

See discussions, stats, and author profiles for this publication at: <https://www.researchgate.net/publication/10943401>

# Raised serum levels of soluble CD40 ligand in patients with familial hypercholesterolemia: Downregulatory effect of statin therapy

ARTICLE in JOURNAL OF THE AMERICAN COLLEGE OF CARDIOLOGY · FEBRUARY 2003

Impact Factor: 16.5 · DOI: 10.1016/S0735-1097(02)02718-3 · Source: PubMed

CITATIONS

96

READS

18

12 AUTHORS, INCLUDING:



Anne Grete Semb

Diakonhjemmet Hospital (Norway)

109 PUBLICATIONS 1,865 CITATIONS

SEE PROFILE



John J P Kastelein

University of Amsterdam

770 PUBLICATIONS 35,860 CITATIONS

SEE PROFILE



Lars Gullestad

Oslo University Hospital

231 PUBLICATIONS 7,394 CITATIONS

SEE PROFILE



Anton F H Stalenhoef

Radboud University Nijmegen

448 PUBLICATIONS 16,258 CITATIONS

SEE PROFILE

# Raised Serum Levels of Soluble CD40 Ligand in Patients With Familial Hypercholesterolemia: Downregulatory Effect of Statin Therapy

Anne G. Semb, MD, PhD,\* Sanne van Wissen, MD,‡ Thor Ueland, BS,† Tineke Smilde, MD, PhD,‡ Torgun Waehre, MD,† Mieke D. Tripp, MD, PhD,§ Stig S. Frøland, MD, PhD,|| John J. P. Kastelein, MD, PhD,‡ Lars Gullestad, MD, PhD,¶ Terje R. Pedersen, MD, PhD,\* Pål Aukrust, MD, PhD,†|| Anton F. H. Stalenhoef, MD, PhD§

*Oslo and Baerum, Norway; and Amsterdam and Nijmegen, the Netherlands*

<b>OBJECTIVES</b>	In the present study, we investigated the effects of statins on serum levels of soluble CD40 ligand (sCD40L) in patients with familial hypercholesterolemia (FH).
<b>BACKGROUND</b>	Atherosclerotic disease seems to involve inflammatory and immunologic mechanisms, and sCD40L has recently been identified as one of the key players in the atherosclerotic process. HMG-Co A reductase inhibitors, statins, have been recognized as immunomodulators and reduce cardiovascular events and mortality, but the effects of statins on sCD40L has not been clarified.
<b>METHODS</b>	In a randomized, double-blind, clinical trial, as part of the Atorvastatin versus Simvastatin on Atherosclerosis Progression (ASAP) trial, 110 patients with FH were given atorvastatin 80 mg/daily (n = 57) or simvastatin 40 mg/daily (n = 53) for two years.
<b>RESULTS</b>	Our main findings were: 1) at baseline patients with FH had significantly higher (approximately 27-fold) serum levels of sCD40L than healthy controls; 2) statin therapy markedly decreased serum levels of sCD40L (approximately 40% reduction); 3) this decrease in sCD40L was found during both "aggressive" (i.e., atorvastatin) and "conventional" (i.e., simvastatin) statin therapy and was not correlated with the degree of reduction in cholesterol levels.
<b>CONCLUSIONS</b>	Our findings may suggest enhanced CD40L-CD40 interaction in FH and that this inflammatory response may be downregulated by statins. (J Am Coll Cardiol 2003;41:275-9) © 2003 by the American College of Cardiology Foundation

Inflammation and immunological processes are considered to have essential roles for the initiation and progression of atherosclerosis (1). Thus, activated T cells, monocytes, and granulocytes have been reported in patients with cardiovascular disease (CVD) with particular enhanced activation in cells isolated from coronary sinus (2,3). Moreover, infiltration of blood-derived macrophages and T cells into the vessel wall seems to be an important feature of the active stages of atherosclerosis. However, the sequence of immunomodulatory steps has not been identified, and it is not clear which molecule(s) play a key role early in the development of atherosclerosis.

