

STATE-OF-THE-ART PAPER

The CD40/CD40 Ligand System

Linking Inflammation With Atherothrombosis

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The role of CD40/CD40 ligand (CD40L) in atherothrombosis is now widely accepted. However, the exact mechanisms linking the CD40/CD40L system and the soluble form of CD40 ligand (sCD40L) with atherothrombosis are currently a topic of intensive research. CD40L and sCD40L belong to the tumor necrosis factor superfamily, and they are molecules with a dual prothrombotic and proinflammatory role. They are expressed in a variety of tissues such as the immune system (in both B and T cells), the vascular wall, and activated platelets. Soluble CD40L has multiple autocrine, paracrine, and endocrine actions, and it may trigger key mechanisms participating in atherothrombosis. CD40/CD40L may participate in the development of coronary atherosclerosis and the triggering of acute coronary syndromes, while sCD40L seems to have a prognostic role not only in subjects with advanced atherosclerosis but also in the general population. Although conventional cardiovascular medication such as antiplatelet therapy, statins, angiotensin-converting enzyme inhibitors, and many others have been shown to reduce both sCD40L and cardiovascular risk, it is still unclear whether specific treatments targeting the CD40/CD40L system will prove to be beneficial against atherothrombosis in the near future. (J Am Coll Cardiol 2009;54:669–77) © 2009 by the American College of Cardiology Foundation

During the last decade, the links between the immune system and atherothrombosis have been well documented, in a way that atherosclerosis is now considered to be an inflammatory disease (1). Therefore, as atherosclerosis has become an epidemic in the Western world, the investigation of the key inflammatory mechanisms involved in atherogenesis is crucial for the design of therapeutic strategies targeting cardiovascular disease.

Over the recent years, it has been well established that interactions between CD40 and its immunomodulating ligand (CD40L), expressed in a variety of cell types including platelets, vascular wall cells, and immune cells, are actively involved in atherogenetic and thrombotic mechanisms and may serve as a link between inflammation, atherosclerosis, and thrombosis (2).

Structure of CD40/CD40L and Regulation of its Expression

CD40 is a type I transmembrane protein receptor and a member of the tumor necrosis factor superfamily, and its gene is located in chromosome 20 (q12-q13.2) (2). Despite the initial suggestion that CD40 exists as a dimer that is

being trimerized after CD40L binding, recent studies described CD40 as a constitutional trimer complex on cell surface (Fig. 1) (3).

The B cells are the main cell type expressing CD40; however, CD40 is also expressed on other immunity cells, epithelial cells, fibroblasts, and vascular wall cells such as endothelial cells (ECs) and smooth muscle cells (SMCs), as well as in platelets (4). In addition, the majority of these cells also coexpress the CD40L receptor. Expression of CD40 is induced by proinflammatory stimuli, such as interleukin (IL)-1, -3, and -4, tumor necrosis factor- α , and interferon- γ . CD40 is regularly up-regulated within 6 to 12 h after the initial stimulation, remaining on the cell surface for 24 to 72 h (4). Transcriptional factors, such as nuclear factor-kappa B and activator of transcription kinases, are known to regulate CD40 expression (4). Similarly, proinflammatory cytokines and oxidized low-density lipoprotein induce the expression of CD40L gene, which is located at chromosome X (q26.3-q27.1) (5).

After its ligation, CD40 is activated and the receptor is internalized into the cell (2). The activated receptor then binds to members of the tumor necrosis factor receptor-associated factor family and stimulate downstream signaling pathways, including nuclear factor-kappa B, with subsequent up-regulation of proinflammatory and proatherogenic genes (6).

Platelets express CD40L after stimulation with a wide range of platelet activators, such as thrombin and thrombin

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**Abbreviations
 and Acronyms**

- ACS** = acute coronary syndrome
- AMI** = acute myocardial infarction
- CAD** = coronary artery disease
- CD40L** = CD40 ligand
- EC** = endothelial cell
- GP** = glycoprotein
- IL** = interleukin
- sCD40L** = soluble CD40 ligand
- SMC** = smooth muscle cell

receptor agonists, for example, collagen, phorbol myristate acetate, and so forth (7). The first studies reported that CD40L expression on platelet surface is dependent on intracellular calcium (Ca²⁺) concentrations and protein kinase C activation (7). Elevated release of soluble CD40 ligand (sCD40L) has also been observed in platelets from diabetic patients after stimulation with thrombin or thrombin receptor activation peptide (8). It was also found that insulin resistance as well as glucose and advanced glycation end products

induce sCD40L release from platelets and increase CD40L expression in murine megakaryocytes (8). Finally, platelet CD40/CD40L expression is also partly regulated by nitric oxide signaling (9). Indeed, as reported by Schafer *et al.* in an elegant clinical study (9) and in an animal model (10), inhibition of nitric oxide synthase in humans results in reduced phosphorylation of platelet vasodilator-stimulated phosphoprotein, and induces platelet activation that overexpress CD40. A similar overexpression of CD40 was also observed in streptozotocin-induced diabetic mice, suggesting that diabetes may result in platelet activation and CD40

overexpression partly by decreasing nitric oxide bioavailability (10).

