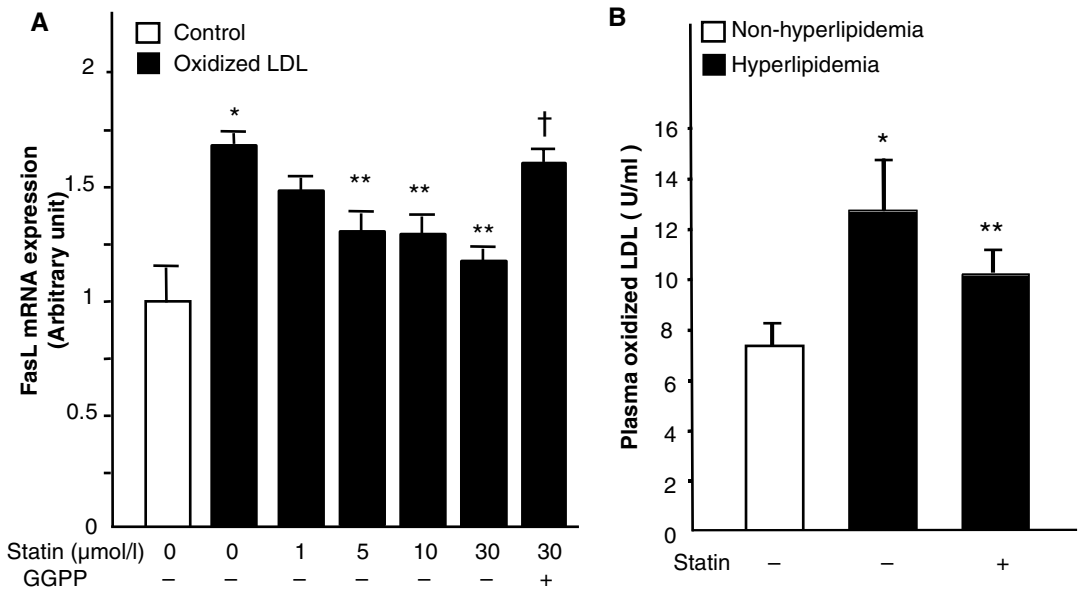

Fig. 4. *Simvastatin suppressed the levels of FasL mRNA expression in cultured leukocytes and decreased plasma OxLDL levels in hyperlipidemic patients. A: Leukocytes were incubated for 2 h with simvastatin at concentrations of 1, 5, 10 and 30 μmol/l, and OxLDL (200 μg/ml) was administered for 1 h in the presence or absence of 5 μmol/l GGPP. FasL mRNA levels were measured as described in the text. Data are represented as the mean±SEM from 4 experiments.* *$p < 0.05$, *significantly different from control cells.* **$p < 0.05$, *significantly different from cells treated with OxLDL alone.* †$p < 0.05$, *significantly different from cells treated with 30 μmol/l simvastatin and OxLDL. B: Plasma OxLDL levels were measured in patients with or without hyperlipidemia.* *$p < 0.05$, *significantly different from the data in non-hyperlipidemic patients.* **$p < 0.05$, *significantly different from the data in hyperlipidemic patients before statin therapy.*

Improvement of Endothelial Function by Statin Therapy Was Greater in Patients Showing a Decrease in FasL mRNA than in Those Not Showing a Decrease

As shown in Fig. 3A, statin therapy did not significantly change the mean levels of FasL mRNA in total hyperlipidemic patients. However, when we classified these patients into two groups according to the degree of decrease in FasL mRNA levels after statin therapy (decreased group: a more than 10% decrease from the baseline level; non-decreased group: a less than 10% decrease from the baseline level), improvement of endothelial function was significantly greater in the decreased group as compared with the non-decreased group (Fig. 3B). These results suggest that there is a strong correlation between FasL mRNA levels of leukocytes and endothelial dysfunction in hyperlipidemic patients.

Simvastatin Directly Suppressed the Upregulation of FasL mRNA Expression by OxLDL in Cultured Leukocytes

To elucidate the molecular mechanism by which statin therapy decreases FasL mRNA levels of leukocytes, we next examined the direct effect of simvastatin on FasL mRNA expression in cultured leukocytes. As shown in Fig. 4A, incubation with simvastatin at concentrations from 1 to 30 μmol/l induced a dose-dependent decrease in the levels of FasL mRNA upregulated by OxLDL. In addition, we analyzed the importance of isoprenoids related to the mevalonate pathway in the FasL expression. Co-incubation with GGPP (5 μmol/l) reversed the inhibitory effect of simvastatin on FasL mRNA expression, suggesting that an intermediate product modified by geranylgeranylation is required for the FasL expression in leukocytes.

