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REVIEW ARTICLE

The CD40-CD40L system in cardiovascular disease

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Abstract
The CD40-CD40L system is a pathway which is associated with both prothrombotic and proinflammatory effects. CD40 and its ligand were first discovered on the surface of activated T cells, but its presence on B cells, antigen-presenting cells, mast cells, and finally platelets, is evident. The soluble form of CD40L (sCD40L) is derived mainly from activated platelets and contributes to the pathophysiology of atherosclerosis and atherothrombosis. Indeed, sCD40L has autocrine, paracrine, and endocrine activities, and it enhances platelet activation, aggregation, and platelet-leucocyte conjugation that may lead to atherothrombosis. It has even been suggested that sCD40L may play a pathogenic role in triggering acute coronary syndromes. Conversely, blockade of this pathway with anti-CD40L antibodies may prevent or delay the progression of atherosclerosis. Concentrations of sCD40L also predict risk of future cardiovascular disease in healthy women and clinical outcomes in patients with acute coronary syndromes. However, there are controversial and uncertain points over the application of this biomarker to clinical cardiology. In this review, we provide an overview of potential implications of CD40-CD40L signalling and sCD40L as a biomarker in patients with atherosclerotic vascular diseases.

Key words: *Atherosclerosis, CD40, CD40L, coronary artery disease, inflammation, sCD40L, thrombosis*

Introduction

CD40 (gene locus 20q12-q13.2) is a member of the tumour necrosis factor (TNF) receptor superfamily that can be activated by its ligand CD40L (1,2). CD40, CD40L (also known as CD154), and soluble CD40L (sCD40L) are components of a pathophysiological pathway intimately involved in inflammation and atherogenesis.

Various immune cells including B lymphocytes, T cells, monocytes, macrophages, dendritic cells, eosinophils, basophils, and mast cells, as well as smooth muscle cells, epithelial cells, and activated platelets have capability to express CD40 (3–7). Its ligand, CD40L (chromosome X, gene loc us q26.3-q27.1), is a type II transmembrane protein that also belongs to the TNF superfamily (8,9). However, the presence of CD40L as a surface molecule was first determined on activated T lymphocytes (3), and subsequent studies have established its presence on apparently all cell types that express CD40 (4–7). The free and soluble form of CD40L (sCD40L) is released from the cell surface into the circulation and appears to preserve the biological activity of CD40L.

The aim of this article is to overview the current state of knowledge on the biology of the CD40-CD40L system and its role in the pathogenesis of cardiovascular disorders with focus on the involvement of this system in the processes of atherogenesis, inflammation, and thrombosis.

CD40-mediated signal transduction

Cellular CD40 expression can be induced by different proinflammatory stimuli, such as interleukin (IL)-1, IL-3, IL-4, TNF-α, and interferon-γ (10,11). CD40 usually appears on the cell surface within 6–12 hours following stimulation, where it remains for 24–72

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Key message

- The CD40-CD40L system has autocrine, paracrine, and endocrine activities, and it is actively involved in the regulation of platelet activation, aggregation, and platelet-leucocyte conjugation that may lead to prothrombotic, proinflammatory, and atherogenic effects.

Abbreviations

ACS	acute coronary syndromes
AF	atrial fibrillation
CD40L	CD40 ligand
GP	glycoprotein
IL	interleukin
LDL	low-density lipoproteins
NF-κB	nuclear factor κB
sCD40L	soluble form of CD40 ligand
TNF	tumour necrosis factor
TRAF	TNF receptor associated factor

h before shedding into circulation (10). After ligation by CD40L, CD40 triggers transcription of proinflammatory and proatherogenic genes (11).

The interaction between CD40 and CD40L initiates intracellular signalling via TNF receptor-associated factors (TRAFs) (12). Subsequently, cytoplasmic kinases and effector proteins become activated. CD40 is able to bind TRAF-1, -2, -3, -5, and -6, depending on cell type and function. CD40-TRAF interaction is followed by internalization of the CD40-CD40L complex and propagation of CD40 signalling.

CD40L enhances expression of TRAF-1, -2, -3, and -6 but not TRAF-5 in endothelial cells (ECs) (13). TRAF-1, -3, and -6 deficiency was found to be associated with increased CD40L-induced IL-6 and/or MCP-1 expression by endothelial cells indicating their anti-inflammatory potential. Indeed, TRAF-3 inhibits (essentially proinflammatory) CD40 signalling in the vascular wall (13–15). In contrast TRAF-2 and -5 deficiency is linked to reduced cytokine expression (13). However TRAF-5 deficiency is also associated with increased expression of adhesion molecules and potentiated macrophage lipid uptake and foam cell formation (16).