CD40L in Stable and Acute Coronary Syndromes (ACS): Insights From Basic Science

Atherogenesis and plaque stability. CD40/CD40L expression is known to be up-regulated in atheroma-associated cells but the exact regulating mechanisms remain largely unknown (5,11). In vitro studies have shown that CD40/CD40L interactions on the EC surface result in endothelium and SMC activation and subsequent adhesion molecules expression, an initiating step in atherogenesis (5,11). In addition, recent evidence suggests that CD40/CD40L has a role in dendritic cells and T-lymphocytes interactions inside the vascular wall (12). Indeed, mapping of activated dendritic cells in human carotid and coronary atheroma demonstrated that CD40L affects dendritic cell maturation; activated and fully mature dendritic cells regulate T-cell infiltration, characteristic for unstable coronary atheroma (13). Furthermore, sCD40L has an unfavorable effect on the vascular redox state and endothelium-dependent relaxation, through activation of specific redox-sensitive intracellular pathways. Cell and animal studies demonstrate that CD40L destabilizes endothelial nitric oxide synthase messenger ribonucleic acid and increases vascular oxygen production (14). Therefore, proinflammatory cytokines, reduced nitric oxide bioavailability, and overexpression of adhesion molecules promote leukocyte recruitment and migration into tunica media, participating in atheroma formation (Fig. 2) (11). In addition, interactions between CD40L and CD40 also affect the release of adipokines from adipocytes, contributing to atherogenesis (15).

Further to the interaction between CD40L and CD40, Zirlik *et al.* (16) demonstrated that sCD40L also interacts with the monocyte/macrophage integrin Mac-1, resulting in Mac-1-dependent adhesion and migration of inflammatory cells. In addition, CD40L^{-/-} mice show significantly reduced invasion of inflammatory cells into the peritoneal cavity compared with CD40^{-/-} and wild-type controls, suggesting the CD40L promotes atherogenesis partly independently of its classic receptor CD40 (Fig. 2) (16).

Evidence suggests that CD40/CD40L interactions induce the expression of matrix metalloproteinases that degrade interstitial collagen and the thin fibrous cap of atheromatous plaques, leading to plaque instability and rupture (11). Indeed, inhibition of CD40L signaling enhances atheroma collagen content in mice (11), whereas it has been suggested that CD40L triggers apoptosis and induces the formation of necrotic core in atherosclerotic plaque (11). Furthermore, recent evidence suggests that CD40/CD40L interactions promote in vivo angiogenesis by up-regulating local vascular endothelial growth factor and basic fibroblastic growth factor expression, affecting in

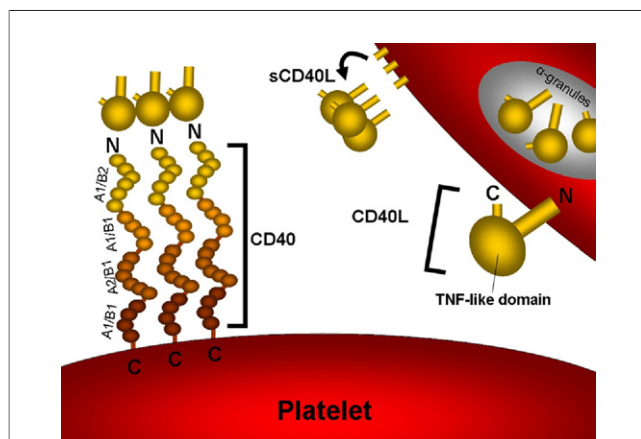


Figure 1 Structure of CD40 Receptor, CD40L, and sCD40L

The CD40 protein, which is a type I transmembrane receptor, exists in trimers, and this trimerization is crucial for its activation. The extracellular region of CD40 is cysteine-rich (consisting of 20 residues). The CD40 protein consists of 4 cysteine-rich domains, and every domain is divided into 2 cysteine modules (A1, A2, B1, B2). The CD40 ligand (CD40L) protein is a type II membrane protein with an intracellular amino terminus and an external carboxy terminus. Its extracellular region belongs to the tumor necrosis factor (TNF) superfamily. The CD40L is stored in α -granules in unstimulated platelets but rapidly translocates to the platelet surface when platelets are activated. The surface-expressed CD40L is then cleaved and shed from the platelet surface as soluble CD40 ligand (sCD40L). C = C-terminal; N = N-terminal.