Statin Therapy Decreased the Levels of Plasma OxLDL in Patients with Hyperlipidemia

Since OxLDL induced upregulation of FasL mRNA expression in leukocytes, as shown in Fig. 4A, we next examined the levels of plasma OxLDL in patients with hyperlipidemia before and after statin therapy. Plasma OxLDL levels were elevated in hyperlipidemic patients compared with non-hyperlipidemic patients. Statin therapy significantly decreased the levels of plasma OxLDL in these patients (Fig. 4B).

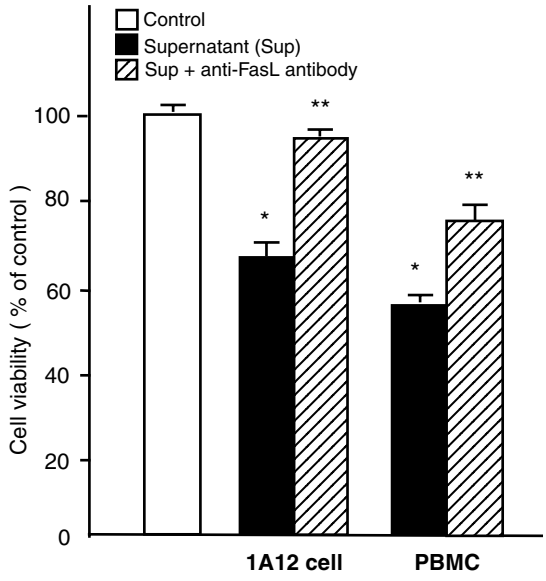

Fig. 5. *The supernatant of leukocytes isolated from hyperlipidemic patients inducesd cell death in Jurkat T cells. Jurkat T cells were cultured in 96-well plates with 200 μl of RPMI1640 medium supplemented with 10% FCS and 100 μl of the supernatant of leukocytes or 1A12 cells in the presence or absence of a neutralized antibody against FasL (4H9), and then the viable cells were quantified by MTT assay as described in the text. Data are represented as the mean±SEM from 4 experiments.* *$p < 0.05$, *significantly different from control cells.* **$p < 0.05$, *significantly different from cells treated with the supernatant in the absence of a neutralizing antibody.*

The Supernatant of Cultured Leukocytes Isolated from Hyperlipidemic Patients Induced Cell Death in Jurkat T Cells

FasL, a member of the TNF-α family, is shed by a metalloproteinase from the membrane to become a soluble form (sFasL), and sFasL induces apoptosis in Jurkat T cells, which are sensitive cells to the Fas-mediated apoptosis (*6*). We cultured Jurkat T cells with the supernatant of leukocytes isolated from hyperlipidemic patients who were classified in the FasL mRNA–higher group and then determined the levels of cell death. As shown in Fig. 5, the supernatant of cultured 1A12 cells, FasL-expressing cells, as a positive control, induced cell death in Jurkat T cells, and this cell death was neutralized with a neutralizing antibody against human FasL (4H9). Importantly, 4H9 also partially neutralized the cell death induced by the supernatant of leukocytes isolated from hyperlipidemic patients. These results indicate that FasL expressed in leukocytes isolated from hyperlipidemic patients is physiologically functional.

Discussion

In this study, we reported the following novel findings. FasL mRNA levels of circulating leukocytes were negatively correlated with the endothelial function in hyperlipidemic but not in non-hyperlipidemic patients. The degree of restoration of endothelial function and suppression of hs-CRP levels by statin therapy was greater when FasL mRNA levels were elevated in hyperlipidemic patients. Additionally, the improvement of endothelial function by statin therapy was greater in the group showing a decrease in FasL mRNA than in that not showing such a decrease.

FasL, a member of the TNF-α superfamily, is a membrane protein that induces apoptosis by binding to its receptor, Fas. Fas is expressed in almost all cells, whereas FasL is mainly expressed in circulating mononuclear cells, including leukocytes. It has been shown that monocytes/macrophages are able to induce apoptosis in vascular cells *in vivo* (*24*). Recent direct evidence shows that increased EC apoptosis correlates with an impaired vasodilator response to acetylcholine in old monkeys (*25*) and that *in vivo* induction of EC apoptosis leads to vessel thrombosis and endothelial erosion (*5*), suggesting that EC loss by apoptosis may contribute to the development of endothelial dysfunction. In the present study, we demonstrated that a negative correlation was observed between FasL mRNA levels of circulating leukocytes and endothelial function in hyperlipidemic patients. In addition, the degree of the improvement of endothelial function was greater in the group showing a decrease in FasL mRNA than in that not showing a decrease, suggesting a strong correlation between FasL, an apoptosis-inducing factor, expressed in leukocytes and endothelial dysfunction in hyperlipidemic patients. In contrast, no correlation was observed between FasL mRNA levels of circulating leukocytes and endothelial function in non-hyperlipidemic patients. In addition, endothelial function was more impaired in the group with higher levels of FasL mRNA than in those with lower levels of FasL mRNA in hyperlipidemic but not in non-hyperlipidemic patients, suggesting that FasL-mediated endothelial dysfunction is specific to hyperlipidemic patients. Notably, plasma OxLDL levels were higher in hyperlipidemic patients compared with non-hyperlipidemic patients, and OxLDL induced an increase in FasL mRNA levels in cultured leukocytes. Importantly, normal ECs are resistant to Fas-mediated apoptosis. These cells, however, are sensitized to Fas-mediated apoptosis in the presence of OxLDL (*7*), suggesting that FasL may be able to induce EC apoptosis in hyperlipidemic but not in non-hyperlipidemic patients when FasL expression is upregulated in circulating leukocytes. OxLDL is also a direct inducer of endothelial dysfunction through downregulation of endothelial NO synthase (eNOS) expression and NO inactivation *via* increases in superoxide anion production (*26*, *27*). Additionally, OxLDL influences macrophage function and inhibits phagocytosis of apoptotic cells, and defects in apoptotic clearance promote

