Seminars in Immunology 21 (2009) 308–312

Contents lists available at ScienceDirect

Seminars in Immunology

journal homepage: www.elsevier.com/locate/ysmim

Review

The immunobiology of CD154–CD40–TRAF interactions in atherosclerosis

David Engel[a,1], Tom Seijkens[a,1], Marjorie Poggi[a], Maryam Sanati[b], Larissa Thevissen[b], Linda Beckers[a], Erwin Wijnands[a], Dirk Lievens[a], Esther Lutgens[a,b,*]

[a] Dept of Pathology, Cardiovascular Research Institute Maastricht (CARIM), University of Maastricht, The Netherlands
[b] Institute for Molecular Cardiovascular Research (IMCAR), University Hospital Aachen, Medical Faculty, Rheinisch-Westfälische Technische Hochschule (RWTH) Aachen, Germany

ARTICLE INFO

Keywords:
Atherosclerosis
Inflammation
Co-stimulation
CD40
CD154

ABSTRACT

Atherosclerosis is a chronic disease of the large arteries that is responsible for the majority of cardiovascular events. In its pathogenesis, the immune system plays a pivotal role. The effectuation of the immune response through interactions between immune cells that is mediated by co-stimulatory molecules, determine atherosclerosis severity. This review will highlight the role of one of the most powerful co-stimulatory dyads, the CD154 (also known as CD40 *ligand*, CD40L)–CD40 dyad, in atherosclerosis. Its cell-type specific actions, signal transduction cascades and its therapeutic potentials will be discussed.

1. Introduction

Atherosclerosis is the most common underlying pathology of cardiovascular diseases like myocardial infarction, stroke and peripheral arterial disease [1]. In histology, atherosclerosis is characterized by the accumulation of lipid, calcifications, extracellular matrix and immune cells in the arterial wall, the so-called 'plaque'. Atherosclerotic plaques predominantly exist in large- and medium-sized arteries [2]. Atherosclerosis is considered as normal 'wear and tear' of the arteries, and becomes symptomatic when plaques that contain a high amount of inflammatory cells and have low levels of extracellular matrix, the 'thin fibrous cap atheromas', have formed. This plaque-stage is prone to 'plaque rupture'. Rupture of an atherosclerotic plaque exposes its thrombogenic components to the blood and results in thrombosis and often in acute occlusion of the respective artery [2].

Although retention and modification of low-density lipoprotein in the arterial wall form the basis of the onset of atherosclerosis, the subsequent activation of the immune system has been proven to play a pivotal role in its pathogenesis. All cells of the innate and adaptive immune system, such as macrophages, mast cells, natural killer cells, T- and B-lymphocytes and dendritic cells, have been detected in the atherosclerotic plaque, and can exert pro- or anti-atherogenic functions [1,3,4].

Especially the interactions between different immune cells, and their bidirectional signaling define the inflammatory activity within the atherosclerotic plaque. These interactions also mediate the differentiation of different T-lymphocyte subsets, like Th1 (TNF-α, IL-1, IFN-γ, IL-12), Th2 (IL-4, IL-5, IL-10), Tregs (regulatory T-cells) (TGFβ, IL-10) and Th17 (IL-17, IL-6, IL-23), which have been shown to minutely regulate immune responses in the plaque. The Th1 and Th17 subsets are generally pro-atherogenic, while the Th2 and Treg subsets exert anti-atherogenic functions [5]. Polarization of naïve T-cells into effector T-cells is regulated by interaction with antigen presenting cells (APCs: DCs, macrophages, B-lymphocytes), both in the plaque and in lymphoid organs. Upon interaction with antigen presenting cells, naïve T-cells undergo clonal expansion and differentiate into effector T-cells. Moreover, APCs undergo maturation, and start producing pro-atherogenic cytokines like IL-12.

Antigen dependent activation of naïve T-cells, and their differentiation into pro-atherogenic effector T-cells are enhanced by co-stimulation or regulated through co-inhibition. Not surprisingly, most of the characterized co-stimulatory and co-inhibitory receptor–ligand pairs are expressed in atherosclerotic lesions [6,7]. Interestingly, they all have a divergent role in atherosclerotic plaque formation [8] (Table 1).

