Introduction

CD40L is gaining much attention for its role in the initiation and progression of atherosclerosis. Several distinct lines of investigation in the context of atherosclerosis dealing with low-grade inflammation, oxidative stress and platelet activation are now emerging, with CD40/CD40L system as the missing link. CD40L appears as a multiplayer of several cell types in the inflammatory network. The peculiarity of CD40L as an inflammatory mediator derived from platelets expands the functional repertoire of platelets from players of haemostasis and thrombosis to powerful amplifiers of inflammation by promoting the release of cytokines and chemokines, cell activation and cell–cell interactions. The multifunctional role of CD40L, as a simultaneous activator of all these systems, further blurs the intricate relationship between such events both in the physiological systems and the pathological derangement occurring in atherothrombosis.

Keywords sCD40L • Inflammation • Atherosclerosis • Thrombosis
CD40 leading to further proteolysis of membrane-bound CD40L, with consequent generation of further sCD40L [6].

There are conflicting data as to whether sCD40L stimulates resting platelets by binding to constitutively expressed CD40 during direct cell–cell contact, thus eliciting proinflammatory responses. Inwald et al. demonstrated granule release and enhanced P-selectin expression after incubation of platelets with trimeric sCD40L [11], suggesting that the biological activity of sCD40L may depend on its existence in a biologically active trimeric structure (as in the case of membrane-bound CD40L) [12]. The prothrombotic activity of sCD40L may be attributable to its KGD peptide sequence as infusion of sCD40L with an altered KGD sequence did not reverse the normal phenotype in CD40L-deficient mice [7]. This sequence in turn allows its binding to glycoprotein Ib/IIa, with consequent stabilisation of arterial thrombi [7]. Proof that sCD40L is a GPIIb/IIIa ligand was obtained through experiments of direct binding of sCD40L to purified glycoprotein Ib/IIa [7]. More recently, in vitro studies showed that GPIIb/IIIa antagonists (epifibatide, abciximab and tirofiban) are capable of inhibiting the release of sCD40L in a dose-dependent manner [8, 10, 13], although translocation of CD40L from intraplatelet stores to the surface was unmodified [10]. Taken together, these observations strongly implicate CD40L in the triggering and perpetuation of platelet activation.

CD40/CD40L interactions have also been involved in inflammation and thrombosis. In fact, CD40 and CD40L are coexpressed by virtually all of the cells involved in the processes of atherosclerosis at all stages, such as vascular endothelial cells, smooth muscle cells, macrophages, activated T lymphocytes and platelets [14]. CD40/CD40L interaction on these cellular types triggers a series of events occurring in the vascular wall and in the circulation during the ongoing inflammatory response, events which taken together identify the inflammatory and prothrombotic phenotype observed in both the early and late stages of atherosclerosis. Ligation of CD40 on ECS and VSMCs induces the expression of adhesion molecules such as vascular cell adhesion molecule-1 (VCAM-1), intercellular cell adhesion molecule-1 (ICAM-1) and P-selectin, which in turn promote the recruitment and extravasation of monocytes and lymphocytes at the site of vascular injury [14]. Further recruitment of lymphocytes is elicited by CD40L-induced secretion of cytokines such as interleukin-1 (IL-1), interleukin-6 (IL-6) and tumour necrosis factor-α (TNF-α) by several cells, thus fostering a predominantly Th1 cytokine-driven immune reaction, characteristic for atherogenesis, and of chemokines, such as macrophage inflammatory protein-1α (MIP-1α), MIP-1β regulated upon activation normal T-cell expressed and secreted (RANTES), stromal derived factor-1 (SDF-1) and monocyte chemoattractant protein-1 (MCP-1) [5]. CD40/CD40L signalling in endothelial cells results in the production of reactive oxygen species (ROS), which antagonise endothelial NO production thus promoting endothelial dysfunction. In addition, this signalling also results in upregulation of the interstitial collagenases MMP-1, MMP-8 and MMP-13, contributing to impairment of plaque stability, and in inhibition of endothelial cell migration, thus preventing reendothelialisation of plaque erosion. Finally, CD40 signalling induces tissue factor (TF) expression [15–17], which, while promoting blood coagulation, is also able to activate platelets, which in turn enhance further CD40L shedding with consequent amplification of the inflammatory reaction. In addition, sCD40L increases stimulation-induced platelet release of ROS through activation of Akt and p38 MAP kinase signalling pathways [18]. Recent in vitro and in vivo experiments in a mouse model elucidated a novel alternative pathway for CD40L-mediated inflammation, by interaction with the monocyte/macrophage integrin MAC-1. This interaction enhances adhesion and migration of inflammatory cells and myeloperoxidase release in vitro, and inhibition of MAC-1 in low-density lipoprotein (LDL) receptor-deficient mice attenuates lesion development and decreases macrophages accumulation [19].

All these events may potentially induce or facilitate an acute thrombotic event (Fig. 1).

**Fig. 1** Role of CD40L in the complex interplay between inflammation, endothelial activation/dysfunction and platelet/coagulative activation. Soluble CD40L, shed from platelets upon platelet activation, triggers monocyte activation, leading to release of inflammatory cytokines and TF expression with thrombin generation, and activation of the endothelium, resulting in further inflammation and platelet activation.

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**CD40L and vascular disease**

**Coronary artery disease**

The pivotal role of platelet activation in atherothrombosis, coupled with the finding that most of sCD40L is derived from platelets, has made CD40L an interesting subject in the setting of cardiovascular disease and of acute coronary syndrome (ACS).