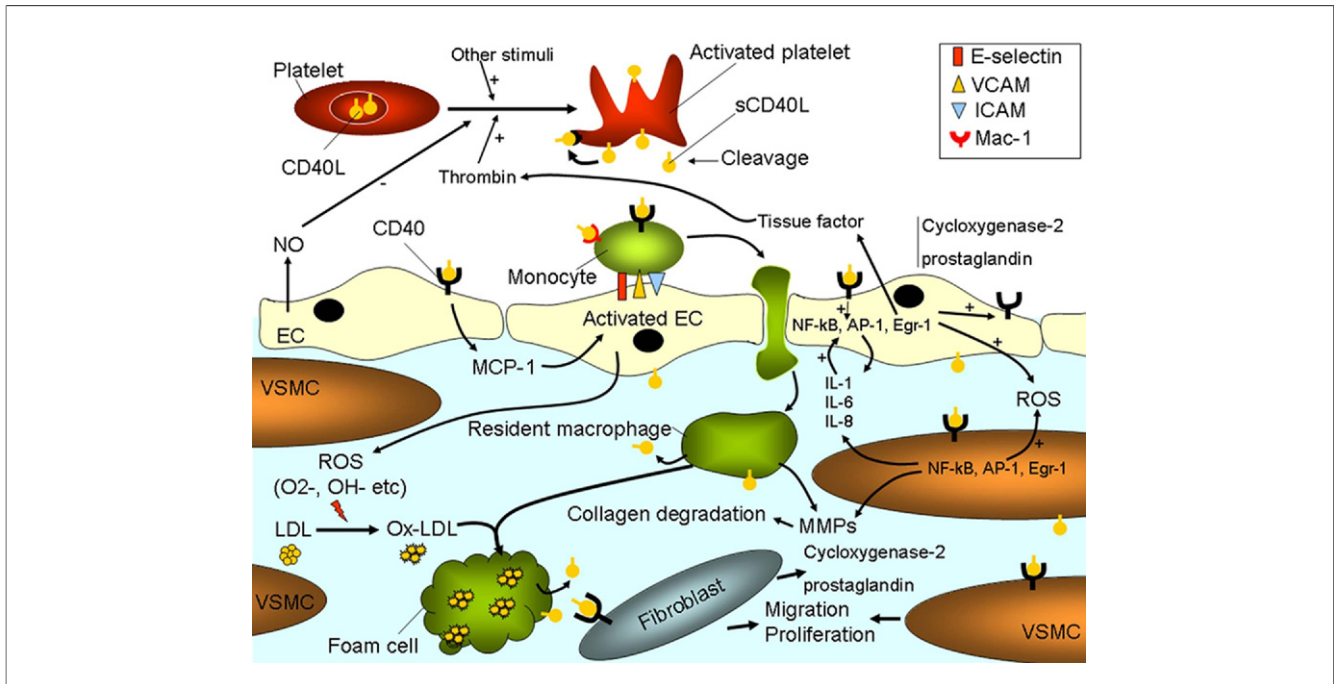


Figure 2 CD40/CD40L and Atherothrombosis

Activated platelets express CD40 ligand (CD40L), which is then cleaved and released as soluble CD40 ligand (sCD40L). The sCD40L binds to circulating monocytes through both its receptor CD40 and through monocyte/macrophage integrin Mac-1, promoting their adhesion to vascular endothelium. The sCD40L also binds to CD40 on endothelial cell (EC) surfaces, which are then activated (by triggering the overexpression of transcriptional factors such as nuclear factor-kappa B [NF- κ B], activator protein [AP]-1, and others) to express adhesion molecules such as E-selectin, vascular cell adhesion molecule-1 (VCAM-1), and intercellular adhesion molecule-1 (ICAM-1) on EC surfaces. These molecules mediate the adhesion and transmigration of monocytes to subendothelial space. Activated ECs and smooth muscle cells (SMCs) also produce proinflammatory molecules such as monocyte chemoattractant protein (MCP)-1 and interleukins (ILs); they release prothrombotic mediators such as tissue factor; and they overexpress enzymes generating reactive oxygen species (ROS). The ROS oxidize low-density lipoprotein (LDL) to oxidized low-density lipoprotein (Ox-LDL) which is up-taken by activated macrophages, which are turned into foam cells. CD40L also activates SMCs and fibroblasts, which are proliferated and migrate to further induce atherogenesis. **Red rectangles** = E-selectin; **red receptors** = Mac-1; **yellow triangles** = VCAM; and **blue triangles** = ICAM. MMP = matrix metalloproteinase; NO = nitric oxide; VSMC = vascular smooth muscle cells.

this way intraplaque neovascularization and plaque vulnerability (17).

Platelet activation and thrombus formation. Further to atheroma formation and plaque weakening, CD40/CD40L interactions have an eminent role in thrombotic events after plaque rupture. The CD40/CD40L interactions induce tissue factor expression on macrophages and ECs and diminish thrombomodulin expression, favoring a local procoagulant and prothrombotic status (18). In addition, the inhibitory role of sCD40L on EC-derived nitric oxide bioavailability may further promote local prothrombotic state (14). Finally, CD40/CD40L interaction inhibits EC migration at the site of the plaque erosion, blocking the re-endothelization process (11).