TRAF-mediated signal transduction and modulation of response to CD40-CD40L

Impaired neointima formation and vascular remodelling has been reported in CD40 receptor-deficient mice as well as in mice with defects in CD40-TRAF-6 but not in CD40-TRAF-2, -3, and -5 (17). This impairment has been linked to decreased inflammatory cell infiltration and matrix-degrading protease activity in which CD40-TRAF-6 signalling plays a key regulator role (17). CD40 deficiency in an atherosclerotic mouse model was associated with a reduction in atherosclerotic burden and a stable atherosclerotic plaque phenotype (i.e. less inflammatory but more fibrotic). In the absence of CD40-TRAF-6 interaction, but not CD40-TRAF-2, -3, or -5 interactions, atherogenesis appears to be completely abrogated in mice. At atherosclerotic lesion sites, TRAF-6 triggers activation of Src/ERK1/2 and IKK/NF-κB proinflammatory pathway monocytes and macrophages (18). Also, CD40-TRAF-6 signalling-deficient mice display reduced blood counts of inflammatory $Ly6C^{high}$ monocytes and impaired recruitment of proatherogenic Ly6C + monocytes to the arterial wall (19).

Taken together, these data indicate that the specific type of CD40-TRAF interaction represents a critical modulator of CD40 pathway proatherogenic activity. Interactions with TRAF-3 and -5 produce predominantly inhibitory effects on the CD40 signalling, whilst TRAF-6-mediated signalling promotes inflammatory responses, CD40-mediated atherosclerotic progression, and appears to play a pivotal role in neointima formation and vascular remodelling. A summary of the complex interactions within the CD40-CD40L signalling pathway is shown in Figure 1.

Release of soluble CD40L into circulation

Among the different cells, platelets are the major source of sCD40L in the blood (20). Recent studies indicate that CD40L is cleaved from the surface of activated platelets to release sCD40L by an enzymatic reaction (21). Although this release process (which leads to sCD40L shedding) has not been precisely elucidated, some evidence points towards a role of matrix metalloproteinase-2 in CD40L cleavage from the platelet surface (13).

The structure of sCD40L has been a subject of debate since some researchers suggested that sCD40L might have inactive monomeric and active trimeric forms, whilst others argued that it only exists in a trimeric form (21,23).

The regulation of membrane-bound and soluble CD40L forms is different and depends on the activator stimulus. For example, in T cell receptor-activated cells, both forms are expressed, and CD28 co-stimulation is shown to increase their expression. The induction of sCD40L preferentially recruits a protein kinase C-dependent mechanism and potentially involvement of the ADAM-10 protease which