Familial hypercholesterolemia (FH) is a lipid disorder that predisposes to premature CVD. Clinical sequelae of CVD are preceded by silent changes with deposition of oxidized low-density lipoprotein (LDL) in the vessel wall. Changes in the arterial wall of the carotid intima and media, measured as carotid intima media thickness (IMT), can

predict coronary artery disease (4,5) and are directly associated with risk of myocardial infarction and stroke (6).

Overwhelming evidence from clinical studies has demonstrated that reducing LDL cholesterol (LDL-C) level with statins results in a lower risk of cardiovascular events (7-11). Moreover, the ASAP study (12), comprising 325 patients with FH, showed that aggressive LDL-C reduction by atorvastatin was accompanied by regression of IMT, whereas conventional LDL-C reduction with simvastatin only retarded progression. However, while the reduction of cardiovascular events by statin therapy is associated with a marked reduction in LDL-C, recent studies have suggested that statins may have several other biological effects beyond that of lipid lowering, such as anti-inflammatory and antioxidatory properties (13). It is, therefore, possible that the beneficial effect of statins in atherosclerotic disease, at least partly, is relayed through its immunomodulatory properties.

To further elucidate these issues, we measured serum levels of soluble CD40 ligand (sCD40L), a ligand in the tumor necrosis factor superfamily, in patients with FH during "aggressive" (i.e., atorvastatin) and "conventional" (i.e., simvastatin) statin therapy as a substudy in the ASAP trial. Raised circulating levels of sCD40L have been reported in angina patients with particularly high levels in those with unstable disease (14-16), and enhanced CD40L-CD40 interaction seems to be involved in athero-

From the \*Cardiologic Department, Aker Hospital, Oslo, Norway; †Research Institute for Internal Medicine, ‡Section of Clinical Immunology and Infectious Diseases, Rikshospitalet, Oslo, Norway; §Medical Department, Baerum Hospital, Baerum, Norway; ¶Academic Medical Center, Amsterdam, the Netherlands; §Department of Medicine, University Medical Center, Nijmegen, the Netherlands. Supported by a grant from Pfizer, Inc., New York, New York. Dr. Pedersen is receiving research grants and consultant honoraria from Pfizer, from Merck, and from Schering Plough.

Manuscript received April 29, 2002; revised manuscript received September 20, 2002, accepted October 4, 2002.

#### Abbreviations and Acronyms

CVD	= cardiovascular disease
EIA	= enzyme immunoassay
FH	= familial hypercholesterolemia
hsCRP	= high-sensitivity C-reactive protein
IMT	= intima media thickness
LDL	= low-density lipoprotein
LDL-C	= LDL cholesterol
sCD40L	= soluble CD40 ligand

genesis (1,17). However, the role of CD40L in early and asymptomatic atherosclerotic disease is mostly unknown.

## METHODS

**Patient population and controls.** The study design and baseline characteristics of the patient population have been described elsewhere (12). Briefly, 110 patients with FH, participating in the ASAP trial, were included in a randomized, double-blind clinical trial. Patients were given 80 mg atorvastatin ( $n = 57$ ) or 40 mg simvastatin ( $n = 53$ ) daily on an intention-to-treat basis. We examined serum levels of sCD40L at baseline and after one and two years on statin treatment. There were no significant differences in demographic or lipid parameters between the full ASAP cohort ( $n = 325$ ) and the patients in this substudy (data not shown). Most of the patients with FH were asymptomatic with no clinical evidence of CVD, but 31 had diagnosed CVD based on the presence of one of the following: previous myocardial infarction, angina pectoris, peripheral artery disease, or previous transient ischemic attack. However, none of these patients had unstable angina, and patients with myocardial infarction within three months before the study were not included. For comparison, age- and gender-matched healthy blood donors ( $n = 20$ ) were used as controls. The characteristics of the study population are given in Table 1. The Institutional Review Boards of both centers in the Netherlands approved the protocol, and written consent was obtained.