Platelets are the main source of sCD40L, being responsible for >95% of circulating sCD40L levels (Fig. 1) (19). CD40L stabilizes arterial thrombi by a beta-3 integrin-dependent mechanism that results in platelet activation by outside-in signaling (Fig. 2) (20). Indeed, apart from its receptor, sCD40L also binds to the glycoprotein (GP) IIb/IIIa platelet receptors, inducing their activation, and GP IIb/IIIa antagonists reduce sCD40L release from activated platelets in vitro (21). As we have recently shown, GPIa

platelet receptor may also regulate sCD40L release from activated platelets (22). Carriers of the 807T polymorphism on the GPIa gene, which regulates surface density of the receptor, express higher sCD40L levels during the acute phase of myocardial infarction (22). Noticeably, healthy carriers of the 807T allele also express higher levels of sCD40L in the presence of endothelial injury (22). However, there is evidence that sCD40L may not directly promote atherogenesis (23), because previous studies in mice suggested that CD40L modulates atherogenesis primarily by its expression on nonhematopoietic cell types rather than monocytes, T lymphocytes, or platelets (23). Therefore, as circulating sCD40L is mainly derived from activated platelets, its association with atherothrombosis could be primarily due to its ability to reflect platelet activation and less because of a direct impact in atherothrombosis.

Finally, CD40L (and possibly sCD40L) play a key role in atherothrombosis by being a link between platelets, inflammation, thrombosis, and atherogenesis (18). The most important mechanisms by which the CD40/CD40L axis affects atherothrombosis are summarized in Figure 2.

Table 1 CD40L in Stable and Unstable Coronary Syndromes

Study, First Author (Ref. #)	Population	Measures	Outcome/Conclusion
Aukrust et al. (26)	26 patients with UA, 29 with stable angina, and 19 controls	Serum levels of sCD40L and T-cell expression of CD40L in UA; sCD40L after platelet and T-cell stimulation; sCD40L induction of MCP-1 synthesis from PBMCs	Higher sCD40L levels and CD40L expression on T cells in patients with UA; sCD40L enhances MCP-1 release in PBMCs; enhanced sCD40L release after platelet activation
Wang et al. (27)	20 patients with UA, 10 patients with stable angina, and 10 controls who underwent coronary angiography	Transcoronary concentration gradient of sCD40L and hsCRP in patients with UA (blood samples from coronary sinus, aortic root, and femoral vein)	Higher sCD40L levels in UA and stable angina; higher transcoronary gradient of sCD40L in UA; higher sCD40L in patients with complex lesions
Yan et al. (25)	20 patients with UA, 24 with stable angina, 12 with AMI, and 16 controls	Serum levels of sCD40L and CD40L expression in PBMCs before and after PTCA	Elevated levels of sCD40L in stable angina and ACS; higher CD40L expression in PBMCs in UA/AMI patients; marked increase in sCD40L after PTCA
Peng et al. (28)	15 patients with AMI, 12 with UA, 23 with stable angina, and 30 controls	Comparison of serum levels of sCD40L in ACS patients; correlation between sCD40L levels and adhesion molecules	Higher levels of sCD40L, sICAM-1, and sVCAM-1 in ACS; sCD40L positively correlated with sICAM-1, sVCAM-1, triglycerides, and apolipoprotein B, and negatively correlated with HDL-C
Garlichs et al. (29)	15 patients with AMI, 25 with UA, 15 with stable angina, and 12 controls who underwent coronary angiography or PCI	Serum levels of sCD40L and platelet CD40L at baseline and after 6 months of follow-up	Higher sCD40L levels and CD40L platelet expression in UA/AMI; lower CD40L expression after 6 months of follow-up
Tousoulis et al. (31)	109 patients with AMI, 201 patients with stable CAD, and 286 controls	Circulating levels of sCD40L, VCAM-1, sICAM-1, and IL-6	Higher sCD40L and IL-6 levels in AMI; higher sICAM-1 and VCAM-1 levels in AMI and stable angina group; IL-6 was independently associated with sCD40L only in controls
Ko et al. (35)	18 patients with AMI scheduled for PCI	Levels of sCD40L in culprit coronary artery and relation to circulating levels	sCD40L levels were significantly higher in culprit coronary artery compared with femoral vein
Malarstig et al. (30)	24-month follow-up of 2,359 patients with NSTEMI from the FRISC II trial, randomized to receive early invasive or conservative treatment with dalteparin or placebo	Prognostic value of sCD40L levels in ACS, effect of –3459A>G single nucleotide polymorphism, and treatment with dalteparin on sCD40L levels	In placebo group, sCD40L levels above median (>290 pg/ml) were associated with increased AMI risk; dalteparin reduced AMI risk in patients with sCD40L levels above median –3459A>G polymorphism associated with increased sCD40L levels
Ohashi et al. (34)	17 patients with AMI and 10 patients with UA who underwent PCI	Coronary sinus and arterial levels of sCD40L, association with end-diastolic index or ejection fraction; CD40L expression in intracoronary thrombi	Transcardiac gradient of sCD40L increased at 9 h in both groups; high CD40L expression in thrombi; association between sCD40L and MMP-9 activity in AMI; aspirating thrombectomy reduced sCD40L levels
Tayebjee et al. (32)	204 patients who attended for cardiac catheterization for investigation of suspected stable CAD	Plasma levels of sCD40L in patients with CAD, possible relation to angiographically-proven severity of the CAD	Higher sCD40L in CAD; no relation with severity of the CAD
Rondina et al. (36)	909 patients undergoing angiography; 303 patients with CAD with a cardiac event within 1 year, 303 with CAD without event, 303 without CAD or event	Prognostic value of sCD40L	Higher sCD40L levels associated with decreased risk of CAD in non-ACS patients
Martins et al. (37)	865 patients with chest pain, with or without angiographically confirmed CAD, with or without AMI	Plasma concentrations of CD40L	There was no difference in sCD40L levels between CAD and non-CAD patients
Antoniades et al. (22)	219 patients with premature AMI and 389 controls; followed up for 1 year	Circulating sCD40L and vWF levels measured at baseline (during acute phase of AMI) and after 1 year; impact of genetic polymorphisms C807T and A1648G on platelets glycoprotein Ia on sCD40L levels was evaluated	Patients with AMI had higher sCD40L levels compared with controls; sCD40L levels were significantly reduced after 1 year in the same subjects; C807T was an independent predictor of sCD40L levels during AMI and after 1 year; C807T was associated with sCD40L levels in healthy controls only with high vWF levels