2. Co-stimulatory molecules in atherosclerosis

Two major families of co-stimulatory molecules include the B7 and tumor necrosis factor (TNF) families [9–12]. These molecules bind to receptors of the CD28 and TNF-receptor family. In the B7/CD28 family, genetic deficiency or inhibition of B7-1, B7-2, ICOS and PD-L1/2 affected atherosclerosis [8]. Deficiency of B7-1 and B7-2 in $LDLR^{-/-}$ mice was shown to inhibit early atherosclerotic lesion development, and reduced the amount of MHCII expression in

* Corresponding author at: Dept of Pathology, Cardiovascular Research Institute Maastricht (CARIM), P. Debeyelaan 25, 6229 HX Maastricht, The Netherlands. Tel.: +31 433876629/+49 241 80 88352; fax: +31 433876613/+49 241 80 82716.
E-mail addresses: E.Lutgens@path.unimaas.nl, elutgens@ukaachen.de (E. Lutgens).
[1] Equal contribution.

1044-5323/$ – see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.smim.2009.06.004

Table 1
Co-stimulatory and co-inhibitory molecules in vascular biology.

	Ref.	Experiment	Plaque inflammation	Plaque fibrosis	Plaque area
B7/CD28 family					
B7-1/2	[13]	B7-1/2$^{-/-}$LDLR$^{-/-}$ mice	–	–	Decreased
	[14]	BMT B7-1/2$^{-/-}$ > LDLR$^{-/-}$ mice	?	?	Increased
CD28	[14]	BMT CD28$^{-/-}$ > LDLR$^{-/-}$ mice	–	+	Increased
ICOS	[15]	Immunized ApoE$^{-/-}$ mice with ICOS-Ig	+	?	Increased
	[16]	BMT ICOS$^{-/-}$ > LDLR$^{-/-}$ mice	+++	+	Increased
PD1/PD2-L	[17]	PD-L1/2$^{-/-}$LDLR$^{-/-}$ mice	+++	+	Increased
TNF/TNFR family					
Ox40L	[18]	Ox40L$^{-/-}$ in C3H/He mice	?	?	Decreased
	[18]	Ox40L tg in C3H/He mice	?	?	Increased
	[19]	Inhibiting mAb in ApoE$^{-/-}$ mice	–	?	Decreased
CD137	[20]	Agonistic mAb in ApoE$^{-/-}$ mice	++		Increased
CD40L	[26]	CD40L$^{-/-}$ApoE$^{-/-}$ mice	– – –	+++	Decreased
	[27]	Antagonistic mAb in ApoE$^{-/-}$ mice	– – –	+++	No effect
	[25,28]	Antagonistic mAb in LDLR$^{-/-}$ mice	– – –	+++	Decreased
	[29,30]	BMT CD40L$^{-/-}$ > LDLR$^{-/-}$ mice	No effect	No effect	No effect
CD40	[38]	CD40$^{-/-}$LDLR$^{-/-}$ mice	No effect	No effect	No effect
	[39]	CD40$^{-/-}$ mice with neointima	– –	++	Decreased
CD40–TRAF	[39]	CD40–TRAF-2/3/5$^{-/-}$ mice with neointima	No effect	No effect	No effect
	[39]	CD40–TRAF-6$^{-/-}$ with neointima	– –	++	Decreased

Overview of the effects of co-stimulatory and co-inhibitory molecules of the B7 and TNF-family in atherosclerosis, with special focus on the plaque area and plaque phenotype. BMT: bone marrow transplantation; . . .> LDLR$^{-/-}$: transplantation of respective bone marrow into lethally irradiated LDLR$^{-/-}$ recipients; –: decrease; – –: stronger decrease; – – –: strong decrease; +: increase; ++: stronger increase; +++: strong increase; ?: unknown, data not available; mAb: monoclonal antibody.

atherosclerotic plaques, and their CD4$^+$ T-cells produced less IFN-γ [13]. However, different results were obtained when B7-1/B7-2$^{-/-}$ or CD28$^{-/-}$ bone marrow was given to irradiated LDLR$^{-/-}$ mice. These chimeric mice developed more atherosclerosis and this was attributed to their impaired Treg development [14]. Similar contradictory results were obtained by studying inhibition of ICOS, a positive co-stimulatory molecule for CD4$^+$ cells. Instead of the expected reduction in atherosclerosis, both immunization with ICOS as well as bone marrow transplantation of ICOS$^{-/-}$ bone marrow into LDLR$^{-/-}$ mice showed an aggravation of atherosclerosis, which was also due to an impaired Treg function [15,16]. Moreover, deficiency of PD–PD-L1/2 interactions, a co-inhibitory dyad, aggravated atherosclerosis, and induced a pro-inflammatory plaque phenotype [17].

