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Statins reduce endothelial cell apoptosis via inhibition of TRAIL expression on activated CD4 T cells in acute coronary syndrome

Kayoko Sato, MD, PhD¹, Toshiyuki Nuki, MD¹, Keiko Gomita, MD¹, Cornelia M. Weyand, MD, PhD², and Nobuhisa Hagiwara, MD, PhD¹

¹Department of Cardiology, Tokyo Women's Medical University, 8-1 Kawada-cho, Shinjyuku-ku, Tokyo, Japan, 162-8666

²Division of Immunology and Rheumatology, Stanford University, CCSR Building, Room 2215, Mail Code 5166, 269 Campus Drive West, Stanford, CA, USA, 94305-5166

Abstract

Objective—Statins reduce cardiovascular-related morbidity and mortality, but their effects on inflammation in atherosclerosis are not fully understood. We investigated whether statins can modulate cytotoxic functions of CD4 T cells in acute coronary syndrome (ACS).

Methods and Results—Fresh CD4 T cells were isolated from 55 patients with ACS without statin treatment on admission and from 34 age-matched controls. CD4 T cells collected from ACS patients induced endothelial cell apoptosis significantly more than control T cells. The TNF-related apoptosis-inducing ligand (TRAIL) receptor DR5 was strongly upregulated on endothelial cells, and TRAIL-specific antibodies effectively blocked CD4 T cell-mediated apoptosis, indicating that T cell-mediated endothelial death was dependent on the TRAIL pathway. Expression of both the activating antigen CD69 and TRAIL was enhanced on ACS T cells. In in-vitro assays rosuvastatin, fluvastatin, and pitavastatin directly blocked CD4 T cell-mediated endothelial cell apoptosis and reduced T cell-expression of CD69 and TRAIL through TCR-induced Extracellular signal-Regulated Kinases (ERK) activation.

Conclusions—Activated CD4 T cells expressing TRAIL are enriched in the blood of ACS patients and induce endothelial injury, which may contribute to the destabilization of the plaque. Early statin therapy may suppress T cell-mediated endothelial cell damage in atherosclerotic plaques and thus prevent cardiovascular events.

1. Introduction

Acute coronary syndrome (ACS) can occur even if coronary artery stenosis is not severe. Acute complications of coronary atherosclerosis are caused by sudden thrombotic occlusion that is superimposed on atherosclerotic lesions. Both rupture and erosion of vulnerable plaques are important underlying pathomechanisms involved in ACS^{1–3}. Vulnerable plaques have a number of characteristic features, including a thin fibrous cap overlying a large lipid core, an inflammatory infiltrate consisting of macrophages, T cells, dendritic cells in the shoulder, a

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Disclosures

None.

high incidence of apoptotic cells, and neo-angiogenesis as signs of inflammation. On the other hand, endothelial cells (ECs) comprise the inner lining of all blood vessels, maintain vascular tone, and exert anticoagulant effects. Therefore, EC dysfunction and apoptosis also play key roles in the development of atherosclerosis⁴. Accordingly, vascular inflammation and EC apoptosis are critical events in the transition of a stable atherosclerotic lesion to a vulnerable plaque.

TNF-related apoptosis-inducing ligand (TRAIL) belongs to the TNF super-family and is an important apoptotic signaling molecule⁵. TRAIL binds to its receptors, TRAIL-R1 (DR4) and TRAIL-R2 (DR5)⁶, and activates caspase-8 through Fas-associated death domain (FADD). Proteolytically activated caspase-8 mediates caspase-3 activation and promotes cell death. Recently, we reported that CD4 T cells from ACS patients build sustained cytotoxic immunologic synapses with vascular smooth muscle cells (VSMCs) and induce VSMC apoptosis⁷. CD4 T cells derived from ACS patients highly express TRAIL on their surface and induce apoptosis in DR5-expressing VSMC⁸. TRAIL expression on CD4 T cells is enhanced by IFN α , which is produced by activated plasmacytoid dendritic cells (pDCs) upon TLR9 stimulation in atherosclerotic lesions⁹. VSMC apoptosis renders plaques vulnerable and prone to rupture.