**Table 1.** Clinical Characteristics of the Study Population

	Atorvastatin	Simvastatin	Controls
n	57	53	20
Age, yrs	47 $\pm$ 1	48 $\pm$ 1	45 $\pm$ 3
BMI, kg/m <sup>2</sup>	26 $\pm$ 3	26 $\pm$ 4	25 $\pm$ 3
Gender, M/F	36/21	35/18	15/5
Smoking, %	69	81	73
CVD, %	24	32	0**
Total cholesterol, mmol/l	10.27 $\pm$ 1.79	10.80 $\pm$ 2.06	5.23 $\pm$ 1.05**
HDL cholesterol, mmol/l	1.14 $\pm$ 0.34	1.11 $\pm$ 0.28	1.15 $\pm$ 0.21
LDL cholesterol, mmol/l	8.30 $\pm$ 1.74	8.92 $\pm$ 2.01	3.76 $\pm$ 0.06**
TG, mmol/l	1.92 $\pm$ 0.92	1.86 $\pm$ 1.03	1.12 $\pm$ 0.10*
IMT, mm	0.96 $\pm$ 0.25	0.92 $\pm$ 0.20	—

Data are given as mean  $\pm$  SD unless otherwise specified. \* $p < 0.05$ ; \*\* $p < 0.001$  versus patients with familial hypercholesterolemia.

BMI = body mass index; CVD = cardiovascular disease; HDL = high-density lipoprotein; IMT = intima media thickness; LDL = low-density lipoprotein; TG = triglycerides.

**Blood sampling protocol.** Serum was collected as previously described (18) using pyrogen-free tubes without additives (BD Vacutainer Systems, Belliver Industrial Estate, Plymouth, United Kingdom). Immediately after blood collection, the tubes were immersed into melting ice and allowed to clot for 1 h before centrifugation at 1,000  $g$  for 10 min. Serum samples were stored at  $-80^{\circ}\text{C}$  in multiple aliquots until analysis. Samples were thawed only once.

**Enzyme immunoassays (EIA).** Serum levels of sCD40L were determined by EIA (detection limit, 0.03 ng/ml; Bender Medsystems, Vienna, Austria) according to the manufacturer's instructions (intra- and interassay coefficient of variation  $<10\%$ ). Analysis was performed in duplicates in a blinded fashion. All samples from a given patient were analyzed in the same microtiter plate to minimize run-to-run variability.

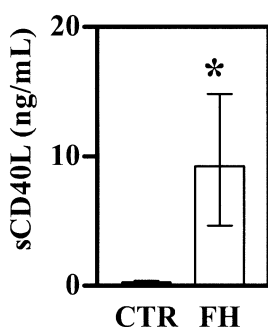
**Miscellaneous.** Cholesterol and triglyceride concentrations were determined with commercially available enzymatic methods (Boehringer Mannheim, FRG, no. 237574, and Sera-PAK, Miles, Italy, no. 6639, respectively) (12). To determine high-density lipoprotein cholesterol, the polyethylene glycol 6000 precipitation method was used (12). Low-density lipoprotein cholesterol was calculated by the Friedewald formula. Intima media thickness was determined in laboratories at the two University Medical Centers participating in the ASAP trial (12). High-sensitivity C-reactive protein (hsCRP) was analyzed at TNO Gaubius Laboratory, Leiden, Netherlands (19).

**Statistics.** Differences between groups were compared by the Mann-Whitney  $U$  test for unpaired data. For sCD40L, repeated measures analysis of variance was performed a priori. If the outcome was significant, Wilcoxon's rank-sum test for paired data was performed a posteriori. Kolmogorov-Smirnov tests of normality with Liliefors significance correction was used to test the distribution of the measured parameter at baseline. Relations between variables were tested using Spearman's rank-correlation test. The level of statistical significance was chosen as  $p < 0.05$  (two-sided). The values are given as mean  $\pm$  SEM.