These studies with sometimes opposing results illustrate the complexity of co-stimulatory and co-inhibitory pathways which can influence functions of both pro-inflammatory effector T-cells and Treg suppression.

For the TNF and TNF-R family members, the results are more consistent. Inhibition of Ox40–Ox40L signaling results in an impaired atherosclerosis development while mice over-expressing Ox40L have accelerated atherosclerosis [18,19]. The same is true for CD137–CD137L (4-1BB/4-1BBL), where treatment with an agonistic CD137 antibody results in accelerated atherosclerosis and the development of an inflammatory, vulnerable plaque phenotype [20].

One of the most elaborately studied co-stimulatory molecules in atherosclerosis is the CD154–CD40 dyad. Inhibition of CD154–CD40-signaling results in a reduction of atherosclerotic plaque size, but also in the induction of a plaque that is extremely low in inflammation and high in fibrosis (Fig. 1). This makes this intervention one of the most powerful plaque stabilizing therapeutics in a laboratory setting, and therefore a potential therapeutic target for the treatment of human atherosclerosis [21].

In this review, we will highlight the mechanisms how CD154–CD40 interactions affect atherosclerosis in different stages of its pathogenesis. Moreover, we will emphasize the cell-type specific effects of the CD154–CD40 signal transduction cascade. Finally, we will discuss the potential of the CD154–CD40 system as therapeutic target in atherosclerosis.

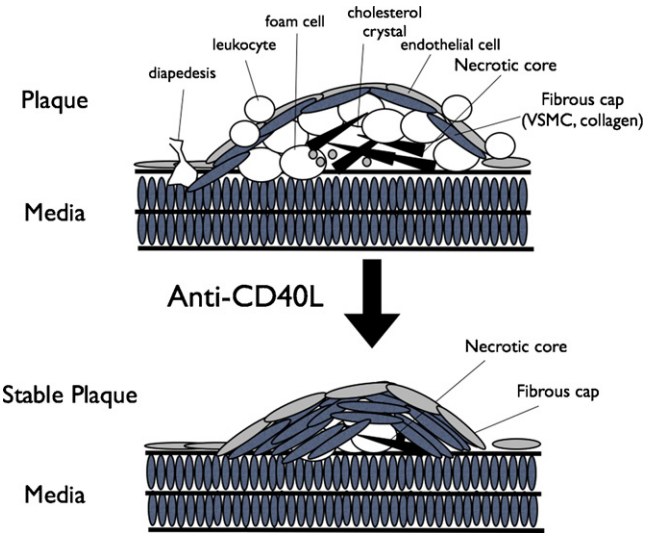

Fig. 1. Inhibition of CD40L reduces atherosclerosis and induces a stable plaque phenotype. The upper panel shows a normal advanced atherosclerotic plaque. The atherosclerotic plaque consists of macrophage foam cells, a necrotic core, VSMCs in the fibrous cap and is covered by endothelial cells. In the atherosclerotic plaque, continuous leukocyte recruitment takes place. The lower panel shows an atherosclerotic plaque in a mouse treated with an anti-CD40L antibody. Inhibition of CD40L reduces atherosclerosis and confers a stable plaque phenotype that contains few inflammatory cells and a high extracellular matrix (ECM) content, the equivalent of a stable atherosclerotic plaque.

3. CD154–CD40 in atherosclerosis

The CD154–CD40 pathway is a special co-stimulatory dyad. It is not strictly a mediator of T-cell co-stimulation, but functions mainly to activate APCs by T-cells. Then, CD40-signaling in the APC induces the expression of other co-stimulatory molecules and thus also activates T-cells [22]. Interestingly, the expression of CD154 is not restricted to T-cells and the expression of CD40 is not confined to the APC, suggesting other functions of the CD154–CD40 dyad than pure co-stimulation [21].