Inhibitors of 3-hydroxy-3-methylglutaryl A (HMG-CoA) reductase, or statins, are widely used lipid-lowering agents. After the Scandinavian Simvastatin Survival Study (4S), multiple large primary and secondary prevention trials demonstrated that statin treatment reduces cardiovascular-related morbidity and mortality. In addition to their lipid-lowering effects, statins have pleiotropic effects and protect the cardiovascular system from damages. Moreover, statins are able to modulate immune responses by inhibiting the expression of MHC II molecules¹⁰ and adhesion molecules in activated endothelial cells and leukocytes^{11, 12}.

In this study, we examined whether TRAIL-expressing CD4 T cells from ACS induce apoptosis of vascular endothelial cells. We further investigated whether the immunomodulatory properties of statins could be attributed to their effects on TRAIL-expressing CD4 T cells which induced endothelial apoptosis and atherosclerotic plaque stability.

2. Materials and Methods

For detailed description of this section please see online supplementary “Materials and Methods”.

2.1. Study population

Total of 55 patients with ACS (68% male, 65 ± 11 yrs old) who had not received statin treatment were included in this study (Supplementary Table I). Blood was drawn at the time of admission to the Coronary Care Unit of Tokyo Women's Medical University. ACS was defined according to the AHA Guideline criteria. Patients with infectious, autoimmune, or neoplastic diseases were ineligible. Thirty-four healthy individuals (62% male, 50 ± 9 yrs old) without cardiovascular disease or other diseases served as controls (NC). The Tokyo Women's Medical University Institutional Review Board approved all protocols, and appropriate consent was obtained from study subjects.

2.2. Cells

Peripheral blood mononuclear cells (PBMCs) and CD4 T cells were isolated from fresh blood. Human umbilical vein endothelial cells (HUVECs), human coronary artery smooth muscle cells (CASMCs), and human aortic smooth muscle cells (AoSMCs) were obtained from Cambrex.

2.3. Real-time PCR

Total RNA was purified from HUVECs, CASCs, and AoSCs. cDNA was amplified with the primer sets for DR4, DR5, and β -actin, as described elsewhere⁸. cDNA copy numbers were expressed relative to 2×10^5 β -actin copies.

2.4. Western blot analysis

To investigate DR4 and DR5 protein expression, HUVECs, CASCs, and AoSCs were analyzed by Western blot analysis.

2.5. Apoptosis assays

Apoptosis of HUVEC was assessed as previously described^{8, 9}. To inhibit TRAIL-mediated apoptosis, T cells were treated with a goat anti-human TRAIL mAb or IgG goat control Ab before apoptosis assay. To examine the effects of statins on CD4 T cell induced cytotoxicity, freshly isolated T cells were pretreated with 10 μ M rosuvastatin (ROS), 10 μ M fluvastatin (FLV), or 10 μ M pitavastatin (PIT).

2.6. Flow cytometry

Cytometric analysis was performed by staining PBMCs and T cells with mouse anti-human CD4-PE-Cy5 mAb, mouse anti-human TRAIL-PE mAb, anti-CD69-FITC mAb, and the respective isotype control Abs. Cells were analyzed using a FACSCalibur flow cytometer and WinMDI software. To analyze the effects of statins on phosphorylation of Extracellular signal-Regulated Kinases (ERK), ERK1 (p44 MAPK) and ERK2 (p42 MAPK), PBMCs were treated with or without rosuvastatin, fluvastatin, or pitavastatin (each at 10 μ M). Then, PBMCs were stimulated with anti-CD3 mAb, followed by anti-mouse IgG. PBMCs were stained with mouse anti-human CD4-PerCP mAb, mouse anti-CD69-PE mAb, mouse anti-human ERK1/2 (pT202/pY204)-Alexa Fluor 488 mAb, and their respective isotype control Abs prior to flow cytometric analysis.

2.7. Statistical analysis

Normally distributed data were analyzed using Student's t-test for independent samples or t-tests for paired samples. In experiments with skewed distribution, corresponding non-parametric tests (Mann-Whitney U tests) were used. One-way ANOVA and Tukey-Kramer method were used to analyze TRAIL mediated endothelial apoptosis. Results are shown as means $\pm$ SD for parametric tests and as box plots with medians and percentiles when nonparametric testing was used.