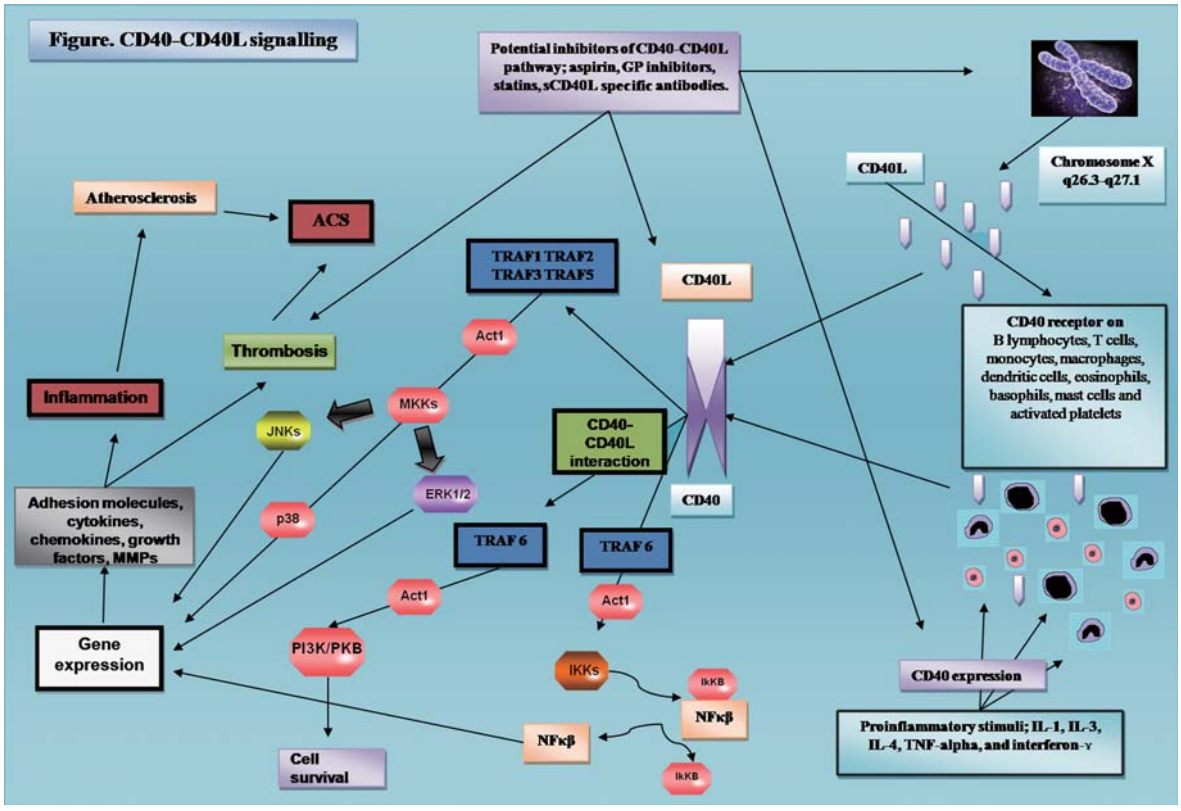

Figure 1. Proinflammatory stimuli increasing expression of both CD40 and CD40L. Once CD40 is expressed on cell surface it interacts with its ligand sCD40L, and intracellular pathways conduct the signal. MKK, NF-κB, and PI3K/PKB (Akt) pathways induce transcription of many inflammatory and prothrombotic mediators. (Act1 = nuclear factor κB activator 1; GP = glycoprotein; IkK = inhibitory κB kinase; IL = interleukin; MKKs = mitogen-activated protein kinase complex; NF-κB = nuclear factor κB; PI3K/PKB (Akt) = phosphoinositide 3 kinases/protein kinase B; TNF-α = tumour necrosis factor α; TRAF = TNF receptor-associated factor.)

was subsequently shown to cleave membrane CD40L to generate sCD40L (24). There are limited data on the precise mechanisms that control the production of sCD40L. Although the main source of sCD40L appears to be activated platelets, it is also cleaved from T lymphocytes with a similar proteolytic mechanism of the cleavage of membrane CD40L shown for platelets (25,26). CD40L triggers expression of adhesion molecules and various cytokines and chemokines from different types of CD40L (27). Beyond its autocrine, paracrine, and endocrine activities, the proinflammatory sCD40L enhances also platelet activation, aggregation, and platelet-leucocyte conjugation which may lead to atherothrombosis.

Soluble CD40L in thrombosis, inflammation, and atherosclerosis

Platelets are pivotal to haemostasis but also have the potential to initiate an inflammatory response of the vascular wall. Once activated, platelets promptly express CD40L and the appearance of CD40L on the platelet surface induces endothelial cells to secrete chemokines and express adhesion molecules, thereby promoting recruitment and extravasation of leucocytes at the site of injury (27). CD40L can be quickly released from the platelet membrane as a soluble form, and, in fact, platelets provide the main source of sCD40L (27,28). Both CD40L and sCD40L have prothrombotic and proinflammatory activities, enhance platelet activation, aggregation, and platelet-leucocyte conjugation, as well as increase the release of reactive oxygen and nitrogen species from stimulated platelets (29).

Although the presence of sCD40L is not essential for platelet aggregation and haemostasis, an experimental study indicated defective platelet-platelet interactions and prolonged bleeding times in CD40L knock-out mice (30,31). Once sCD40L is released from platelets, it triggers activation of various inflammatory and prothrombotic pathways and may serve as a molecular 'bridge' between hypercholesterolaemia, inflammation, and a prothrombotic state (32,33). For example, increased concentrations of sCD40L in hypercholesterolaemic subjects correlate with up-regulation of factor VIIa and prothrombin fragment 1+2 as well as increased platelet activation (reflected by 11-dehydro-thromboxane B2 and P-selectin

expression) (32). Additionally, the reduction of sCD40L by statins parallels a decrease in factor VIIa and prothrombin fragment 1+2 levels (32). One recent study has even shown that activated glycoproteins (GP)Ib, IX, and V induce sCD40L release via thromboxane A_2 from human platelets (33). Importantly, this process was significantly up-regulated in patients with atherosclerosis (33).