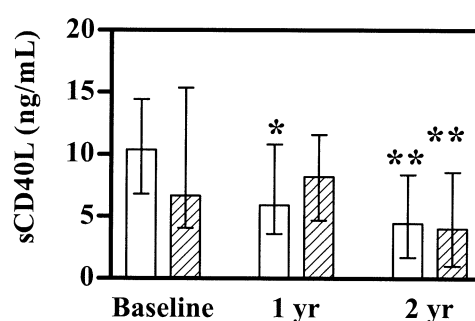
## RESULTS

**Baseline levels of CD40L.** As can be seen in Figure 1, serum levels of sCD40L were significantly elevated in hypercholesterolemic patients compared with healthy controls. In fact, while sCD40L was nearly undetectable in healthy controls, the mean level was 10 ng/ml in the patients with FH (approximately 27-fold higher), with no overlap between patients and controls. Patients with CVD ( $n = 31$ ) did not have significantly higher sCD40L ( $10.7 \pm 0.77$  ng/ml) levels than those without CVD ( $n = 79$ ) ( $8.4 \pm 1.2$  ng/ml). In fact, the patients with the six highest levels of sCD40L did not have CVD.

**Statin-induced changes of sCD40L.** In the ASAP trial, we have previously reported significant changes in lipid parameters, hsCRP, and IMT during statin therapy, with



**Figure 1.** Serum levels of soluble CD40 ligand (sCD40L) in 110 patients with familial hypercholesterolemia (FH) compared with 20 gender- and age-matched healthy controls (CTR). Data are given as median and 25th to 75th percentiles. \* $p < 0.001$  versus controls.



**Figure 2.** Serum levels of soluble CD40 ligand (sCD40L) in 110 patients with familial hypercholesterolemia receiving atorvastatin 80 mg ( $n = 57$ ) or simvastatin 40 mg ( $n = 53$ ) daily for two years (yr). Data are given as median and 25th to 75th percentiles. **Open bar** = atorvastatin; **hatched bar** = simvastatin. \* $p < 0.01$ ; \*\* $p < 0.001$  versus baseline.

the most pronounced changes in the atorvastatin group (12,19). As can be seen in Table 2, similar patterns were also observed in this substudy, and, notably, these changes in lipid parameters were accompanied by a marked reduction in sCD40L levels after two years of therapy (approximately 40% reduction) (Fig. 2). There were no differences between atorvastatin and simvastatin treatment after two years, but after one year, the reduction was significantly greater in the atorvastatin group. However, sCD40L levels were lower in the simvastatin group at baseline, and because the reduction in sCD40L was inversely correlated with baseline levels ( $r = -0.75$ ,  $p < 0.001$ , and  $r = -0.68$ ,  $p < 0.001$ , after one and two years of therapy, respectively), this may represent a bias when comparing changes between the two treatment groups. The change in sCD40L after two years was not correlated with the change in IMT ( $r = 0.06$ ), LDL ( $r = -0.07$ ) or hsCRP ( $r = 0.01$ ) in either of the two treatment groups. The statin effects were similar in those with or without CVD (data not shown).

## DISCUSSION

In the present study, we show that patients with FH are characterized by significantly raised serum levels of sCD40L compared with carefully matched healthy controls. Moreover, we found that both “aggressive” (i.e., atorvastatin) and “conventional” (i.e., simvastatin) statin therapy significantly reduced serum levels of sCD40L with no difference between

the two treatment groups. Our findings may suggest enhanced CD40L-CD40 interaction in FH and that this inflammatory response may be downregulated by statins.

We and others have previously demonstrated raised serum levels of sCD40L in angina patients with particular high levels in those with unstable disease (14,15), and the present results suggest that such an increase is not restricted to symptomatic atherosclerotic disease. In fact, markedly raised serum levels of sCD40L (approximately 25-fold higher) were also found in this study in patients with FH, mostly without evidence of CVD. Recently, Garlicks et al. (20) showed a trend toward raised serum levels of sCD40L in asymptomatic patients with moderate hypercholesterolemia, but few patients were studied. Moreover, it was recently reported that, in healthy middle-aged women, plasma level of sCD40L  $>3.71$  ng/ml was associated with significantly increased relative risk of developing future cardiovascular events (21). However, based on leakage from platelets, measurements of sCD40L should preferably be performed in serum or platelet-free plasma, and the results obtained from “ordinary plasma” may be unreliable (15).