3. Experimental Results

3.1. CD4 T cells from ACS patients induce apoptosis of endothelial cells

We investigated whether CD4 T cells from patients with ACS can mediate endothelial cell damage by inducing apoptosis. CD4 T cells from ACS patients induced apoptosis in 50% to 55% of human umbilical vein endothelial cells (HUVECs), a significant increase compared to NC ($P < 0.0001$, Figure 1A, 1B). Furthermore, there were no significant differences in CD4 T cells-induced apoptosis between patients with and without dyslipidemia in ACS (Supplementary Figure I).

3.2. CD4 T cells induce endothelial cell apoptosis through the TRAIL pathway

To examine whether CD4 T cells also induce endothelial apoptosis through the TRAIL pathway, we examined mRNA expression of TRAIL receptors in human coronary artery smooth muscle cells (CASCs), human aortic smooth muscle cells (AoSCs), and HUVECs.

Transcripts for DR5 were abundant in both VSMCs and HUVECs, whereas DR4 transcript levels were low (Figure 2A). These results were confirmed by protein levels using Western blots analysis (Figure 2B). To investigate whether HUVECs are sensitive to TRAIL/DR5 mediated apoptosis, CD4 T cells were pre-incubated with goat anti-human TRAIL Ab or IgG goat control Ab before apoptosis assay. Treatment with anti-TRAIL Ab inhibited apoptosis of HUVECs ($P < 0.001$), making it comparable to levels of NC in ACS T cell-treated cultures, but it had no effect on apoptosis induced by NC T cells (Figure 2C).

3.3. TRAIL-expressing activated CD4 T cells are more abundant in patients with ACS

In order to induce apoptosis in target cells, CD4 T cells must be activated. CD4 T cells derived from ACS patients strongly expressed an early activation marker, CD69, at significantly higher levels than NC T cells ($P < 0.05$, Figure 3A), but TRAIL expression in ACS patients was not significantly higher than that of NC patients prior to TCR triggering. After activating CD4 T cells by incubation on CD3-coated plates, TRAIL expression on CD4 T cells increased significantly in ACS patients compared to NC T cells ($P < 0.005$, Figure 3B).

3.4. Statins protect endothelial cells from CD4 T cell-induced apoptosis and TCR triggering intracellular ERK1/2 phosphorylation

Next, to assess whether statins have protective effects against cytotoxic CD4 T cells, freshly isolated CD4 T cells from ACS patients who had not received statin treatment were cultured in the absence (control) or presence of different statins (rosuvastatin, fluvastatin, or pitavastatin) at various doses (each at 1, 10, 50 μM) before testing their ability to induce apoptosis in HUVECs. All three statins most effectively inhibited apoptosis of HUVECs each at 10 μM dose (Supplementary Figure II). To assess whether these statins have high ability to protect CD4 T cell-induced endothelial apoptosis, a cohort of 16 ACS patients were studied. Rosuvastatin, fluvastatin, and pitavastatin all significantly inhibited the ability of CD4 T cells to induce apoptosis in HUVECs ($P < 0.0001$, $P < 0.0001$, and $P < 0.0001$, respectively, Figure 4A).

TCR signaling induced the mitogen-activated MAP kinase cascade consisting of ERK1/2¹³. To investigate the immunomodulating effects of statins for CD4 T cell activation, we isolated PBMCs from ACS patients and tested TCR triggering intracellular ERK1/2 phosphorylation. Pretreatment with rosuvastatin, fluvastatin, or pitavastatin decreased intracellular ERK1/2 phosphorylation in fresh ACS CD4 T cells (Figure 4B).