Accumulating evidence indicates the connective role of the CD40-CD40L system between inflammation, atherosclerosis, and thrombosis. In a mouse model, CD40L-deficient platelets failed to form platelet-leucocyte aggregates (34). The injection of CD40L-positive platelets contributes to atherogenesis, whilst CD40L-deficient platelets had no effect on atherosclerotic lesion formation.

CD40L appears to promote atherogenesis in a complex way, via activation of leucocytes and increase of platelet-leucocyte and leucocyte-endothelium interactions (34). Moreover, sCD40L induces tissue factor expression on macrophages and endothelial cells and down-regulates endothelial thrombomodulin expression (35). In addition, sCD40L has the ability to bind the GPIIb/IIIa receptors on platelets and induce GPIIb/IIIa activation (36). Vice versa, GPIIb/IIIa antagonists inhibit the release of sCD40L from activated platelets (37). Of note, GPIIb/IIIa antagonists, such as abciximab, eptifibatide, or tirofiban are unable to inhibit this activation-dependent translocation of CD40L from intraplatelet compartment to the platelet surface. However, the release of sCD40L from activated platelets seems inhibited in a dose-dependent manner by abciximab, eptifibatide, and tirofiban (37). Thus, GPIIb/IIIa inhibitors may obviate the proinflammatory and prothrombotic effects of sCD40L. Indeed, GPIIb/IIIa antagonists, when used at doses that optimally inhibit platelet aggregation, can reduce the inflammatory and prothrombotic state driven by sCD40L (36). Nonetheless, regulation of sCD40L release from platelets is a complex process that is only partly mediated by GPIIb/IIIa but also involves actin polymerization and a matrix metalloproteinase inhibitor-sensitive pathway as mentioned above (37).

The CD40-CD40L pathway and inflammation

Activation of the CD40-CD40L system promotes a chronic inflammatory state in the vascular wall, and it contributes to atherogenesis and development of its complications such as acute coronary syndromes (ACS) (12,38). In addition to its activity in blood, the CD40-CD40L system probably executes cell type-specific signalling in the atherosclerotic plaque. Indeed, sCD40L levels are significantly higher in samples collected from the atherosclerotic lesions compared to peripheral samples (39). Increased CD40L levels in atherosclerotic sites significantly correlated with enhanced local inflammatory burden (39). The contribution of the CD40-CD40L system to the atherosclerotic process is complex, and evidence indicates both early and late-phase contributions. CD40L may also promote inflammation independently from CD40 via an interaction with Mac-1 (40).

Activation of the pathway may be triggered by various cardiovascular risk factors. For example, elevated levels of sCD40L have been reported in subjects with several different diseases, including diabetes, impaired glucose tolerance, metabolic syndrome, obesity, insulin resistance, and systemic hypertension (41). In patients with diabetes mellitus, strong correlations between sCD40L and both IL-6 and tissue factor have been established (42). Of note, an intensified medical therapy and life-style modification programme can reduce elevated plasma sCD40L levels in patients with diabetes, with and without overt cardiovascular disease (42). A recent study indicated that even cigarette smoking could induce both the up-regulation of the CD40-CD40L pathway and the formation of platelet-monocyte aggregates (43). Oxidized low-density lipoprotein (LDL) molecules promote the expression of CD40 and CD40L in human atheroma, whilst statins may limit the expression of both markers directly (44). Accordingly, some of the anti-inflammatory pleiotropic actions of statins may be attributed to their impact on reduced CD40 signalling (45).

Interactions between CD40 on antigen-presenting cells and CD40L on T lymphocytes drive the T cell expression of proatherogenic interferon-γ, TNF-α, IL-1, and IL-18 (by Th1 subset) but also potentially antiatherogenic IL-4, IL-5, and IL-10 (by Th2 subset) (46). Generally, CD40L-related activities contribute local vascular inflammation and endothelial dysfunction. The CD40-CD40L system also induces expression of adhesion molecules (vascular cell adhesion molecule, intercellular adhesion molecule, and E-selectin) by the endothelium-facilitating migration of atherogenic leucocytes into the vascular wall (47). However, interactions of leucocyte CD40-CD40L with innate regulatory T lymphocytes had no significant impact on atherogenesis, thus once again demonstrating the complexity of biological roles of the pathway (48).