Continued on next page

Table 1 Continued

Study, First Author (Ref. #)	Population	Measures	Outcome/Conclusion
Plaikner et al. (33)	1,089 consecutive patients in an emergency department for medical or neurologic emergencies	Diagnostic value of plasma sCD40L levels for AMI or ischemic stroke	In adults presenting to emergency department, sCD40L is not useful as a diagnostic marker for acute cardiac, cerebrovascular ischemic, or thromboembolic events

ACS = acute coronary syndrome; AMI = acute myocardial infarction; CAD = coronary artery disease; CD40L = CD40 ligand; FRISC II = Fragmin and fast Revascularization during InStability in Coronary artery disease II; HDL-C = high density lipoprotein cholesterol; hsCRP = high sensitivity C-reactive protein; IL = interleukin; MCP = monocyte chemoattractant peptide; MMP = matrix metalloproteinase; NSTEMI = non-ST-segment elevation myocardial infarction; PBMC = peripheral blood monocyte cell; PCI = percutaneous coronary intervention; PTCA = percutaneous transluminal coronary angioplasty; sCD40L = soluble CD40 ligand; sICAM = soluble intercellular adhesion molecule; sVCAM = soluble vascular cell adhesion molecule; TF = tissue factor; UA = unstable angina; VCAM = vascular cell adhesion molecule; vWF = von Willebrand factor.

CD40L in Stable and ACS: Insights From Clinical Studies

After the initial observations from basic science supporting that CD40L perpetuates inflammation and induces thrombus formation in experimental models (1), many studies have examined the potential role of CD40/CD40L interactions in ACS and stable coronary artery disease (CAD) at a clinical level. These studies have demonstrated that circulating sCD40L levels are significantly higher in patients with acute myocardial infarction (AMI) or unstable angina, compared with healthy persons (22,24–33) (Table 1). Indeed, it appears that a gradual increase in sCD40L levels takes place with ACS progression (26,28). Transcoronary sCD40L levels exhibit an early peak just 9 h after onset of AMI or unstable angina (27,34). However, there is no significant difference between AMI and unstable angina patients in circulating sCD40L levels (24). Interestingly, intracoronary CD40L levels are higher in the culprit coronary artery than in the peripheral circulation, reflecting the activation of a potent local inflammatory process (27,31,35).

As we have recently shown, sCD40L levels are independent from the underlying proinflammatory state in AMI or CAD patients, as assessed by circulating levels of IL-6, soluble intercellular adhesion molecule-1, and soluble vascular cell adhesion molecule-1 (24). Conversely, in healthy subjects, sCD40L is strongly associated with IL-6 serum levels (24). Therefore, it seems that in patients with AMI or CAD, circulating sCD40L may be dominantly dependent on sCD40L release from activated platelets and to a lesser extent on inflammatory status, which is a major determinant of sCD40L in healthy subjects (24). Although the clinical importance of circulating sCD40L in ACS is still controversial (28), it seems that measurement of sCD40L can be evaluated either as an inflammatory marker or as a marker of platelet activation, depending on the underlying disease state.