In the present study, we found that a significant reduction in IMT during statin therapy was accompanied by a marked reduction in sCD40L. Although the degree of changes was not statistically correlated, this finding may further support a link between atherogenesis and CD40L. In fact, it has become clear that the dyad CD40-CD40L plays a pivotal role in the pathogenesis of atherosclerosis. CD40 is ex-

**Table 2.** Lipid Parameters and IMT in Patients With Familial Hypercholesterolemia Receiving 80 mg Atorvastatin ( $n = 57$ ) or 40 mg Simvastatin ( $n = 53$ ) Daily for 2 Years

	Atorvastatin			Simvastatin			Differences in Changes
	Baseline	1 Year	2 Years	Baseline	1 Year	2 Years	
Total cholesterol (mmol/l)	$10.27 \pm 1.79$	$5.73 \pm 1.11\ddagger$	$5.69 \pm 1.03\ddagger$	$10.80 \pm 2.06$	$7.11 \pm 1.04\ddagger$	$6.79 \pm 1.08\ddagger$	$<0.05$
HDL cholesterol (mmol/l)	$1.14 \pm 0.34$	$1.24 \pm 0.41\ddagger$	$1.23 \pm 0.36\ddagger$	$1.11 \pm 0.28$	$1.25 \pm 0.32\ddagger$	$1.20 \pm 0.32\ddagger$	0.647
LDL cholesterol (mmol/l)	$8.30 \pm 1.74$	$3.94 \pm 1.02\ddagger$	$3.92 \pm 0.97\ddagger$	$8.92 \pm 2.01$	$5.26 \pm 1.06\ddagger$	$4.97 \pm 1.02\ddagger$	$<0.05$
Triglycerides (mmol/l)	$1.92 \pm 0.92$	$1.31 \pm 0.76\ddagger$	$1.31 \pm 0.68\ddagger$	$1.86 \pm 1.03$	$1.42 \pm 0.72\ddagger$	$1.48 \pm 0.94\ddagger$	$<0.05$
IMT (mm)	$0.96 \pm 0.25$	$0.90 \pm 0.22\ddagger$	$0.87 \pm 0.21\ddagger$	$0.92 \pm 0.20$	$0.92 \pm 0.20$	$0.98 \pm 0.22\ddagger$	$<0.001$
hsCRP	$4.1 \pm 5.7$	$2.4 \pm 3.3\ddagger$	$2.1 \pm 2.3\ddagger$	$3.7 \pm 5.3$	$2.0 \pm 2.1^*$	$2.1 \pm 2.2\ddagger$	0.534

\* $p < 0.05$ ; † $p < 0.01$ ; ‡ $p < 0.001$  versus baseline. Data are given as mean  $\pm$  SD.

HDL = high-density lipoprotein; hsCRP = high sensitivity C-reactive protein; IMT = intima media thickness; LDL = low-density lipoprotein.

pressed on a variety of cells (e.g., lymphocytes, macrophages, vascular smooth muscle cells, and endothelial cells) and is present in almost all cell types in human atherosclerotic lesions with enhanced expression in advanced, rupture-prone, and ruptured plaques (22,23). Moreover, binding of CD40L to its receptor on cell membranes (i.e., CD40) induces a diversity of responses with relevance to atherogenesis such as enhanced synthesis of inflammatory cytokines, chemokines, and tissue factor, upregulation of adhesion molecules as well as activation of matrix metalloproteinases (22-28). In fact, interruption of CD40L-CD40 interaction has been shown to impair atherogenesis in LDL receptor-deficient mice consuming a high-cholesterol diet, significantly reducing the size and lipid content of atherosclerotic lesions (28). Moreover, studies using CD40L/low-density lipoprotein receptor or CD40L/apolipoprotein E double-deficient mutant mice confirmed the crucial role for CD40 signaling during the initiation and progression of atherosclerotic lesions in two different mouse strains (22,29).