3.5. Statins inhibit CD4 T cell activation and TRAIL expression on CD4 T cells

TCR signal activates ERK pathway, followed by up-regulation of CD69 in T cells¹³. We investigated which statins inhibit CD4 T cell-induced HUVEC apoptosis by pretreating fresh PBMCs from ACS patients with statins each at 10 μM (rosuvastatin, fluvastatin, or pitavastatin) or medium alone (control) and analyzing the T cell activation marker CD69 expression on CD4 T cells. Pretreatment with rosuvastatin, fluvastatin, or pitavastatin decreased CD69 expression on CD4 T cells ($P < 0.01$, $P < 0.001$, and $P < 0.0005$, respectively, Figure 5A). To examine whether statin treatment decreased TRAIL expression on CD4 T cells, we isolated CD4 T cells from ACS patients and treated them with or without rosuvastatin, fluvastatin, or pitavastatin on anti-human CD3 coated plates and then measured TRAIL expression on activated CD4 T cells. Cells treated with rosuvastatin, fluvastatin, and pitavastatin all showed reduced TRAIL expression after activation compared with untreated controls ($P < 0.05$, $P < 0.01$, and $P < 0.005$, respectively; Figure 5B), strongly suggesting that statins inhibit CD4 T cell-induced apoptosis by inhibiting TRAIL expression on activated CD4 T cells.

4. Discussion

Since the “response-to-injury” hypothesis was proposed by Ross (1999), it has been widely accepted that the progression of atherosclerosis occurs due to chronic inflammation. Activated CD4 T cells are often observed in unstable atherosclerotic plaque lesions^{14, 15}. CD4 T cells in atherosclerotic plaques were previously shown to induce VSMC apoptosis through the TRAIL/DR5 pathway in human carotid artery-SCID chimera mice⁸. Here, we demonstrated that ACS CD4 T cells also strongly induced EC apoptosis. ECs abundantly expressed DR5, and were effectively killed by activated TRAIL-expressing CD4 T cells derived from ACS patients. This effect could be specifically blocked by anti-TRAIL Ab, indicating that ACS-derived CD4 T cell-induced apoptosis depends on the TRAIL/DR5 pathway in ECs. EC apoptosis induces stable endothelialized plaques to undergo thrombotic plaque erosion¹⁶, and 20% to 40% of coronary sudden death is caused by plaque erosion^{2, 3}. These data indicate that induction of apoptosis in ECs by TRAIL-expressing CD4 T cells may be a critical step in the erosion of vulnerable plaques in ACS.

Statins have pleiotropic effects and can reduce the incidence of cardiovascular events, independent of their lipid-lowering effects. CD4 T-helper (Th)1/Th2 polarization is associated with decreased STAT4 and enhanced STAT6, which inhibit Th1 development and augment Th2 development, and Th1/Th2 polarization improves disease progression in animal models^{17, 18}. Recently, it was shown that rosuvastatin induces a rapid reduction of Th1 cytokines, TNF α and IFN γ , by T cells from patients with ACS stimulated with phorbol-2-myristate-13-acetate and ionomycin¹⁹. Moreover, atorvastatin inhibits T cell responses by decreasing geranylgeranylated RhoA and farnesylated Ras at the plasma membrane, which inhibits ERK and p38 phosphorylation in an experimental autoimmune encephalomyelitis (EAE) model²⁰.

However, it is not clear how statins regulate the cytotoxic activity of CD4 T cells in ACS. In this study, we examined patients with ACS who did not receive statins at the time of hospitalization in order to remove the influence of statin effects on CD4 T cells at disease onset. We showed that dyslipidemia with high LDL and with low HDL did not affect CD4 T cell-induced EC apoptosis. In addition, rosuvastatin, fluvastatin, and pitavastatin all inhibited CD4 T cell-induced EC apoptosis, although these protective effects did not differ among patients with or without dyslipidemia (data not shown). These studies indicate that statins have direct immunomodulatory effects that protect ECs from CD4 T cell-induced injury and plaque erosion.

TRAIL is stored in cytoplasmic vesicles and is immediately expressed on the surface of T cells triggered by TCR-mediated signals and IFN α ^{9, 21}. Human atherosclerotic plaques contain large amounts of MHC class II antigen-presenting cells (APCs), including ECs, VSMCs, and macrophages. Certain kinds of ligands, such as oxidized LDL, and heat shock protein, are thought to activate infiltrated T cells in the atherosclerotic plaque lesion^{22–24}; as a result, it is possible that TRAIL expression is induced on the surface of T cells. In this study, we investigated the inhibition of CD4 T cell-induced EC apoptosis by statin treatments with a specific focus on T cell activation and TRAIL expression. We showed that CD69, an early activation marker, was strongly expressed on CD4 T cells in ACS.