The vast majority of data regarding stable CAD are derived from clinical studies comparing the effect of CD40/CD40L interactions in ACS with the effect in stable CAD and healthy subjects. As we (24) have shown, plasma sCD40L is higher in patients with stable angina than in healthy subjects, but lower compared with patients with

ACS (24). Indeed, multiplicity of risk factors is associated with a summing effect, leading to an even higher increase in sCD40L levels in CAD patients (24), although there is a wide variation between different clinical studies (Table 1) (29,36,37).

Circulating sCD40L levels in stable or unstable coronary syndromes are also affected by genetic determinants. Genetic polymorphism A3459G on sCD40L has been reported as a determinant of sCD40L levels, with carriers of 3459G allele having elevated sCD40L levels during ACS (30). We have previously demonstrated that genetic polymorphisms affecting the expression of GPIa on platelets' surface (such as C807T polymorphism on the GPIa gene) may affect sCD40L levels in clinical conditions such as stable CAD or AMI, whereas this effect is not observed in healthy persons, in whom platelet activation is not the driving force of circulating sCD40L (22).

Taken together, these complex mechanisms regulating circulating sCD40L levels in humans make the interpretation of this biomarker extremely difficult, limiting its clinical use.

Methodological Considerations Regarding Measurement of sCD40L in Humans

A number of methodological issues regarding the measurement of circulating sCD40L levels in humans may partly explain the controversial results provided by the various clinical studies. In more detail, a recent study reported that sample preparation may substantially influence sCD40L levels, suggesting that increased sCD40L levels may result from *in vitro* platelet activation (38). Therefore, it has been suggested that plasma samples are more suitable for sCD40L measurements because serum sCD40L levels are correlated with platelet count and reflect their *in vitro* activation status (36). Furthermore, whole blood should be assessed as soon as possible after blood sampling. Prolonged keeping (3 h) at room temperature before freezing may lead to a false increase in sCD40L levels, whereas temporary storage at 4°C has minimal effects on plasma sCD40L (39). Importantly, sCD40L levels appear to have diurnal variations (being elevated during the light phase compared with the dark phase) (40), indicating the need for standardizing time of blood sampling in clinical studies.

Table 2 CD40L and Risk Prediction in Coronary Artery Syndromes

Study, First Author (Ref. #)	Population	Measures	Outcome/Conclusion
Schonbeck et al. (47)	130 women who had AMI, stroke, or death during 4 years follow-up and 130 controls, from WHS	Prognostic risk value of plasma sCD40L levels in patients with AMI	High sCD40L (>95th percentile) levels associated with increased risk for future cardiac events
Heeschen et al. (19)	1,088 patients with ACS, 626 with acute chest pain (controls) from the CAPTURE trial	Prognostic risk value of sCD40L levels; possible improvement of cardiac risk after the use of abciximab	Higher sCD40L associated with increased risk of death or AMI in ACS group and controls. Abciximab reduced cardiac risk in patients with higher sCD40L levels
Kinlay et al. (48)	2,908 subjects from the MIRACL study	Plasma sCD40L levels	High sCD40L associated with increased cardiac risk. Atorvastatin reduced cardiac risk but did not affect sCD40L levels
Dominguez-Rodriguez et al. (41)	96 patients with STEMI	sCD40L/IL-10 ratio as a better prognostic marker in patients with STEMI	sCD40L/IL-10 ratio was a better predictor of in-hospital adverse events in patients with STEMI
Liang et al. (50)	66 patients with CAD progression in follow-up and 124 patients with no CAD progression on repeated angiography	Predictive role of sCD40L levels in CAD progression	sCD40L did not have a predictive role in CAD progression
Manenti et al. (42)	172 patients with non-ST-segment elevation ACS	Improvement of TIMI risk score prognostic value with the use of CD40L	CD40L could not add prognostic value to the TIMI risk score for patients with non ST-elevation ACS
Olenchock et al. (52)	2,403 ACS patients from placebo arm of the OPUS-TIMI 16 trial	Association between sCD40L levels and cardiovascular outcomes in patients with ACS	There was no association between sCD40L levels and cardiac events such as death or AMI
Tanne et al. (51)	203 patients with CAD who had AMI, sudden cardiac death, or ischemic stroke and 203 control subjects	Association between sCD40L and risk of coronary events in patients with CAD	Increased sCD40L levels were not associated with increased risk of future coronary events in patients with CAD
Cipollone et al. (43)	70 patients who underwent PTCA	Possible relation of pre-procedural sCD40L levels and restenosis risk during 6 months of follow-up	Increased sCD40L levels were associated with restenosis in patients who underwent PTCA after 6 months follow up
Turker et al. (44)	36 patients with stable angina who underwent PCI and stent implantation	Assessing the association between sCD40L levels and in-stent restenosis risk during 6 months of follow-up	Increased pre-procedural sCD40L levels is independent predictor of in-stent restenosis in patients with stable angina
Apple et al. (45)	455 patients presenting on admission with ACS	Prognostic value of sCD40L levels for adverse cardiac events	No association between sCD40L levels and risk of cardiac events in patients with ACS was observed
Morrow et al. (53)	1,524 patients presenting within 24 h of onset of non-ST-segment elevation ACS in the TACTICS-TIMI 18 trial	Individual and combined with traditional cardiac biomarkers (B-type natriuretic peptide, hsCRP, cardiac troponin-I) prognostic value of sCD40L for ischemic events	No association between sCD40L plasma levels and the risk for recurrent ischemic events in patients with non-ST-segment elevation ACS treated with GP IIb/IIIa receptor antagonist
L'Allier et al. (49)	53 patients who underwent balloon angioplasty	sCD40L levels before intervention and at 1- and 6-month follow-up and relation to angiographic restenosis	Sustained elevation of serum CD40 ligand levels 1 month after coronary angioplasty predicts angiographic restenosis
Ferro et al. (46)	231 patients with permanent or persistent atrial fibrillation, and 54 with paroxysmal atrial fibrillation	Plasma levels of sCD40L and relation to vascular events (stroke or AMI) during follow-up (mean 27.8 ± 8.8 months)	Enhanced soluble CD40L level is a predictor of vascular events in patients with nonvalvular atrial fibrillation