The immunomodulatory effects of statin therapy are extensively investigated, and we report a marked downregulation of serum levels of sCD40L during such therapy. The decrease in sCD40L was found during both "aggressive" (i.e., atorvastatin) and "conventional" (i.e., simvastatin) statin therapy and was not correlated with the degree of reduction in cholesterol levels. The cholesterol-independent effects of statins appear to be well documented in vitro and in a growing number of experimental models (13,30,31), and the results in the present study suggest that downregulation of sCD40L could be added to the nonlipid effects of statins. Whatever the mechanisms, the ability of statins to decrease sCD40L levels in serum in FH, combined with their previously reported downregulatory effects on CD40 expression on monocytes in patients with moderate hypercholesterolemia (20), suggests that statins could impair CD40L-CD40 interaction in vivo. Thus, based on the potential pivotal role of CD40L-CD40 in all stages of atherogenesis, downregulation of this interaction could clearly contribute to the beneficial effects of statins in cardiovascular disease.

We report that asymptomatic FH patients are characterized by markedly raised serum levels of sCD40L and, even more importantly, concentration of this inflammatory mediator significantly decreased during both "aggressive" and "conventional" statin therapy. These findings suggest enhanced CD40L-CD40 interaction in familial hypercholesterolemia, and our results further support the notion that the beneficial effects of statins in cardiovascular disease are partly relayed through immunomodulatory pathways.

**Reprint requests and correspondence:** Dr. Pål Aukrust, Section of Clinical Immunology and Infectious Diseases, Medical Department, Rikshospitalet, N-0027 Oslo, Norway. E-mail: pal.aukrust@rikshospitalet.no.