It is known that TCR signaling induces the mitogen activated MAP kinase cascade consisting of ERK1/2, and that Ras/ERK pathway up-regulates CD69 in Jurkat T cells^{13, 25}. In addition, Ras, Rho, and Rab small GTPases in T cell lipid rafts are important components of signal transduction pathways that regulate the immune response²⁶. These small GTPases are prenylated and activated by mevalonate-derived isoprenoid compounds such as farnesylpyrophosphate (FPP) and geranylgeranylpyrophosphate (GGPP). Lovastatin and

simvastatin inhibition of TCR-triggered CD69 expression is Ras-dependent in Jurkat cell lines^{27, 28}. In this manuscript we showed that statin treatment inhibited intracellular ERK1/2 phosphorylation in fresh ACS CD4 T cells after TCR stimulation. Statin treatment also inhibited CD69 expression and inhibited TRAIL expression after activation by TCR triggering in ACS patients. It is possible that statins act as direct inhibitors of MHC II in APCs and subsequently in T cell activation. Therefore, these statins may suppress the activation of CD4 T cells by inhibiting small GTPase prenylation, along with intracellular ERK1/2 phosphorylation and CD69 expression and TRAIL expression in patients with ACS.

In conclusion, our data demonstrate that patients with ACS have elevated levels of active CD4 T cells, which can be easily induced to express TRAIL on their surfaces after TCR triggering. TRAIL-expressing CD4 T cells can induce endothelial injury through EC apoptosis and may contribute to plaque instability, such as erosion, and incidence of ACS. It is important to consider that vulnerable plaques contain a population of activated T cells when treating patients with ACS. In this study, we have shown that statins have pleiotropic immunomodulatory effects and inhibit the cytotoxicity of CD4 T cells towards ECs in ACS by suppressing T cell activation and TRAIL expression. Therefore, statin therapy in the acute phase of ACS may reduce plaque instability, improve disease prognosis, and lower the incidence of cardiovascular mortality.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. van der Wal AC, Becker AE, van der Loos CM, Das PK. Site of intimal rupture or erosion of thrombosed coronary atherosclerotic plaques is characterized by an inflammatory process irrespective of the dominant plaque morphology. *Circulation* 1994;89:36–44. [PubMed: 8281670]
2. Kolodgie FD, Burke AP, Farb A, Gold HK, Yuan J, Narula J, Finn AV, Virmani R. The thin-cap fibroatheroma: a type of vulnerable plaque: the major precursor lesion to acute coronary syndromes. *Curr Opin Cardiol* 2001;16:285–292. [PubMed: 11584167]
3. Virmani R, Burke AP, Farb A, Kolodgie FD. Pathology of the vulnerable plaque. *J Am Coll Cardiol* 2006;47:C13–C18. [PubMed: 16631505]
4. Nakajima T, Schulte S, Warrington KJ, Kopecky SL, Frye RL, Goronzy JJ, Weyand CM. T-cell-mediated lysis of endothelial cells in acute coronary syndromes. *Circulation* 2002;105:570–575. [PubMed: 11827921]
5. Wiley SR, Schooley K, Smolak PJ, Din WS, Huang CP, Nicholl JK, Sutherland GR, Smith TD, Rauch C, Smith CA, et al. Identification and characterization of a new member of the TNF family that induces apoptosis. *Immunity* 1995;3:673–682. [PubMed: 8777713]
6. Schneider P, Thome M, Burns K, Bodmer JL, Hofmann K, Kataoka T, Holler N, Tschopp J. TRAIL receptors 1 (DR4) and 2 (DR5) signal FADD-dependent apoptosis and activate NF-kappaB. *Immunity* 1997;7:831–836. [PubMed: 9430228]
7. Pryshchep S, Sato K, Goronzy JJ, Weyand CM. T cell recognition and killing of vascular smooth muscle cells in acute coronary syndrome. *Circ Res* 2006;98:1168–1176. [PubMed: 16601227]
8. Sato K, Niessner A, Kopecky SL, Frye RL, Goronzy JJ, Weyand CM. TRAIL-expressing T cells induce apoptosis of vascular smooth muscle cells in the atherosclerotic plaque. *J Exp Med* 2006;203:239–250. [PubMed: 16418392]