CAPTURE = C7E3 Fab Anti-Platelet Therapy in Unstable Refractory Angina; GP = glycoprotein; MIRACL = Myocardial Ischemia Reduction with Aggressive Cholesterol Lowering; OPUS = Orbofiban in Patients with Unstable coronary Syndromes; STEMI = ST-segment elevation myocardial infarction; TACTICS = Treat angina with Aggrastat and determine Cost of Therapy with an Invasive or Conservative Strategy; TIMI = Thrombolysis In Myocardial Infarction; WHS = Women's Health Study; other abbreviations as in Table 1.

CD40L and CAD Prognosis

A growing body of evidence has shed light on many aspects of the role of CD40L in cardiovascular disease, and it has been supported that it may have a predictive value (41–46) (Table 2). In the Women's Health Study, high sCD40L plasma levels were associated with a higher risk of major

cardiovascular events (47). In the CAPTURE (c7E3 Fab Anti-Platelet Therapy in Unstable Refractory Angina) trial (19), patients with high sCD40L levels had an almost 3-fold higher risk for cardiovascular death or AMI (19), whereas in the MIRACL (Myocardial Ischemia Reduction with Aggressive Cholesterol Lowering) study, high

sCD40L was an independent risk factor for recurrent cardiovascular events (19,48). Indeed, it has been suggested that combined assessment of CD40L and troponin-T levels has better predictive value regarding AMI risk (30). Moreover, carriers of the $-3459A>G$ polymorphism in the CD40L gene are at higher AMI risk, indicative of a causal role of sCD40L in the pathophysiology of ACS (30). Notably, a small prospective study suggested that high sCD40L levels predict angiographic restenosis in patients who undergo coronary angioplasty (49). In contrast, other clinical studies suggested that CD40L has no predictive value in patients with CAD (50,51) or ACS (52,53) (Table 2).

In conclusion, given the lack of a standardized methodological approach and the complexity of CD40L pathophysiology, clinical studies have yielded contradictory results (Table 2). Therefore, additional large prospective trials are needed to clarify the possible predictive value of sCD40L in cardiovascular disease.

CD40/CD40L as a Therapeutic Target in Atherosclerosis

Antiplatelet/antithrombotic agents. As activated platelets are the main source of circulating sCD40L, antiplatelet agents were among the first drugs studied for their CD40L-lowering properties. Clopidogrel, which acts via adenosine diphosphate receptor inhibition, completely inhibits sCD40L release from adenosine diphosphate-stimulated platelets; patients under clopidogrel treatment exhibit reduced CD40L platelet expression and circulating sCD40L levels (54). Moreover, it was also found that both short- and long-term treatment with clopidogrel significantly reduces sCD40L levels in patients with stable CAD (55), although a recent study demonstrated enhanced expression of CD40L on platelets surface after 1 year of treatment with clopidogrel 75 mg/day plus aspirin 325 mg/day in patients with CAD (56).

Cyclooxygenase-2 inhibition may also be a potential target. Patients receiving aspirin exhibit decreased sCD40L release from platelets after platelet activation with collagen (21). The GP IIb/IIIa receptor inhibitors, such as eptifibatid, abciximab, and tirofiban, inhibit platelet aggregation as well as sCD40L release in vitro (21). The beneficial effect of GP IIb/IIIa inhibitors has been also demonstrated in clinical studies. The GP IIb/IIIa antagonists appear to be particularly beneficial for the subgroup of high-risk ACS patients with increased sCD40L levels; abciximab significantly reduced cardiovascular risk in patients with elevated sCD40L levels in the CAPTURE trial (19). In addition, as increased sCD40L levels reflect a prothrombotic state, the FRISC II (Fragmin and fast Revascularization during InStability in Coronary artery disease II) study demonstrated that low-molecular-weight heparin (dalteparin) reduces the risk of AMI in non-ST-segment elevation ACS patients with high sCD40L levels (30,57).