## REFERENCES

- Ross R. Atherosclerosis—an inflammatory disease. *N Engl J Med* 1999;340:115-26.
- Serani GGN, Prisco D, Martini F, et al. Acute T-cell activation is detectable in unstable angina. *Circulation* 1997;95:1806-12.
- Mazzone A, DeSeri S, Ricevuti G, et al. Increased expression of neutrophil and monocyte adhesion molecules in unstable coronary artery disease. *Circulation* 1993;88:358-63.
- Chambless LE, Heiss G, Folsom AR, et al. Association of coronary heart disease incidence with carotid arterial wall thickness and major risk factors: the Atherosclerotic Risk in Communities (ARIC) study: 1987 to 1993. *Am J Epidemiol* 1997;146:483-94.
- Hodis HN, Mack WJ, Labree L, Selzer RH, Liu CR, Azen SP. The role of carotid arterial intima media thickness in predicting clinical coronary events. *Ann Intern Med* 1998;128:262-9.
- O'Leary DH, Polak J, Kronmal RA, Manolio TA, Burke GL, Wolfson SK. Carotid artery intima and media thickness as a risk factor for myocardial infarction and stroke. *N Engl J Med* 1999;340:14-22.
- Scandinavian Simvastatin Survival Study Group. Randomized trial of cholesterol lowering in 4444 patients with coronary heart disease: the Scandinavian Simvastatin Survival Study (4S). *Lancet* 1994;344:1383-9.
- The Long-Term Intervention with Pravastatin in Ischaemic Disease (LIPID) Study Group. Prevention of cardiovascular events and death with pravastatin in patients with coronary heart disease and a broad range of initial cholesterol levels. *N Engl J Med* 1998;339:1349-57.
- Shepherd J, Cobbe SM, Ford I, et al. Prevention of coronary heart disease with pravastatin in men with hypercholesterolemia. *N Engl J Med* 1995;333:1301-7.
- Sacks FM, Pfeffer MA, Moye LA, et al. The effect of pravastatin on coronary events after myocardial infarction in patients with average cholesterol levels. *N Engl J Med* 1996;335:1001-9.
- Downs JR, Clearfield M, Weis S, et al. Primary prevention of acute coronary events with lovastatin in men and women with average cholesterol levels: results of AFCAPS/TexCAPS. *JAMA* 1998;279:1615-22.
- Smilde TJ, van Wissen S, Wollersheim H, Trip MD, Kastelein JJP, Stalenhoef AJP. Effect of aggressive versus conventional lipid lowering on atherosclerosis progression in familial hypercholesterolemia (ASAP): a prospective, randomised, double-blind trial. *Lancet* 2001;357:577-81.
- Farmer JA. Pleiotropic effects of statins. *Curr Atheroscler Rep* 2000;2:208-17.
- Garlichs CD, Eskafi S, Raas D. Patients with acute coronary syndromes express enhanced CD40L/CD154 on platelets. *Heart* 2001;86:649-55.
- Aukrust P, Müller F, Ueland T, et al. Enhanced levels of soluble and membrane-bound CD40L in patients with unstable angina: possible reflection of T lymphocyte and platelet involvement in the pathogenesis of acute coronary syndromes. *Circulation* 1999;100:614-20.
- Peng DQ, Zhao SP, Li YF, Li J, Zhou HN. Elevated soluble CD40 ligand is related to the endothelial adhesion molecules in patients with acute coronary syndrome. *Clin Chim Acta* 2002;319:19-26.
- Robbie L, Libby P. Inflammation and atherothrombosis. *Ann NY Acad Sci* 2001;947:167-79.
- Aukrust P, Liabakk NB, Müller F, Lien E, Espevik T, Frøland SS. Serum levels of tumor necrosis factor- $\alpha$  (TNF  $\alpha$ ) and soluble TNF receptors in human immunodeficiency virus type 1 infection—correlates to clinical, immunologic and virologic parameters. *J Infect Dis* 1994;169:1186-7.
- van Wissen S, Trip MD, Smilde TJ, de Graaf J, Stalenhoef AFH, Kastelein JJP. Hs-CRP reduction with statin therapy is associated with regression of carotid IMT in patients with familial hypercholesterolemia. *Atherosclerosis*. In Press.
- Garlichs CD, Smeier A, Eskafi S, et al. Upregulation of CD40 and CD40L (CD154) in patients with moderate hypercholesterolemia. *Circulation* 2001;104:2395-400.
- Schönbeck U, Varo N, Libby P, Buring J, Ridker PM. Soluble CD40 and cardiovascular risk in women. *Circulation* 2001;104:2266-8.
- Schönbeck U, Libby P. CD40 signaling and plaque stability. *Circ Res* 2001;189:1092-103.
- Schönbeck U, Libby P. The CD40/CD154 receptor/ligand dyad. *Cell Mol Life Sci* 2001;58:4-43.

24. Alderson MR, Armitage RJ, Tough TJ, Strockbine L, Fanslow WC, Spriggs MK. CD40 expression by human monocytes: regulation by cytokines and activation of monocytes by ligand for CD40. *J Exp Med* 1993;178:669-74.
25. Reul RM, Fang JC, Denton MD, et al. CD40 and CD40 ligand (CD154) are coexpressed on microvessels in human cardiac allograft rejection. *Transplantation* 1997;64:1765-74.
26. Mach F, Schönbeck U, Sukova GK, et al. Functional CD40 ligand is expressed on human vascular endothelial cells, and macrophages: implications for the CD40-CD40 ligand signalling in atherosclerosis. *Proc Natl Acad Sci USA* 1997;94:1931-6.
27. Henn V, Slupsky JR, Grafe M, et al. CD40 ligand on activated platelets triggers an inflammatory reaction. *Nature* 1998;391:591-4.
28. Mach F, Schönbeck U, Sukhova GK, Atkinson E, Libby P. Reduction of atherosclerosis in mice by inhibition of CD40 signaling. *Nature* 1998;394:200-3.
29. Lutgens E, Gorelik L, Daemen MJ, et al. Requirement for CD154 in the progression of atherosclerosis. *Nat Med* 1999;5:1313-6.
30. Kwak B, Mulhaupt F, Mylly S, Mach F. Statins as a newly recognized type of immunomodulator. *Nat Med* 2000;6:1399-402.
31. Altieri DC. Statins begin to sprout. *J Clin Invest* 2001;108:365-6.