Vascular implications of the CD40-CD40L pathway

A recent study established that sCD40L promotes neointimal formation after arterial injury and may impair the function of peripheral blood angiogenic outgrowth endothelial progenitors (49). In contrast, therapeutic blockade of sCD40L may accelerate endothelial

regeneration and attenuate neointimal remodelling (49). Plasma levels of sCD40L increase after arterial injury from percutaneous coronary interventions (PCI) (50), and high sCD40L concentrations have been shown to be associated with late restenosis after PCI in which neointimal proliferation and inflammation play a pivotal role (51). These observations indicate a negative impact of the pathway activation in the vascular structure function. Indeed, inhibition of the CD40-CD40L pathway reduces vascular inflammation and might potentially serve as a therapeutic target in cardiovascular disease.

In addition to providing an inflammatory background essential for the initiation of the atherosclerotic process, CD40-CD40L activity is distinctly enhanced inside the plaque, perhaps playing an important role in plaque growth and remodelling. The detrimental role of the pathway in the vascular wall is supported by the observations that CD40-CD40L signalling in macrophages induces expression and release of proinflammatory, proteolytic, and prothrombotic mediators (i.e. monocyte chemoattractant protein-1, MIP-1α, MIP-1β, RANTES, IL-1, IL-6, IL-8, interferon-γ, TNF-α, matrix metalloproteinases-1, -2, -3, -9, -11, -13, and tissue factor) (52). Also, activation of CD40 signalling on vascular smooth muscle cells and endothelial cells increases the expression of inflammatory and prothrombotic mediators (53).

Reflecting the systemic nature of atherosclerosis, elevated sCD40L levels have been found in patients with different localization of the disease including coronary, carotid, and peripheral vascular beds (54). However, in patients with rheumatoid arthritis, despite a significant increase in sCD40L, there was no significant relation between its levels and the carotid intima-media thickness (55). One may speculate that the relative impact of the pathway on the atherogenesis may be smaller in the presence of other concomitant severe inflammatory changes.

CD40-CD40L pathway and cardiovascular complications

Plasma levels of sCD40L are higher in patients with unstable compared with stable coronary disease (38,56). Clinical studies have shown that sCD40L concentrations predict the risk of future cardiovascular disease in healthy women and clinical outcome in patients with ACS (57,58). Increased levels of sCD40L could therefore be considered as a new cardiovascular risk factor. Indeed, sCD40L has been shown to be involved in atherosclerotic plaque destabilization and thrombus formation during the acute phase of myocardial infarction (14,47,52,56). High sCD40L concentrations are linked to clinical outcomes in hospitalized patients with ACS (58). And sCD40L predicted adverse cardiac outcomes in patients with ACS both independently of troponin-I levels and synergistically when biomarkers are used in combination (50). In a recent prospective study, a 48% recurrence of vascular events in diabetic patients with stroke was predicted by increased sCD40L levels (59). Accordingly it seems plausible that distinctive proinflammatory changes associated with activation of the pathway result in accelerated growth and destabilization of atherosclerotic plaques.

The effects of several medications on the CD40-CD40L pathway in cardiovascular pathology have been assessed in parallel with clinical outcomes. For example, the GPIIb/IIIa receptor antagonist, abciximab, has been shown to ameliorate increased risk of recurrent cardiovascular events in ACS patients with high sCD40L levels undergoing PCI (58). Similarly, a substudy of the Myocardial Ischemia Reduction with Aggressive Cholesterol Lowering (MIRACL) trial demonstrated that atorvastatin was able to reduce prominently the risk of recurrent cardiovascular events associated with high sCD40L in patients with ACS (60).

However, results of clinical trials showing a positive link between sCD40L levels and cardiovascular complications are not universal. Several studies have failed to establish a significant relationship between cardiovascular events and sCD40L levels. A poor correlation has been reported between the sCD40L and the individual components or the calculated Framingham Coronary Heart Disease Risk Scores (61). A substudy of the TACTICS-TIMI-18 trial found no association of sCD40L levels with non-fatal recurrent ischaemic event risk in non-ST-elevation ACS patients treated with a platelet GPIIb/IIIa receptor antagonist (62). Tables I and II summarize data on the role of sCD40L in patients with ACS, stroke, and stable coronary artery disease, where some of the diverse observations could reflect the complexity of the CD40L pathway and its biological functions.