Statins. Multiple pleiotropic effects have been attributed to statins; recent in vitro studies have demonstrated that statins can also reduce oxidized low-density lipoprotein-induced or cytokine-induced CD40L expression on human umbilical vein ECs, SMCs, and mononuclear phagocytes in a concentration-dependent way (5). Additionally, incubation of activated platelets with atorvastatin leads to decreased platelet-induced cyclooxygenase-2 expression in human umbilical vein ECs (58). Therefore, statins may be actively implicated in platelet-endothelium interaction. Indeed, at a clinical level, statin treatment reduces plasma sCD40L levels in patients with CAD (59). Notably, the MIRACL study (48) demonstrated that early atorvastatin treatment initiation after ACS reduces the risk for future cardiovascular events associated with high sCD40L levels; nevertheless, atorvastatin treatment only slightly affected sCD40L levels.

Thiazolidinediones. Thiazolidinediones are peroxisome proliferator-activated receptor- γ agonists, widely used in type 2 diabetes mellitus treatment. Evidence from in vitro studies suggests that different cell types, such as platelets or ECs, treated with thiazolidinediones exhibit reduced expression of CD40L messenger ribonucleic acid or protein levels (60). Rosiglitazone treatment for 12 weeks significantly reduced serum sCD40L levels in patients with type 2 diabetes mellitus and CAD compared with placebo (61), although short-term rosiglitazone treatment failed to affect sCD40L levels in healthy persons (62), highlighting the complex mechanisms regulating sCD40L release in different disease states.

Other strategies. Several studies have also investigated the sCD40L-lowering properties of various nutritional and pharmaceutical agents. Patients with AMI receiving n-3 fatty acids on a daily basis for 12 months had significantly reduced sCD40L levels (63). Additionally, in vitro data support a potent effect of antioxidants on CD40L expression, even though it is still unknown if this efficacy confers any clinical benefits. Specifically, CD40L expression is inhibited in activated platelets treated with antioxidants such as vitamin C, superoxide dismutase, or phospholipase A2 inhibitors (57). Other strategies such as angiotensin-converting enzyme inhibitors also reduce sCD40L levels in various subpopulations, as we have previously described (64). Finally, despite the stimulatory effect of homocysteine on sCD40L release, the effect of homocysteine-lowering treatment with folic acid on circulating sCD40L in CAD patients remains controversial (65).

Despite these important findings, the role of CD40/CD40L as a direct therapeutic target in atherothrombosis has been questioned, as both CD40L^{-/-} mice (66) and humans receiving antibodies against CD40L (67) appear to have higher rates of thromboembolic complications, suggesting that inhibition of platelet CD40L may render platelet plugs unstable. Indeed, Langer et al. (66) have suggested that the interactions between CD40 and CD40L-immune complexes may derange platelet func-

tion and be responsible for the thromboembolic complications noted.

Conclusions

CD40L and its soluble counterpart, sCD40L, are molecules with prothrombotic and proinflammatory properties, expressed in a variety of tissues such as immunity cells, vascular wall, and most importantly, platelets. Multiple autocrine, paracrine, and endocrine effects have been attributed to CD40/CD40L, triggering all together atherothrombosis. Patients with both stable and unstable coronary artery disease exhibit higher circulating sCD40L levels, and it is likely that sCD40L may have a predictive value. However, a number of questions need to be resolved in this area of research in the coming years. The protocols measuring serum or plasma sCD40L need to be standardized in clinical practice, as technical issues may explain the huge variation of the findings across the literature. Importantly, evidence suggests that reducing high sCD40L levels by the use of conventional drugs such as antiplatelet agents and statins may lead to improved clinical outcome. However, it is still unclear whether sCD40L can be used as a therapeutic target in cardiovascular disease, as circulating sCD40L levels reflect mainly platelet activation, and the direct involvement of sCD40L (as opposed to membrane-bound CD40L) in atherogenesis has been questioned. Therefore, more clinical studies in different cohorts, using standardized methods for measuring sCD40L, are required to elucidate these issues.

Although the development of therapeutic strategies specifically targeting CD40/CD40L seems promising, the use of antibodies against CD40L has been associated with thromboembolic events, and these data have weakened the enthusiasm for future studies using CD40L/CD40 as a direct therapeutic target in atherosclerosis. Upcoming results of long-awaited large clinical trials would hopefully elucidate the potential use of CD40L (and/or sCD40L) as a reliable biomarker and therapeutic target in cardiovascular disease.

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