9. Niessner A, Sato K, Chaikof EL, Colmegna I, Goronzy JJ, Weyand CM. Pathogen-sensing plasmacytoid dendritic cells stimulate cytotoxic T-cell function in the atherosclerotic plaque through interferon- α . *Circulation* 2006;114:2482–2489. [PubMed: 17116765]
10. Kwak B, Mulhaupt F, Myit S, Mach F. Statins as a newly recognized type of immunomodulator. *Nat Med* 2000;6:1399–1402. [PubMed: 11100127]
11. Rezaie-Majd A, Prager GW, Bucek RA, Scherthaner GH, Maca T, Kress HG, Valent P, Binder BR, Minar E, Baghestanian M. Simvastatin reduces the expression of adhesion molecules in circulating monocytes from hypercholesterolemic patients. *Arterioscler Thromb Vasc Biol* 2003;23:397–403. [PubMed: 12615677]
12. Eccles KA, Sowden H, Porter KE, Parkin SM, Homer-Vanniasinkam S, Graham AM. Simvastatin alters human endothelial cell adhesion molecule expression and inhibits leukocyte adhesion under flow. *Atherosclerosis* 2008;200:69–79. [PubMed: 18486135]
13. Villalba M, Hernandez J, Deckert M, Tanaka Y, Altman A. Vav modulation of the Ras/MEK/ERK signaling pathway plays a role in NFAT activation and CD69 up-regulation. *Eur J Immunol* 2000;30:1587–1596. [PubMed: 10898494]
14. Hansson GK, Holm J, Jonasson L. Detection of activated T lymphocytes in the human atherosclerotic plaque. *Am J Pathol* 1989;135:169–175. [PubMed: 2505620]
15. Zhou X, Stemme S, Hansson GK. Evidence for a local immune response in atherosclerosis. CD4+ T cells infiltrate lesions of apolipoprotein-E-deficient mice. *Am J Pathol* 1996;149:359–366. [PubMed: 8701976]
16. Durand E, Scoazec A, Lafont A, Boddaert J, Al Hajzen A, Addad F, Mirshahi M, Desnos M, Tedgui A, Mallat Z. In vivo induction of endothelial apoptosis leads to vessel thrombosis and endothelial denudation: a clue to the understanding of the mechanisms of thrombotic plaque erosion. *Circulation* 2004;109:2503–2506. [PubMed: 15148270]
17. Youssef S, Stuve O, Patarroyo JC, Ruiz PJ, Radosevich JL, Hur EM, Bravo M, Mitchell DJ, Sobel RA, Steinman L, Zamvil SS. The HMG-CoA reductase inhibitor, atorvastatin, promotes a Th2 bias and reverses paralysis in central nervous system autoimmune disease. *Nature* 2002;420:78–84. [PubMed: 12422218]
18. Hakamada-Taguchi R, Uehara Y, Kuribayashi K, Numabe A, Saito K, Negoro H, Fujita T, Toyo-oka T, Kato T. Inhibition of hydroxymethylglutaryl-coenzyme A reductase reduces Th1 development and promotes Th2 development. *Circ Res* 2003;93:948–956. [PubMed: 14563711]
19. Link A, Ayadhi T, Bohm M, Nickenig G. Rapid immunomodulation by rosuvastatin in patients with acute coronary syndrome. *Eur Heart J* 2006;27:2945–2955. [PubMed: 17012299]
20. Dunn SE, Youssef S, Goldstein MJ, Prod'homme T, Weber MS, Zamvil SS, Steinman L. Isoprenoids determine Th1/Th2 fate in pathogenic T cells, providing a mechanism of modulation of autoimmunity by atorvastatin. *J Exp Med* 2006;203:401–412. [PubMed: 16476765]
21. Kayagaki N, Yamaguchi N, Nakayama M, Eto H, Okumura K, Yagita H. Type I interferons (IFNs) regulate tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) expression on human T cells: A novel mechanism for the antitumor effects of type I IFNs. *J Exp Med* 1999;189:1451–1460. [PubMed: 10224285]
22. Palinski W, Tangirala RK, Miller E, Young SG, Witztum JL. Increased autoantibody titers against epitopes of oxidized LDL in LDL receptor-deficient mice with increased atherosclerosis. *Arterioscler Thromb Vasc Biol* 1995;15:1569–1576. [PubMed: 7583529]
23. Stemme S, Faber B, Holm J, Wiklund O, Witztum JL, Hansson GK. T lymphocytes from human atherosclerotic plaques recognize oxidized low density lipoprotein. *Proc Natl Acad Sci U S A* 1995;92:3893–3897. [PubMed: 7732003]
24. Zal B, Kaski JC, Arno G, Akiyu JP, Xu Q, Cole D, Whelan M, Russell N, Madrigal JA, Dodi IA, Baboonian C. Heat-shock protein 60-reactive CD4+CD28null T cells in patients with acute coronary syndromes. *Circulation* 2004;109:1230–1235. [PubMed: 14993140]
25. Zha Y, Marks R, Ho AW, Peterson AC, Janardhan S, Brown I, Praveen K, Stang S, Stone JC, Gajewski TF. T cell anergy is reversed by active Ras and is regulated by diacylglycerol kinase- α . *Nat Immunol* 2006;7:1166–1173. [PubMed: 17028589]
26. Cantrell DA. GTPases and T cell activation. *Immunol Rev* 2003;192:122–130. [PubMed: 12670400]