The relation between sCD40L and clinical outcomes has been also investigated in clinical settings other than atherosclerosis but is generally not supportive of a significant role for sCD40L in disorders such as atrial fibrillation (AF) or heart failure. Indeed, plasma sCD40L levels were only marginally increased in patients with AF (63). A lack of clear correlation with relevant plasma markers (soluble P- and E-selectins, von Willebrand factor, etc.) suggested that the source of the stimulus was unlikely to be the endothelium or platelets alone (63). Increased sCD40L levels have been shown to have moderate predictive value for stroke and myocardial infarction in patients with non-valvular AF (64,65). Preoperative platelet activation, assessed by sCD40L levels, appears to be predictive for postoperative AF (66). A recent study failed to find

Table I. Impact of sCD40L on cardiovascular outcomes in acute coronary syndromes and stroke.

Authors	Study population	Samples	Main findings/impact on cardiovascular outcomes
Wykrzykowska JJ et al. (80)	98 non-diabetic CAD patients treated with drug-eluting stents	Plasma	sCD40L increased after clopidogrel cessation
Morrow DA et al. (62)	1524 patients with non-ST-elevation ACS	Plasma	No increase in risk of non-fatal recurrent ischaemic events with higher sCD40L in patients treated with glycoprotein inhibitors
Olenchock BA et al. (81)	2403 patients with ACS	Plasma	Absence of association between sCD40L and cardiovascular outcomes (risk of death, MI)
Davi G et al. (59)	90 diabetic patients with acute stroke	Plasma	Up-regulation of CD40L and monocyte chemoattractant protein-1 is associated with increased risk of recurrence of cardiovascular events
Ferro D et al. (64)	231 patients with atrial fibrillation	Plasma	Enhanced sCD40L level predicted vascular events in patients with non-valvular atrial fibrillation
Novo S et al. (82)	42 patients with asymptomatic low-grade carotid stenosis	Plasma	High levels of sCD40L predict the risk of cardiovascular events in this patient group
Malarstig A et al. (83)	2359 patients with non-ST-elevation ACS	Plasma	In a 24-month follow-up, patients with sCD40L levels > 290 pg/mL had increased risk of acute MI; dalteparin reduced MI risk in these patients. A single nucleotide polymorphism, 3459A > G, was associated with increased sCD40L levels
Heeschen C et al. (58)	1088 ACS patients, 626 non-cardiac chest pain patients	Plasma	Higher sCD40L levels were associated with increased risk of death or MI in ACS group and controls. Abciximab reduced cardiac risk in patients with higher sCD40L levels
Manenti ER et al. (84)	172 patients with non-ST-elevation ACS	Serum	No additional prognostic value of CD40L to TIMI risk score
Apple FS et al. (85)	457 patients with ACS	Plasma	No differences in mortality rates were observed between those with increased versus normal concentrations of CD40L

CAD = coronary artery disease; ACS = acute coronary syndrome; MI = myocardial infarction; TIMI = thrombolysis in myocardial infarction.

any association between CD40L and pathophysiology of congestive heart failure as well as any predictive role of sCD40L for clinical events in this condition (67).

The CD40-CD40L pathway as therapeutic target in cardiovascular medicine

The CD40-CD40L system has been investigated as a direct therapeutic target in atherothrombosis in mice and humans by infusion of anti-CD40L antibodies (68,69). Experimental studies have established that blockade of the CD40L pathway can inhibit atherogenesis, and mice lacking CD40L also exhibit delayed lesion progression and inflammatory cell recruitment at sites of atherogenesis (70–73). Such treatment reduces the relative content of macrophages and lipids and increases the proportion of smooth muscle cells and collagen.

Unfortunately, therapeutic administration of anti-CD40L antibodies increases thromboembolic complications. Interactions between CD40 and CD40L antibodies have been suggested as a possible mechanism to explain increased thromboembolic events associated with impaired platelet function (68). Although recent attempts to block the CD40 system by CD40L antibodies have not successfully provided antithrombotic effects, we believe that more precise targets in this system, for example blockage of a specific element of TRAF-mediated signal transduction, may potentially have value in treatment or prevention of atherosclerosis and thrombosis. For example, the blockage of the CD40-TRAF-6 axis is promising since experimentally it has been associated with reduced atherosclerosis (19).