atherogenesis (*28*). Taken together, these results suggest that OxLDL may play a pivotal role in the mechanism underlying differences observed between hyperlipidemic and non-hyperlipidemic patients. Further studies, however, are necessary to clarify these points.

A growing body of evidence shows that many of the beneficial effects of statin therapy in cardiovascular diseases are endothelium-dependent (*11*). Statins directly suppress the induction of inflammatory cytokines and thrombotic factors by atherogenic factors. For example, statins suppress endothelial CD40 and CD40 ligand expression in the presence of OxLDL (*29*). Statins also prevent endothelial tissue factor expression induced by thrombin (*30*, *31*). Statins, therefore, exert many favorable effects on the endothelium and prevent endothelial dysfunction in the presence of these atherogenic factors. These findings suggest that statin therapy is equally effective on the restoration of endothelial function in any patients with hyperlipidemia. Statin therapy, however, results in a greater clinical benefit when levels of the inflammatory biomarker CRP are elevated (*32*, *33*). In the present study, we also demonstrated that significant improvement of endothelial function and suppression of serum hs-CRP levels by statin therapy was observed in the group with higher levels of FasL mRNA but not in the group with lower levels. Since the degree of decrease in serum cholesterol levels after statin therapy was not different between the groups (data not shown), these differences may be due to cholesterol-independent effects. However, our study may have been limited by the lack of a hyperlipidemic group that was not treated with statins.

Our *in vitro* studies showed that simvastatin directly suppressed FasL mRNA expression in PBMCs stimulated with OxLDL, which was consistent with the data previously reported (*9*). In addition, statin therapy decreased the plasma levels of OxLDL, which induces up-regulation of FasL mRNA expression in leukocytes (Fig. 4B). Thus, down-regulation of FasL mRNA expression by statin therapy may be mediated through both direct suppression of FasL mRNA expression in leukocytes and inhibition of the elevation of plasma OxLDL levels in hyperlipidemic patients. Pharmacokinetic data showed that serum concentrations of statin vary between 0.5 to 5 μmol/l. Thus, the concentrations used in our *in vitro* studies are relevant to the therapeutic concentration ranges.

Although apoptosis is widely recognized as a clean death, this dogma was recently challenged by studies showing Fas-mediated activation of several proinflammatory genes and cytokines (*34*). Chang *et al*. (*35*) reported that oxidized phospholipids of apoptotic cells activate ECs to induce a local inflammatory response by recruiting monocytes *via* monocyte-EC interaction. In a number of experimental models, FasL has been found to initiate a severe inflammatory response (*36*, *37*). Hohlbaum *et al*. (*38*) also reported that FasL engagement of resident peritoneal macrophages *in vivo* induces apoptosis and the production of neutrophil chemotactic factors. In addition, Fas activation induces proinflammatory cytokine responses and reactive oxygen species production from human T cell leukemia Jurkat cells and macrophages (*39*, *40*). Taken together, these findings suggest that the Fas/FasL system also plays a role in the genesis of vascular inflammatory responses as well as endothelial dysfunction. Thus, the anti-inflammatory effects of statin therapy may be partially mediated though suppression of the Fas/FasL pathways.

In conclusion, we demonstrated for the first time that the levels of FasL mRNA in circulating leukocytes correlated with the degree of impairment of endothelial function in hyperlipidemic but not in non-hyperlipidemic patients. In addition, the levels of FasL mRNA predicted the efficacy of statin therapy on the restoration of endothelial function and inflammation in hyperlipidemic patients. These results may provide new insight into the molecular mechanisms underlying the beneficial effects of statin therapy in patients with vulnerable plaques.

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