27. Goldman F, Hohl RJ, Crabtree J, Lewis-Tibesar K, Koretzky G. Lovastatin inhibits T-cell antigen receptor signaling independent of its effects on ras. *Blood* 1996;88:4611–4619. [PubMed: 8977253]
28. Ghittoni R, Patrussi L, Pirozzi K, Pellegrini M, Lazzerini PE, Capecchi PL, Pasini FL, Baldari CT. Simvastatin inhibits T-cell activation by selectively impairing the function of Ras superfamily GTPases. *FASEB J* 2005;19:605–607. [PubMed: 15677697]

Figure 1. Cytotoxic CD4 T cells from ACS patients

A, CD4 T cells derived from peripheral blood of ACS and controls (NC) were incubated on human umbilical vein endothelial cells (HUVECs) monolayers and frequencies of apoptotic HUVECs were assessed after 3h. B, Representative apoptotic nuclear changes of HUVECs induced with ACS T cells. Scale bar = 100 μm .

Figure 2. CD4 T cell-induce EC apoptosis through the TRAIL pathway

A, mRNA was isolated from human coronary artery smooth muscle cells (CASMCs), human aortic smooth muscle cells (AoSMCs), and HUVECs and amplified by real-time PCR for transcripts of the TRAIL receptors DR4 and DR5. B, Expression levels for DR4 and DR5 protein were analyzed by Western blots. C, CD4 T cells from patients with ACS and NC were pretreated with anti-TRAIL antibody or isotype control IgG2a antibody before the T cell apoptosis assay. Apoptosis rates were determined after 3 h. All Data are representative of four experiments.

Figure 3. TRAIL expression is increased on activated CD4 T cells in ACS

A, Peripheral blood mononuclear cells (PBMCs) were isolated and CD69 expression was analyzed by flow cytometry. B, PBMCs were stimulated in anti-human CD3-coated plates (After TCR triggering). Control cells were seeded onto uncoated plates (Control). TRAIL expression on CD4 T cells was analyzed by flow cytometry.

Figure 4. Statins inhibit CD4 T cell-induced EC apoptosis and ERK activation

A, Fresh CD4 T cells were isolated from 16 ACS patients and tested for their ability to induce HUVEC apoptosis pretreated with different statins (rosuvastatin, ROS, fluvastatin, FLV, or pitavastatin, PIT) or without statin (control). **B**, Fresh PBMCs from ACS were pretreated with (dotted line) or without (solid line) statins. Then, PBMCs were stimulated with (After TCR triggering) or without (Control) anti-CD3 mAb, followed by anti-mouse IgG for cross-linking. Intracellular ERK1/2 phosphorylation in CD4 T cells was analyzed by flow cytometry. Isotype control (shaded histogram). Data are representative of five experiments.

Figure 5. Statins inhibit CD4 T cell activation and TRAIL expression

A, CD69 expression on CD4 T cells were pretreated with or without statins (15 ACS) and analyzed. B, PBMCs were pretreated with or without statins and then stimulated in anti-human CD3-coated plates (11 ACS). Expression of TRAIL was assessed by flow cytometry.