Determination of sCD40L in blood samples: serum or plasma?

Another controversial issue related to the potential clinical application of sCD40L as a biomarker of the

Table II. Relationship between sCD40L levels and cardiovascular outcomes in patients with stable coronary artery disease.

Authors	Study population	Samples	Main findings/impact on cardiovascular outcomes
Obradovic SD et al. (86)	52 CAD patients receiving acetyl salicylic acid and thienopyridine	Plasma	sCD40L increased after percutaneous coronary intervention in patients with high adenosine diphosphate-induced platelet aggregation
Rondina MT et al. (87)	606 patients with stable CAD and 303 controls	Serum	sCD40L levels were lower in patients with CAD. Higher sCD40L was associated with decreased risk of CAD
Tousoulis D et al. (88)	201 stable CAD patients, 109 ACS patients, and 286 controls	Plasma	sCD40L levels were increased in both stable CAD and ACS patients
Tanne D et al. (89)	203 patients with CAD and 203 control subjects	Serum	Higher serum CD40L was not associated with increased risk of ischaemic stroke or coronary events in patients with chronic CAD

ACS = acute coronary syndrome; CAD = coronary artery disease.

cardiovascular risk stems from various methodological issues. Recent studies suggest that sample processing and temperature can profoundly affect the results of sCD40L assays. Serum clotted on ice and platelet-poor plasma are thought to minimize release of sCD40L in *in-vitro* conditions and probably represent preferable media to measure *in-vivo* levels of sCD40L (74). Serum and plasma sCD40L levels appear to be similar in patients with normal platelet counts, but measurements performed with serum clotted on ice give significantly higher sCD40L values than plasma in patients with thrombocytosis (74).

Levels of sCD40L measured in platelet-poor plasma did not differ significantly between stable and unstable angina patients; however, significantly lower levels of sCD40L were determined in serum (75). Another study suggested that plasma samples (but not serum samples) were appropriate for sCD40L measurements (76). Measurement of sCD40L, serum P-selectin levels, and light transmission platelet aggregometry were performed in healthy donors and show that citrated plasma samples yielded the most valid estimates of CD40L levels (77). However, the pre-analytical conditions of these studies should be interpreted carefully because *in-vitro* platelet activation during sample preparation may increase sCD40L concentrations, and disease-related *in-vivo* activation might not contribute to the high plasma sCD40L concentrations (78). The source of sCD40L that we aim to determine (e.g. platelet-derived sCD40L) and patients' platelet count may direct the type of the sample media (i.e. serum or plasma) for its eventual quantification.

The controversy regarding the appropriate specimen and assay procedure for the analyses of blood sCD40L has been revisited in 20 healthy volunteers by ELISA. Serum sCD40L levels were determined to be significantly higher than plasma collected in citrate, EDTA, or heparin, without any significant differences between plasma preparation procedures. The main parameters that influence sCD40L levels appeared to be the presence of platelets in the specimen and high levels of bilirubin, haemoglobin, and triacylglycerols (79). Furthermore, increasing centrifuge gravity has also a decreasing effect on sCD40L levels via platelet depletion.

Reported discrepancies in relationships between sCD40L levels and risk of cardiovascular events may reflect diverse biological roles of the CD40-CD40L pathway and differential implication of various cell types that may serve as a source of sCD40L in a specific clinical condition.

Conclusion

The CD40-CD40L system and its soluble mediator sCD40L are involved in the main inflammatory and thrombotic pathways of atherosclerotic cardiovascular diseases. The interactions between CD40L and TRAFs appear to be a major determinant for the signal transduction. The differential implication of the different TRAFs on various cell types mediates and modulates a wide range of biological effects of the CD40-CD40L pathway. Some of these CD40-TRAFs interactions are pivotal for atherogenesis, neointima formation, and vascular remodelling (e.g. TRAF-6). The system contributes in atherogenesis and thrombosis and works as a bridge between inflammation, atherosclerosis, and thrombosis. However, the clinical role of the pathway remains controversial, and even large-scale prospective studies have reported contradictory results. These discrepancies may in part be related to the methodological limitations of sCD40L assessment. Despite some promising data, our current state of the knowledge does now allow justifying markers of the CD40-CD40L pathway as reliable

clinical tools for the evaluation of the risk of atherogenesis and atherothrombosis. Further studies are clearly needed to elucidate the gap between molecular mechanisms and clinical outcomes.

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